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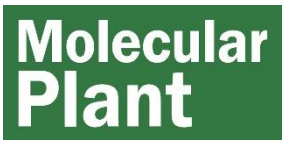
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POSTER ABSTRACTS

Theme 1: Phenotyping on different scales

Components of Non-Photochemical Quenching (NPQ) Detected by Sun-Induced Fluorescence (SIF) and Photochemical Reflectance Index (PRI) in the Field

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Non-photochemical quenching (NPQ) is a phenomenon that is observed in photosystem II (PSII) of plants, algae and cyanobacteria. In the case of land plants, mechanisms of NPQ include energy-dependent quenching (qE) which is triggered by acidification of thylakoid lumen, zeaxanthin-dependent quenching (qZ) which is activated by the xanthophyll zeaxanthin (Z), and photoinhibition quenching (qI) which is associated with photoinactivation of PSII. Although these quenching mechanisms have been intensively studied and well-documented by means of active chlorophyll fluorescence measurements, how they affect sun-induced fluorescence (SIF) in natural environments has not been investigated in the field. If we can use SIF to remotely quantify photosynthetic activity from canopy to ecosystem level, it will offer an alternative approach for field phenotyping, and further, provide the basis for global mapping of vegetation fluorescence and beyond, as envisaged by the Fluorescence Explorer (FLEX) mission of European Space Agency. We analyzed SIF and photochemical reflectance index (PRI) in Arabidopsis mutants lacking the qE (*npq4*) or qZ (*npq1*) component of NPQ in field conditions in summer and winter. Diurnal time courses of SIF and PRI were monitored by measuring hyperspectral reflectance. In parallel, active fluorescence measurements were performed by the light-induced fluorescence transients (LIFT) method. In summer, SIF was able to detect different diurnal responses of NPQ between the mutants and wildtype (WT) while qZ-less *npq1* and qE-less *npq4* were comparable to each other. PRI reflected Z accumulation in WT and *npq4*, but not in Z-deficient *npq1*. Under cold stress in winter, SIF was similarly suppressed in both mutants and WT throughout the day, indicating the effect of strong qI, which was confirmed by low F_v/F_m measured by LIFT. Understanding the diurnal and seasonal contributions of the NPQ components could improve SIF-based photosynthesis models for field phenotyping and remote sensing.

The possibilities and challenges of UAV-borne remote sensing for detection of potato late blight in the field

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In Sweden about one fourth of all fungicides used in agriculture is applied to potatoes (*Solanum tuberosum*), mostly to protect against late blight caused by *Phytophthora infestans*. Currently potato farmers are advised to manually scout their fields, which is time-consuming. It would therefore be of great value to automate this process. Furthermore, automated detection could benefit field trials since it can be carried out more frequently, over larger areas, and be more objective, than if done manually. Photography, or multi-spectral reflectance measurements, using an unmanned aerial vehicle (UAV) is an attractive option.
The aim of the present project was to test the potential to detect, monitor and quantify infestation of late blight in potatoes by UAV-borne remote sensing. The tests were done in potato field trials in Southern Sweden over two seasons with one digital RGB camera and one multispectral sensor with five narrow bands: blue, green, red, red edge and near IR. Images were collected from 8, 14 and 44 m above ground at 8 occasions during 2016 and 2017. Remote sensing data was compaired with the infection levels observed by manual inspection. So far, we are able to associate a visible injury covering 4% of the canopy with a decrease in the red edge reflectance.
In a parallel attempt, we use computer vision and machine learning to detect late blight. To this end, we use the image recognition service Watson in collaboration with IBM. We have evaluated influences of confounding effects such as in field light variation, influence of soil and lesion size, and are creating a pipeline for automated handling of the images. This work is part of the EnBlightMe project (Vinnova 2016-04386), in which we develop an intelligent support system as a prototype app to help the farmer to detect late blight and avoid unnecessary spraying.

Morphological and physiological attributes of Okra against heat stress

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Okra (Abelmoscus esculentum L.) is an important summer vegetable of family Malvaceae. As temperature of the world is rising day by day due to climate change, the physiological behavior of crops is also changing and tolerance for heat is getting minimized. Plant Stress a major concern for agriculture in the era with ever increasing food demands. Among these, heat stress is perhaps the most disturbing one. The aim of this research was to screen different genotypes for high temperature and categoriese them into heat sensitive and tolerant ones. Plants were grown in controlled growth room at institute of Horticulture, University of Agriculture, Faisalabad (28/ 22°C day/ night temperature) for four weeks. Then the heat was gradually increased by 2°C daily to avoid sudden osmotic shock until the desired high temperature (45/35°C day/night) was achieved and genotypes were kept at this temperature for one week. The research findings concluded that heat stress had potentially effected morphological and physiological parameters like photosynthetic measurements, chlorophlyll contents, transpiration rate, sub stomatal CO2, leaf surface temperature and water use efficiency.

Functional analysis of CDPK Related Kinase (CRK) family in Arabidopsis thaliana plant

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The CDPK-SnRK superfamily consists of seven types of serine-threonine protein kinases including the Ca^{2+}/calmodulin-dependent kinase-related kinase (CRK) subfamily, from which the *Arabidopsis thaliana* CRKs are the less characterized. In *Arabidopsis* eight members of the CRK family members have been reported. Previous studies from one of the members of AtCRKs showed that the plasma membrane localized AtCRK5 is required for proper polar localization of PIN2 in *Arabidopsis* roots. Inactivation of AtCRK5 causes root gravitropic defect; reduced root growth and enhanced lateral root formation. In this study, we performed the functional analysis of T-DNA insertion mutants of *Arabidopsis* CRK family members and over-expressing transgenic lines tagged with Green Fluorescent protein (GFP) as well as the characterization of developmental alterations, their response to gravitropic processes in roots/hypocotyls bending. Study of the AtCRK family members with C-terminal GFP tag revealed that they exhibit plasma membrane localization in the roots as it was predicted by their N-terminal myristolyation sites and thus assumed to be important candidates for study of root gravitropic and developmental processes. Delay in the germination rate was also noticed in the AtCRK mutants as compared to wild type. Furthermore, we characterized AtCRK1, which was earlier reported to be thermo-tolerant and salt sensitive member of this family. We found that it had a photo sensible phenotype in continuous light leading to enhanced cell death revealing its potential regulatory role in maintaining of cellular homeostasis during continuous light.
This research was supported by Tempus Public Foundation, Hungary and Biological Doctoral School University of Szeged, Hungary, OTKA PD project No. 115502, PD128055 and OTKA Project No. NN-110962, Ministry for National Economy GINOP-2.3.2-15-2016-00001 and by Hungarian-German TÉT_12_DE-1-2013-0015.

PHENOTYPING PLANT-PATHOGEN INTERACTIONS

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Pathogens usually cause alterations in plant metabolism that can be detected using imaging techniques even in the absence of symptoms. In our previous works we have applied variable chlorophyll fluorescence, multicolour fluorescence imaging and thermography (providing information about photosynthesis, secondary metabolism and transpiration, respectively) to monitor infections on plants caused by virus, bacteria and fungi (Granum et al. 2015 *European Journal of Plant Pathology* 142:625-632; Pérez-Bueno et al. 2015 *Physiologia Plantarum* 153:161-174; Montero et al. 2016 *Physiologia Plantarum* 157:442-452; Pérez-Bueno et al. 2016 *Frontiers in Plant Science* 6:1209; Pérez-Bueno et al. 2016 *Frontiers in Plant Science* 7:1790; Pineda et al. 2017 *Functional Plant Biology* 44:563-572; Pineda et al. 2018 *Frontiers in Plant Science* 9:164).
In the present work, white root rot in avocado trees caused by *Rosellinia necatrix* has been analysed both at lab and field scale using proximal and remote imaging sensors. At lab scale results indicated that changes on plant metabolism only take place at a late stage of the infection. This changes where related to water stress as a consequence of a loss in functionality of the root system. In contrast, the reflectance index NDVI, measured from a remotely piloted aircraft system, proved to be a good predictor of white root rot in avocado orchards, even in the absence of symptoms. Furthermore, we propose the use of a logistic regression analysis fed with NDVI data as a sensitive and reliable method of detection at field scale. This method would be of great help in the control of orchards since current methods to detect *R. necatrix* are based on invasive and time consuming microbial and molecular techniques.
This work was financially supported by CICE-Junta de Andalucía (P12-AGR-0370), MINEICO-CSIC-ERDF funds (RECUPERA 2020/ 20134R060) and AGL14-52518-C2-1-R.

DUS (DISTINCTIVENESS, UNIFORMITY AND STABILITY) CHARACTERIZATION OF SOME MULBERRY (Morus spp.) VARIETIES CULTIVATED IN INDIA.

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Distinctiveness, Uniformity and Stability (DUS) test is carried out following the guidelines provided by Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) of Government of India, New Delhi. The test is conducted for 90 days each of two independent growing cycles per year (June- August and September to November) in on-site testing method on randomly selected six mulberry (Morus spp) varieties cultivated in the agroclimatic region of Malda district of West Bengal. The plants were raised with a crow height of 60 cm and spacing of 150cm × 150 cm in Pit system as per PPV and FRA guidelines. Observations were made on 09 plants equally divided among 03 replications. All the observations on leaf characters were measured using fully expanded mature leaves in the middle portion on the longest shoot. 25 characteristics have been chosen for DUS testing. It is concluded that there is more than one similarity between the triploid variety (Tr-10) and the back cross variety (BC-259) and the wild locally cultivated varieties (Kajli & S1). Distinctiveness is observed in Rotundiloba in intermodal distance, phyllotaxy, leaf angle, leaf shape, leaf hairiness and leaf type as it is a different species, while also have many more similarities with Kajli & S1. Another triploid variety S1635 (hybrid of S1 and Kajli) is found quite interesting in DUS testing as it shows some kind of distinctiveness, also uniformity and similarity in aspect to the 25 DUS characters of mulberry which is helpful in Protection of Plant varieties & Farmers' Rights Authority (PPV & FRA)of Government of India.

Effects of UV-B/gamma radiation on physiological development and DNA damage in Scots pine

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In plants, ambient UV-B radiation has been suggested to prime protective responses towards various stressors. Also, it may be speculated whether UV-B could affect sensitivity towards gamma radiation. Using a ^{60}Co gamma source and fluorescent UV-B-tubes under controlled conditions, we aimed to investigate the effect of UV-B on responses to low levels of gamma radiation in seedlings of Scots pine. After 6 days of gamma exposure (1-540 mGy h^{-1}) plant size was reduced with increasing dose rate ≥40 mGy $h^{-1.}$ Combined UV-B-gamma (≤100 mGy $h^{-1)}$ for 6 days resulted in a slight trend only of additional decrease in plant size. H_2O_2 levels were then increased with increasing gamma dose rate but no significant effect of UV on ROS or total antioxidant capacity was observed. On the other hand, increased DNA damage (Comet assay one hour after light on in the morning), with increasing gamma dose rate was then observed with an additive effect of gamma and UV-B. However, although the gamma dose-rate-dependent growth-inhibiting effect and cell damage (at ≥100 mGy $h^{-1)}$ persisted after termination of exposure there were no clear after-effects of UV-B. To shed further light on interactive UV-gamma effects, experiments with pre-exposure to UV-B before gamma or combined UV-B gamma treatment are conducted and results from these studies where UV-B-induced antioxidants are analysed, will be discussed.

Screening for novel waxy alleles in winter wheat TILLING population

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Starch is a main component of wheat grain and consists of two glucan polymers amylose and amylopectin with the ratio ranging between 25 and 75%, respectively. The amylose/amylopectin ratio has a major influence over the physiochemical properties of starch and determines its optimal application in the industry. Recently, amylose-free (Waxy) and high-amylose wheats, consisting up to 100% apylopectin and 70% amylose, respectively, were produced through the development of new biotechnology techniques. The starches of these wheats provide the unique starch functional properties that are desirable for food and non-food industries.
Targeting Induced Local Lesions in Genomes (TILLING) population of the two winter wheat cultivars (‘Kena DS’ and ‘Gaja DS’) were developed using ethylmethane sulfonate (EMS) in order to induce mutations in genes of interest. Mutation density of one mutation per 37.84 Kb of the DNA was observed in this TILLING population While screening freezing tolerance associated genes. In this study, pilot experiment to evaluate amylose content variation in our material was performed. M3 generation genotypes of TILLING-population and its wild type cultivar ‘Kena DS’ and Waxy wheat breeding line as control were chosen to identify variation for amylose content using traditional Iodine-Potassium Iodide (I2–KI) solution. Low blue colour intensity indicating low amylose content was observed in a subset M3 genotypes. This indicates our TILLING population will serve as a valuable source of novel alleles of starch biosynthesis genes in order to develop winter wheat cultivars with various amylose content. Further work will be: (1) to identify amylose content variation in 756 genotypes of M3 TILLING population using traditional I2–KI dyeing method; (2) to estimate precize amylose content in selected genotypes by spectrometry; (3) to make sequence analysis of key starch biosynthesis genes of selected genotypes in order to identify novel alleles

IDENTIFICATION OF KEY GENES INVOLVED IN VACUOLAR ACIDIFICATION OF APULIAN AUTHOCTONOUS GRAPEVINE VARIETY MARESCO BY A RNA-SEQ APPROACH

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Italian spumante has a long tradition, deeply-rooted in the Piedmont region since the 19th century. The final characteristics of a spumante are strongly influenced by several factors, including the grapevine variety and growing conditions. Moreover, the acidity of berry plays a fundamental role in the final quality and taste. The acidity is strongly influenced by the cell vacuole in which it is determined by the expression of several genes.
The cultivar Pinot Bianco is one of the most famous variety used for spumante production and presents a pretty high level of vacuolar acidity in temperate climate.
The Apulia Region (Sud-Est of the Italian peninsula) promotes the protection, characterization and identification of autochthonous germplasms. In this regard, regional projects aim to support, through integrated multidisciplinary approaches, an effective recovery and study of the grapevine germplasm, as a prerequisite to the exploitation of the regional agro-biodiversity.
With the purpose to study the vacuolar acidification pathway in grapevine and identify varieties of Apulia origin which could be used in the production of spumante, the Maresco variety (characterized by good acidic vacuoles) is currently under investigation by whole transcriptome analysis, using the Pinot Bianco variety as comparison.
The RNA-seq approach is being used for transcriptomic analysis of berries harvested from Maresco and Pinot plants cultivated in the same Apulian fields (CRSFA experimental field in Locorotondo, Bari, Italy). Berries were collected at two time points (t_0 and t_1) corresponding to pre-veraison and 50% of veraison, respectively. Skins and flesh of berries were separated before RNA extraction. RNA purifications were carried out in triplicate for each cultivar and time point.
Purified RNA was used for preparation of libraries using the TruSeq RNA Sample Preparation Kit v2 (Illumina, USA).
RNA-seq sequencing data and identification of highly expressed genes, possibly related to vacuole acidification process, will be presented.

High-throughput proximal remote sensing using LiDAR to examine genotypic variation in radiation use-efficiency, biomass and yield of wheat and triticale

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Wheat yield potential gains need to accelerate fast if the looming increase in worldwide demand for wheat is to be met. Substantial gains in crop biomass will be part of the solution but there has been relatively little long-term progress in biomass. The accumulation of biomass (typically measured as above-ground biomass, AGBM) is a function of (1) the accumulated amount of radiation intercepted by the canopy (Rint) and (2) how effectively that intercepted radiation is converted to AGBM, i.e. the radiation use-efficiency (RUE).
Measuring AGBM and Rint to derive RUE is tedious and time-consuming, conventionally involving destructive, ground-level cuts of AGBM and periodic measures of Rint. Here we introduced proximal remote sensing, using LiDAR, for high-throughput sensing of components of RUE in experiments aimed at assessing causes of genotypic variation in AGBM accumulation in wheat. Using manual and LiDAR-based methods, we measured AGBM and Rint of several near-isogenic wheats varying over a large range in height, then final grain yield (GY). Likewise, we also compared many released and advanced breeding lines of triticale and wheat.
For the wheat height isolines, AGBM production at anthesis was strongly, positively correlated with height but there was no difference in Rint. Hence, RUE was also strongly correlated with height. Tall triticales had greater anthesis AGBM than wheat due to both greater Rint and higher RUE.
Shorter triticales, equivalent in height to semi-dwarf wheats, had higher HI than taller triticales but not higher GY because of lower AGBM. This was partly due to lower RUE associated with shorter stature, as found for wheat.
Despite being substantially taller than wheat, the HI of tall triticale was unexpectedly a little higher than wheat. The HI of shorter-statured triticale was higher still, suggesting potential to improve both biomass and HI of wheat to achieve future yield potential gains.

Leaf surface chemical and optical properties reflect variation based on the genotype and provenance of origin in silver birch

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Leaf surface secondary metabolites of cuticular wax layer on silver birch act as barrier against herbivores and pathogens. The leaf surface structures including the trichomes, wax thickness and texture influence the reflectance, transmittance and absorbance of light on the leaf thereby affecting the leaf optical properties. We study the genotypic and provenance based leaf surface variation of micropropagated trees from southern (60°N), central (62°N) and northern (66°N) latitudes of Finland growing in a common garden field, Joensuu (62°N). Leaves of four genotypes per provenance were sampled. We assessed the leaf surface secondary metabolites with HPLC-MS and leaf spectral reflectance properties with the visible and near infrared (VNIR) and short wave infrared (SWIR) hyperspectral cameras in the laboratory. Variation in the chemical profile was evident in PCA and a good class separation was attained in LDA with an accuracy of 97% for genotype and 94% for provenance. Herbivory indices showed negative correlation with the contents of the secondary metabolites such as flavonoid and triterpenoid aglycones. Significant variation of the provenance and genotype in the reflectance profile of VNIR and SWIR imaging was represented in PCA with a better class separation in LDA.

Our results showed that accumulation of leaf surface secondary metabolites varied more strongly among the genotypes than among the provenance. This study implies that the secondary metabolites on leaf surface are associated with herbivore resistance in silver birch. Thus potential role of surface secondary metabolites needs to be considered in further studies of plant–herbivore interaction and resistance breeding. The results from optical properties of the leaves showed much stronger provenance variation compared to the genotype. We conclude that hyperspectral imaging is a good technique to detect the genotypic and provenance related variation in silver birch non-invasively.

Monilia laxa phenotyping in apricots

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Organic apricot production is currently not profitable. The main obstacle to sustainable profitability is brown rot caused by the fungus *Monilia laxa* (Aderh. & Ruhl). In the current apricot germplasm no source of total resistance has been shown, but some varieties are expressing interesting levels of tolerance. In order to highlight the component of tolerance involved, different phenotyping methods of evaluation of *M. laxa* were carried out and compared in a segregate bi-parental population of 180 descendants between Bakour (tolerant) and Bergeron (susceptible). The first phenotyping consisted in a visual evaluation of Monilia symptoms in the tree (from 0 to 100% of infection) 35 days after full blossom. Out of the calibration between trees with various level of vigour and floribondity, one of the main concern in orchard deals with the phenological stage of the different genotypes and the interaction with climatic conditions during the blooming period in particular the level of humectation. To avoid or reduce part of these risks an experiment under controlled conditions with ideal parameters for Monilia growth has been performed. For the first test, a plug of *M. laxa* mycelium was put on branches (20°C, 80% HR, darkness), and 15 days after the length of the infection was measured. For the second test, a spore suspension of the same strain (10^4 conidia / ml) was sprayed in flowers (20°C, 90% HR, 14 hours day), and percentage of infected flowers was measured 36 hours after inoculation. Different levels of infection were observed within the bi-parental population for the three phenotyping evaluations, but the segregation has been observed only for the visual and branches infections. As field, branch and flower evaluations were not highly correlated, QTL detection will be conducted on all the traits for identifying the components involved in the apricot tolerance to Monilia.

PHYSIOLOGICAL PHENOTYPING OF BREAD WHEAT GENOTYPES UNDER MEDITERRANEAN CONDITIONS

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Physiological phenotyping can help to identify useful traits that are related with crop performance under water-limited conditions. The objectives of this work were to: i) investigate the photosynthetic mechanisms involved in the tolerance to water deficit; ii) analyse the relationship between the assessed physiological traits and grain yield (GY); and iii) identify those characters that could have a greater impact in the determination of tolerant genotypes under field conditions. We studied a set of 14 bread wheat genotypes with contrasting tolerance in terms of yield performance under terminal drought, in Mediterranean environments under well-watered and water-limited conditions. No significant differences were found among genotypes for leaf water potential (Ψ) and leaf gas exchange in 2015 and 2016, except for stomatal conductance (gs) and transpiration rate (E) at grain filling stage. The relationship between Ψ and gs was fitted by an exponential model; at Ψ < -2 MPa the stomatal conductance was lower than 200 mmol m^{-2} s^{-1}. The chlorophyll fluorescence parameters were in general significantly different among genotypes and environments, in 2015 and 2016. The relationship between GY and gs was curvilinear, showing that at gs < 150 mmol m^{-2} s^{-1} there was a strong reduction in GY. Also, a negative and exponential relationship was found between GY and the quantum yield of non-photochemical energy conversion (Y(NPQ)). Finally, positive and linear relationships were found between GY and maximum rate of electron transport rate (ETRmax) and chlorophyll index.
Acknowledgements: To projects Fondecyt 1150353 and Adapting Agriculture to Climate Change (A2C2), Universidad de Talca.

Unravelling internal rhythms in the bryophyte Porella platyphylla

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The circadian clock anticipates to predictable changes, regulating diverse aspects of plant growth and development and usually increases plant fitness. It is synchronized by daily cyclic environmental cues, mainly light and temperature. While angiosperms and green algae clocks have been intensively modelled, it remains challenging to study functional characterization of the clock of the closest relatives to the first land plants, the bryophytes. Their phylogenetic position and ecological importance makes them fundamental organisms to reveal the mechanisms regulating the ancestral clock. Circadian rhythms of monospecific carpets of the bryophyte *P. platyphylla*, exposed to a training phase during five days (light and/or humidity cycles) followed by four days of continuous conditions (light and/or humidity), was measured by high-throughput techniques of delayed fluorescence and imaging fluorescence. These techniques allow temporal variation quantification in a real-time and non-destructive manner. We did not find any variation under continuous light when plants were previously exposed to light/dark cycles, suggesting that light cycles do not set the clock governing endogenous rhythms. However, our results showed that humidity/dryness cycles were the most important environmental cue setting the clock in *P. playphylla.* This conclusion agrees with a recent study[1], which showed that the circadian rhythms of early land plants were weak compared with those in angiosperms, but basic metabolic processes as photosynthesis may be under circadian control. Further research involving a complex set of environmental conditions simulations is needed.

[1] Linde et al. 2017. New Phytologist 216: 576-590.
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A low-cost method for high-throughput phenotyping of transpiration efficiency

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Productivity of wheat in rain-fed environments is frequently restricted by limited available water. When access to water is restricted, yield gains can be unlocked by increasing the efficiency with which plants are able to convert available soil moisture into useable plant biomass (transpiration efficiency, TE). Low-throughput/high-input screening techniques that have been used up-to-now to measure TE restrict the ability to phenotype large numbers of genotypes quickly and accurately in resource-limited programs. A low-cost, low-technology, and easily scalable method is proposed to allow continuously watering of the pots and frequent monitoring of water use. This method was tested for 11 diverse wheat genotypes sown at three different dates. The minimal period required to reliably evaluate genotypes for TE was assessed by harvesting plants at different stages. Significant genotypic variation for TE was only detected once plants had reached a certain size. Slight variations were observed in the ranking of genotypes depending on the phenological stage at harvest and the growing environment. Overall, the TE ranking of the studied genotypes remained generally consistent across growing conditions for harvests occurring after the expansion of the flag leaf. This result indicates that the throughput of TE phenotyping platforms can be increased by reducing the trial duration, thus allowing multiple trials to be performed within a season. The method developed allows relatively fast screening for TE with a low-cost platform.

Using Protective Non-Photochemical Quenching (pNPQ) for Assessing Rice Canopy Productivity

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Plants have a love/hate relationship with light. Plants require light to convert carbon dioxide into food (photosynthesis). However, at increasing light levels, net photosynthesis is reduced and reaches a plateau, resulting in excessive excitation energy in pigment protein complexes. The excess energy would harm the reaction centres resulting in a condition called photoinhibition. Plants remove this excess energy via a harmless pathway called non-photochemical quenching (NPQ). However, it is not known how much of NPQ is actually protective and this is important since it momentarily reduces the quantum yield of photosynthesis resulting in a potential loss in productivity. Studying pNPQ (protective NPQ) at the canopy level is important for crop canopy productivity. So far, pNPQ has only been used for work with model plants such as *Arabidopsis thaliana*.
pNPQ can now be quantified in a non-destructive manner using dark-adapted photochemical quenching (qP_d) (Ruban and Murchie, 2012). qP denotes the number of photosystem II (PSII) reaction centres that are intact and open. In the absence of photoinhibition, qP_d (measured in the dark) is 1. The onset of photoinhibition is denoted by a qP_d value lesser than 1.This allows early detection of onset of photoinhibition during different ontogenetic phases in all plants *in vivo*. Hence, when translated to canopies, it could be used to accurately estimate light tolerance level simultaneously in leaves at different states of light saturation.
Rice canopies with different expression levels of the photoprotective protein PsbS were used. Youngest fully emerged leaves were dark adapted for 45 minutes prior to fluorescence measurements under different actinic light settings using a Walz Junior PAM (Walz Effeltrich, Germany) as described in Ruban and Belgio (2014). Calculations for ϕ_{PSII} and qP_d were made following Ruban & Murchie (2012). Here we show that the qPd method works effectively in rice.

Infection of Norway spruce by needle bladder rust: Quantitative assessment of disease severities by image analysis

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High elevation spruce forests of the European Alps are frequently infected by the fungal pathogen *Chrysomyxa rhododendri* ('needle bladder rust'), which causes remarkable defoliation, reduced tree growth, and limited rejuvenation. Exact and time-efficient quantification of the disease severity on different spatial scales is crucial to improve monitoring, management and resistance breeding activities. However, determination of the disease severity is so far based on visual assessments or laborious counting of diseased needles.

Based on the distinct yellow discoloration of attacked needles during summer, we investigated whether image analysis of digital photographs can be used to quantify the disease severity and to improve phenotyping compared to conventional assessment in terms of time effort and application range. The developed protocol included pre-processing and analysis of digital RGB images, gained in ground surveys and by the use of a semi-professional quadcopter.

Disease symptoms and healthy needle areas were accurately identified by the established method and enabled to calculate the percentage of diseased needle area. Obtained disease severities correlated linearly with results obtained by manual counting of healthy and diseased needles. This applied to all approaches, including images of individual branches with natural background (R^2 = 0.87) and with black background (R^2 = 0.95), juvenile plants (R^2 = 0.94), and top and side views of entire tree crowns of adult trees (R^2 = 0.98 and 0.88, respectively).

Results underline that a well-defined signal related to needle rust symptoms of Norway spruce can be extracted from images recorded by standard digital cameras and by using camera drones. Attention should be paid to prevent artefacts due to suboptimal light conditions, large shadows, and discolouration during advanced disease stages. The presented protocol enables precise and time-efficient quantification of disease symptoms caused by *C. rhododendri* and provides several advantages compared to conventional assessment by manual counting or visual estimations.

Physiological phenotyping of Abies nordmanniana as a basis for developing phytohormone-based strategies to improve Christmas tree production

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Abies nordmanniana is the major tree species cultivated for the production of Christmas trees and is therefore considered to be a high-value crop. The 12 years production time of *A. nordmanniana* trees is quite lengthy and the normal growth pattern results in tree morphologies that are not in favor of the consumers' wishes. During the first years, trees grow relatively compact with limited apical growth, while in later years the top leader elongation rate is too high. Understanding the physiological and metabolic mechanisms causing these growth patterns would allow for directed management to optimize production time and the product quality. In a physiological phenotyping approach, trees of distinctly different growth were analyzed for their phytohormone profiles comprising abscisic acid, auxin, various cytokinins, the ethylene precursor ACC, jasmonic acid, and salicylic acid, in tissue critical for tree growth and development. Profiling of these phytohormones was complemented by analysis of carbohydrate metabolism based on sugar content and central enzyme activities as well as by determination of antioxidative enzyme activities. The integrated data could be correlated with effects such as growth retardation of individual trees. These detailed analyses strongly contribute to our understanding of physiological and biochemical growth control in gymnosperm tree species. The knowledge gained from this physiological phenotyping approach will also directly feed into the development of phytohormone-based strategies to control growth and decrease cultivation time in Christmas tree production; concepts of these strategies will be discussed.

Large scale phenotyping of starch granules

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Plants polymerise simple sugars into starch and store it in form of water insoluble semi-crystalline starch granules. This starch is central to human nutrition, as it is the most important source of carbohydrates in human diet and for feeding our livestock. Further, it has innumerable industrial applications, like in papermaking and brewing. The various industrial sectors utilise starch from a broad variety of plants to cover their specific requirements linked to certain properties of starch granules. These granules differ in shape, size and chemical composition among plant species and tissues. The processes that determine the number and morphology of starch granules are poorly understood from the perspective of starch biosynthesis and plant physiologists are interested in finding mutants with altered starch phenotypes. A comprehensive examination of starch granule shape and size distribution is however time and labour intensive. To overcome these constraints, we developed flow cytometry and image analysis into high-throughput methods to resolve granule size distribution and morphology. Flow cytometry allows measuring the size-distribution of many starch granule samples (96-well format) in a short time. Similarly, the high-throughput image acquisition is performed in a 96-well format using an automated light microscope. The acquisition time for the same sample amount is comparable to flow cytometry and has the advantage to spot morphological differences. That comes at higher costs, as it requires a novel approach to automated image analysis. Both methods can be applied to starch samples from a wide variety of sources, like leaves of the model species Arabidopsis as well as to samples from tubers or cereal endosperms.

Characterization of Carbohydrate Metabolism During the Transition from Primary Root Growth to Taproot Development in Sugar Beet by Physiological Phenotyping

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In sugar beet taproots, an anomalous type of secondary thickening growth via a series of supernumerary cambia is paralleled by sucrose accumulation. The underlying regulatory mechanisms are, however, still poorly understood. Since sugar beet studies to date mainly concentrated on source-sink relations in mature plants with fully developed taproots, the metabolic changes occurring during the initial phase of taproot development have not yet been systematically addressed. An experimental platform for semi-high throughput profiling of activities for 14 key enzymes of carbohydrate metabolism [1] was applied to characterize sugar metabolism during the transition from normal to taproot development within the first 80 days after sowing. In vitro enzyme activity analyses were complemented with in situ enzyme activity staining in taproot sections, as well as soluble sugar and phytohormone profiles.
Distinct temporal and spatial dynamics of activity signatures were found that correlated with certain developmental stages as observed by microscopic evaluation. These signatures could also be robustly reproduced when plants were grown under different conditions in two additional locations. Profiles of phytohormones and soluble carbohydrates also showed distinct developmental patterns.
Our study demonstrates that physiological phenotyping was valuable to characterize sugar beet taproot development, showing that early in development, roots go through three distinct metabolic phases as they complete primary root growth and enter the stage of secondary growth and sucrose storage. These findings support the importance of physiological phenotyping approaches as tools which complement classical and non-invasive phenotyping in basic research as well as in plant breeding and precision agriculture, contributing to bridging the genotype-phenotype gap for elucidation of the complex interaction between genotype, environment, and crop management [2].
The financial support by KWS SAAT SE, Einbeck, Germany is gratefully acknowledged.
[1] Jammer et al. 2015. J Exp Bot 66: 5531–5542.
[2] Großkinsky et al. 2015. J Exp Bot 66: 5429–5440.

Rice a1, 3-Fucosyltransferase Gene (OsFucT) Affects Flower Development and Vegetative Growth

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N-linked glycosylation is one of the key post-translational modifications. α1,3-Fucosyltransferase (OsFucT) is responsible for transferring α1, 3-linked fucose residues to the glycoprotein N-glycan in plants. We characterized an *Osfuct* mutant that displayed pleiotropic developmental defects, such as impaired anther and pollen development, diminished growth, shorter plant height, fewer tillers, and shorter panicle length and internodes under field conditions. In addition, the anthers were curved, the pollen grains were shriveled, and pollen viability and pollen number per anther decreased dramatically in the mutant. Matrix-assisted laser desorption/ionization time-of-flight analyses of the N-glycans revealed that α1, 3-fucose was lacking in the N-glycan structure of the mutant. Mutant complementation revealed that the phenotype was caused by loss of *OsFucT* function. Transcriptome profiling also showed that several genes essential for plant developmental processes were significantly altered in the mutant, including protein kinases, transcription factors, genes involved in metabolism, genes related to protein synthesis, and hypothetical proteins. Recently we obtained transgenic rice expressing a human α1,6-Fucosyltransferase (*HsFucT*) in *Osfuct* mutant. These studies would facilitate a further understanding of the function of genes mediating N-glycan modification in rice.

DEP1 gene in wheat species with different spike phenotypes

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The rice *DEP1 (DENSE AND ERECT PANICLE 1)* locus controls the panicle architecture, number of grains per panicle and grain yield. *DEP1* homologs are present in the other cereals including species of wheat and barley, even though they do not produce panicles but spikes.
We determined spike phenotype for twelwe accessions which belong to seven wheat species, *T. monococcum, T. durum, T. compactum, T. sphaerococcum*, *T. antiquorum*, *T. macha* and *T. spelta*. The full-length sequences of *DEP1* were obtained and characterized for wheat accessions with compact, compactoid and normal spikes. Obtained sequences were species specific. Substitutions within 5th exon similar to rice gain-of-function mutation were not identified. cDNA analysis showed that despite the interspecies diversity, all wheat sequences encoded polypeptides of the same size. We did not identify *DEP1*-related differences between the compact (or compactoid) and normal spike phenotypes in the tested wheat species. Therefore, *DEP1* gene does not directly participate in the control of the spike architecture in wheats. The result of *DEP1* gene expression analysis seems promising.
The comparative and phylogenetic analysis revealed origin of different *DEP1* gene sequences. Thus, tetraploid wheat *T. durum* obtained two variants of the *DEP1* belonged to A and B genomes. In the hexaploid wheats *T. aestivum, T. compactum,* and *T. spelta,* three variants of this gene originating from A, B, and D genomes were detected. *DEP1* genes of the diploid wheats *T. monococcum* and *T. urartu* differ. It seems that a precursor of the *DEP1* gene in *T. monococcum* originates from it's wild progenitor *T. boeoticum*.
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Phenotyping of different winter wheat varieties in a free air CO2 enrichment (FACE) experiment. Assessing physiological parameters by active and passive chlorophyll fluorescence techniques

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Predicted CO_2 concentrations [CO_2] for the year 2050 will be 550 ppm, 140 ppm higher than now. Studies under elevated [CO_2] have shown that plants response with increased yield and biomass, a decline in stomatal conductance and higher rates of photosynthesis. Breeding for future plants under predicted [CO_2] is mandatory to provide us plants with high photosynthetic efficiency, hence resource use efficiency. In BreedFACE, a free CO_2 enrichment facility combined with the latest non-invasive field phenotyping techniques, crops can be grown under elevated [CO_2] and common agricultural practice typical for the region near Bonn, Germany. In this study the photosynthetic response to elevated [CO_2] of 12 winter wheat varieties is determined by active and passive plant fluorescence from winter to spring. During this transient period a strong interaction between environmental change and CO_2 dependent photosynthesis is expected.
The BreedFACE experiment was sown in November 2017 with a historical winter wheat collection including 12 varieties released between 1966 and 2013. Two non-invasive phenotyping sensors were used: the light induced fluorescence transient (LIFT) device for active fluorescence parameters and the Fluorescence Box (FloX Box) for Sun-induced Fluorescence (SIF). Derived from active fluorescence were the PSII photosynthetic efficiency as Fv/Fm' and the Q_A re-oxidation capacity (Fr_2/Fm') both measured non-invasively by LIFT. Results thus far show that both parameters were lower at elevated [CO_2]. From March at higher temperature compared to February both parameters increased. Lower Fv/Fm' and Fr_2/Fm' at elevated [CO_2] may indicate a down regulation of PSII with excess sugars. Results at continuous intervals till June are collected and will be shown along with SIF measurements from same varieties. These data and further detailed analysis will have to confirm the response to elevated [CO_2] of active and passive fluorescence from winter to spring across winter wheat varieties.

Analysis of metal metabolism in plants by X-ray spectroscopic methods

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Various transition metals are essential nutrients, but toxic at elevated concentrations. Many mechanisms of metal uptake, transport, binding and sequestration are shared between animals (incl. humans), fungi, plants and even bacteria, while others are specific for one group. Analyzing metal distribution (compartmentation) and speciation is a crucial step in revealing mechanisms of metal uptake, transpor,t sequestration, deficiency, toxicity and detoxification. The least artifact-prone techniques for this task all belong to the field of X-ray spectroscopy, including EDX, XRF and XAS (XANES+EXAFS), and this contribution will show examples of all mentioned techniques. For minimizing artifacts, element re-distribution and ligand exchanges inside the measured tissues have to be prevented. The most reliable method for reaching this aim is measurement of shock-frozen, hydrated tissues, but alternative sample preparation techniques (e.g. conventional tissue fixation, freeze-drying, freeze-substitution and live measurement) will be discussed as well. Ideally, these samples are analyzed as bulk tissues without physical thin sectioning. Measuring in the semi-microscopic tissue-level domain with benchtop µXRF beamlines means looking at 2D projections of element distribution down to about 15µm resolution; this has the main advantage of convenient measurement of living samples and direct correlation with other in vivo measurements. Synchotron beamlines, in contrast, allow recording of µXRF tomograms with sub-cellular resolution (down to <100nm), but for these high resolutions shock-frozen samples are required. In the ideal case the same samples can be analyzed by µXAS as well, allowing a direct correlation between element distribution and local differences in speciation if the µXAS is done in a confocal or tomographic way. In this contribution, this ideal case will be compared to related techniques of measurement, in particular SEM-EDX and XAS on powdered frozen-hydrated samples. Differences between the techniques, especially in terms of physiological insights that can be obtained as well as artifact risks, will be highlighted.

Field based phenotyping of forage crops using close remote sensing tools

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Cultivated grasslands provide forage for livestock farming and are essential elements in European agriculture (Humphreys et al. 2010). To ensure sufficient biomass production, of good quality, that is stable over several seasons, farmers need excellent varieties of the main component species (mostly grasses and clovers). Furthermore varieties need to be resilient to the abiotic and biotic stresses (e.g. drought, frost, heat, rust, …), and need to be ready for future scenarios in the context of climate change. In the past years, forage crop breeders, traditionally using plot harvesters and visual scores to evaluate their selections, are increasingly implementing technological solutions such as image analysis and drone based phenotyping tools to keep pace with these high expectations. In addition, accurate and reliable phenotyping is essential to allow full exploitation of molecular tools to advance genetic improvement (Lootens et al. 2016).
We have developed several close remote sensing tools to phenotype grass plants/plots/communities in the context breeding. Phenotyping using image analysis under field conditions assures that the genotype (plant/plot/community) under evaluation is developing in an agriculturally relevant environment, and allows an objective evaluation of many selections in parallel in a non-destructive manner. Based on images captured using different sensors (RGB, multispectral and thermal), vegetation indices can be calculated from orthomosaics at a spatial/planimetric resolution of up to 0.5 cm and digital elevation models, built based on the structure from motion (SfM) technique, with an altimetric resolution up to 1.2 cm. This is more than sufficient to assess and screen individual plants and plots. Applications related to the evaluation of winter damage, sod density, persistency, drought tolerance, growth, biomass accumulation, … based on image analysis from close remote sensing will be discussed.

Online 13C18O2 analysis with IRIS for nondestructive plant phenotyping

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Looking at the global demand for feed crops it is predicted to increase almost double by 2050 due to growing world population (Foley et al., 2011). Considering the increase in global temperature and water scarcity, crops in the future need to be more water and nutrient use efficient to sustain food security. Photosynthesis or net canopy CO2 exchange is one of the driving forces of crop yield formation.
Since most commercially available equipment have been designed for single leaf measurements, photosynthesis at a leaf level has been studied more intensively than canopy photosynthesis.
Leaf photosynthesis measurements are often poorly correlated with crop yield, whereas whole plant (canopy) photosynthesis measurements correlate well with crop yield (Kim et al., 2006). Whole canopy measurements bypass the problem of finding a representative leaf and give information about the whole plant physiology and other plant physiological processes. In addition to canopy photosynthesis measurements, non-destructive approaches such as stable isotope measurements via online lasers are excellent tools to study the efficiency in transpiration and photosynthesis in crop plants (Senbayram et al., 2015).
Here, a custom-built phenotyping system attached to the Thermo Scientific™ Delta Ray™ Isotope Ratio Infrared Spectrometer (IRIS) was constructed to impose accurate determination of whole plant photosynthesis, transpiration and water use efficiency (WUE) in a continuous flow whole plant chamber.
In this study, we examined sugar beet genotypes during the wetting-drying cycle and studied their efficiency in water use.

N-fertilization of strawberry (Fragaria ananassa) cv. 'Sonata' plantlets

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Currently, the production of strawberries in Norway is dominated by conventional open field productions. Compared to other countries further south growing seasons in Norway are short with relatively low summer temperatures and long days. Long days favour leaf and runner production and delay flower initiation and crown branching in many cultivars. Therefore, cultivation in semi- and controlled environments in combination with the use of cold-stored large plants with already initiated flower initials (called 'production-ready plants'), is an option to extend the season. The present study was performed at controlled conditions in a phytotrone. Plantlets of cv. Sonata were grown at 18°C, first at 20 h (long days) followed by 10 h (short days) at different fertilization regimes (low-, medium- and high N levels). Weekly registration of chlorophyll content (using SPAD chlorophyll meter), number of leaves, number of runners and crowns in addition to sampling of leaves, petioles, roots and apex. Leaves will be analysed for N content and apex studied for floral initials.

Mutations that potentially disrupt the DNA-binding domain of Arabidopsis SPCH and MUTE produce fertile plants, but with modified stomatal abundance and distribution patterns

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Stomata formation in the leaf epidermis takes place as the organ primordium develops through a sequence of cell divisions and differentiation events leaded consecutively by three closely related bHLH transcription factors. The first one, SPCH, is essential for the asymmetric cell divisions necessary for stomatal lineage initiation and progression, and mutants lacking SPCH function do not produce stomatal lineages. MUTE is the second master bHLHs conducting the process, and its action is needed to exit the asymmetric division program and schedule that the last cell product of the asymmetric divisions (a late meristemoid) becomes a guard mother cell, committed to a final symmetric division. Loss of MUTE function prevents stomata formation, but immature lineages are produced. Finally, FAMA closes the division program and promotes the differentiation of the two guard cells to generate a stoma.
We have described a new *spch-5* allele carrying a point mutation in the bHLH domain that displayed normal growth, but had an extremely low number of sometimes clustered stomata in the leaves, whereas the hypocotyls were stomataless. Two related alleles, *spch-2* and *SPCHPPP*, also behave as hypomorphic mutants: plants are fertile and produce stomata but in altered numbers and spatial distribution patterns. We generated three MUTE versions mimicking the three *SPCH* alleles, and used them to complement the loss-of-function *mute-3* stomataless mutant. We also generated lines for the conditional overexpression of the various alleles. The interest of these lines lie in two main aspects: 1) their potential to modify stomatal abundance –and thence photosynthesis and transpiration, and 2) the fact that they serve as models to understand the molecular mechanisms underlying the action of this special group of bHLH transcription factors, as mutations in residues necessary for DNA binding nevertheless retain functionality.

Heat priming effects on anthesis heat stress In wheat cultivars with contrasting tolerance to heat stress

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We aimed to test if repeated early abiotic stresses could improve heat tolerance during anthesis heat stress in wheat cultivars. Two wheat cultivars, Gladius and Paragon, were subjected to a pre-anthesis high temperature priming process at three and five complete developed leaves stages. Primed and control plants were subjected to either a high temperature stress or non-heat stress temperature for 7 days during anthesis. Gas exchange and chlorophyll fluorescence were used to investigate the physiological performance of plants.
No difference in assimilation rate was observed between treatments for Gladius. Heat stressed Paragon parameters were not measurable due to the premature senescence of plants. No strong dependence was observed to prove the initial assumption of early heat stress being accountable for improving heat tolerance. However; a great difference between cultivars in response to heat stress was observed. Yield parameters of Gladius primed plants did not differ from their respectively control treatment. A distinct result was observed for heat sensitive cultivar Paragon, suggesting a cumulative deleterious effects caused by the repeated heat stress.

DO C3 CEREAL CROPS DIFFER ON LEAF PHYSIOLOGICAL TRAITS AND PRODUCTIVITY IN MEDITERRANEAN ENVIRONMENTS?

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The comparative between cereals and the identification of trait associated to drought tolerance, source limitations, yield, among others, contribute to understanding the physiology and productivity of cereals. In this work we studied the physiological response of bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* var. durum L.), barley (*Hordeum vulgare* L.) and triticale (*Triticosecale Wittmack*), growing in greenhouse and field conditions, with and without water stress during grain filling, in 2016 and 2017. In the greenhouse, barley showed the shortest time of grain filling period (approx. 28 days compared to 50 days of triticale), the lowest harvest index (HI), but the highest grain yield (GY). In field conditions, triticale had the highest GY but HI was not significant different from the other cereals (average 0.45). Leaf gas exchange and chlorophyll fluorescence differed among species and between water conditions, but genotype x water availability interactions were not significant. Triticale showed the highest stomatal conductance (gs), while barley the lowest (in all growing conditions). In the greenhouse, durum wheat presented the highest intrinsic water use (WUE_i) but in the field, it was triticale. In both growing conditions, barley shows the lower WUE_i. In conclusions the four cereals showed differences in the parameters evaluated; the largest differences in physiological parameters were between barley and triticale.

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Dissecting the genetic architecture of salinity tolerance in wild tomato (Solanum pimpinellifolium) using high-throughput longitudinal phenotyping in controlled and field conditions

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Salinity is a serious constraint to global agricultural productivity, affecting 5% of soils, 20% of irrigated areas and 97% of global water reserves. Improving the salinity tolerance of major crop species will help attenuate yield losses and expand irrigation opportunities, offering particular benefits to areas where poverty, food and water scarcity are prevalent. Tomato, as the most important horticultural crop globally with high commercial and nutritional value, is a compelling candidate for improvement. However, salinity tolerance is a complex trait with a limited genetic repertoire in domesticated crop varieties, frustrating attempts to breed and engineer tolerant crop varieties. Here, we present a genome-wide association study that wields the latest phenotyping technologies and the rich genetic resources of the wild, salt-tolerant tomato *Solanum pimpinellifolium* to identify traits that contribute to salinity tolerance and their genetic basis. We used a diversity panel with 230 *S. pimpinellifolium* accessions, performing longitudinal image-based high-throughput phenotyping in controlled and field conditions in young and mature plants. Our results reveal substantial natural variation in various measures of salinity tolerance across a large number of traits. In particular, the use of unmanned aerial vehicle (UAV)-based remote sensing in the field for high-resolution RGB, thermal and hyperspectral mapping offers novel insights into plant growth, photosynthetic performance and other traits that have until recently been difficult to measure in the field. Genotyping-by-sequencing yielded several thousand SNPs across the diversity panel. The association study combining these phenotypic and genotypic datasets is still underway, but is expected to identify novel components of salinity tolerance, and their underlying genetics, towards the development of new salt-tolerant tomato cultivars.

In planta heterologous expression to characterize putative enzymes involved in pyrrolizidine alkaloids biosynthesis identified by subtractive transcriptomics

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Pyrrolizidine alkaloids (PAs) are produced by several angiosperms as part of their chemical defense against herbivores. Homospermidine synthase (HSS) is already known as the first pathway specific enzyme but the remaining steps of the biosynthesis are still far from being understood. Transcriptome analyses have show nthat diamine oxidases (DAOs) are co-expressed with HSS, indicating a probable specific function in Pas metabolism. For functional characterizations, cloning of full-length sequences have been initiated. Previous attempts to express the HiDAO1 in *E.coli* failed because the heterologously expressed enzyme had no bioactivity. This may be due to missing post-translational modifications due to the prokaryotic expression system. Therefore the *in planta* expression seems to be promising to characterize our candidates.

Automated phenotyping of berries - case woodland strawberry (Fragaria vesca)

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Measuring plant growth and development is an important part of plant research. Producing quantitative data is often slow and prone to variability and errors. Therefore, development of digitalization and automated data collection methods is essential for highly accurate data production. We have used the automated imaging system of the Finnish National Phenotyping Infrastructure (https://www.helsinki.fi/en/infrastructures/national-plant-phenotyping) for analyzing berry traits in woodland strawberry (*Fragaria vesca*), the diploid model plant for the octoploid garden strawberry (*F.* x *ananassa*). We have developed and optimized the data collection method as well as established an RGB-based imaging strategy for appropriate shape parameters as well as colour. With this imaging strategy we have phenotyped our woodland strawberry accession collection consisting of 200 accessions collected from all around western Europe. These accessions show high variability in many developmental traits including berries. Furthermore, the genomes of all of these accessions have been fully sequenced. The phenotypic and genomic data are used for genome wide association mapping to identify putative regulatory genes affecting berry traits as potential targets of gene editing and marker development in garden strawberry breeding programs.

The effect of hydric stress on some physiological traits and relationships with alfalfa yield

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In the context of climate change drought stress is one of the main environmental factors limiting crop production. Alfalfa, drought-tolerant crop, generally tolerates water shortages quite successfully, but its yield may fluctuate appreciably under drought conditions.
Therefore, strategies for sustainable use of water and drought resistance improvement based on the physiological traits are important and physiological approaches should be integrated in conventional breeding. Research was performed with 74 alfalfa genotypes under vegetation house conditions at two watering levels.
The objective was to identify the available genetic variation and to establish efficient testing methods for characters which might positively influence alfalfa performance under drought conditions. Our research focused on plant height, number of shoots, stomatal resistance and biomass accumulation.
The results showed that for drought tolerant genotypes the plant height and number of shoots were reduced more than in sensitive ones. The stomatal resistance is higher for sensitive genotypes than for resistant ones, but there are some sensitive genotypes with low stomatal resistance and conversely, which opens new opportunities to improve drought resistance. The results showed that the stomatal resistance of young plants has been correlated with production both under optimal conditions and stress conditions, indicating that stomatal resistance has a very significant impact on production both under stress and optimum conditions.
Key words: *alfalfa, drought susceptibility index, morpho-physiological traits, stomatal resistance*

Overcoming the plant phenotyping bottleneck by integrated approaches

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Quantitative analysis of structure and function of plants has become the major bottleneck in basic research and applied use of plants. Significant interdisciplinary approaches have been started within the recent years to establish research infrastructure able quantitatively assess relevant traits under controlled and field conditions to understand the dynamic interactions between genetic constitution, molecular and biochemical processes with physiological responses leading to the development of phenotypes.
In this presentation, we would illustrate resent developments and results in plant phenotyping approaches across scales from the lab to the field and underline the role of plant phenotyping networks. Recent initiatives such as the EU funded project EPPN2020 aim at enabling the European scientists to access key plant phenotyping facilities across Europe and perform experiments they were not able to do hitherto, while the ESFRI listed project EMPHASIS aims at long- term sustainable development of the plant phenotyping infrastructure in Europe. Finally, the International Plant Phenotyping Network, a non-profit association integrates the plant phenotyping community as a global communication hub.

Graft compatibility characterization of new apricot cultivars grafted onto different Prunus rootstocks

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In recent years, new apricot cultivars (*Prunus armeniaca*) are being introduced in Spain in order to extend the harvest period from May to September. However, several factors should be considered when selecting an apricot cultivar, including local climate, chilling requirements, flowering and ripening as well as the graft compatibility with the most common rootstocks adapted to our soil conditions.
Plant grafting is an important plant propagation technique that has been widely used in agriculture. However, the application of grafting is restricted due to incompatibility problems between the graft partners (scion and rootstock). In particular, when grafting involves two different species or genera, a lack of affinity may occur and in that case, the candidate will be discarded. The mechanisms behind graft incompatibility are not fully understood yet but involve genetically as well as structural aspects.
Thus, the introduction of new varieties requires knowledge of the extent and nature of incompatibility reactions before releasing these cultivars on the market. In this study, 25 different apricot cultivars grafted onto 4 different *Prunus* rootstocks ('Marianna 2624', 'Mirared', 'Miragreen' and 'Montclar') have been evaluated by vegetative parameters and histological analysis one month after grafting. Graft length, number of leaves and thickening at the graft interface were recorded, as well as the development of new vascular connections between the scion and rootstock of the different unions. The results will provide valuable knowledge at an early stage for determining the most suitable scion/rootstock combination to establish in the field according to their graft compatibility.

Botanical-chemical formulations enhanced yield and protection against Bipolaris sorokiniana in wheat by inducing the expression of pathogenesis-related proteins

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Two experiments (pot and field experiments) were conducted in two consecutive years to evaluate the protective effects of botanical-chemical formulations on physiological, biochemical performance and grain yield of wheat inoculated with *Bipolaris sorokiniana.* We compared different formulations comprising *Calotropis procera, Jacaranda mimosifolia, Thevetia peruviana* extracts, chemical fungicide (mefenoxam) and salicylic acid to modulate the defense system of wheat host plants. Among the selected plant species *J. mimosifolia* aqueous and methanolic leaf extracts (1.2% w/v) resulted in 96 to 97% inhibition against *B. sorokiniana*. Both in pot and field experiments, among all the formulations of selected plant extracts the combined application of JAF2 (*J. mimosifolia* 0.6%)+MFF2 (mefenoxam 0.1%) lowered the dose of chemical fungicide required while reducing leaf spot blotch disease. The same combined formulation induced resistance in wheat apparently through the accumulation of peroxidase, polyphenol oxidase, protease, acid invertase, chitinase and phenylalanine ammonia lyase. This formulation also stimulated the defense-related gene expression of PR-proteins. The same treatment gave even more increase (48%, 12% and 22%) in no. of grains/spike, grains weight and grain yield, than the MFF1 (mefenoxam 0.2%). We conclude that foliar application of *J. mimosifolia* leaf extract with very low dose of chemical fungicide (*J. mimosifolia* 0.6%+mefenoxam 0.1%) is a promising approach for the management of leaf blight and spot blotch in wheat.

Integration of physiological phenotyping into a holistic phenomics approach

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Plant responses are affected by the interaction of genome x environment x management that determines phenotypic plasticity and explains the variability of genetic components. Whereas great advances have been made in the cost efficient and high through-put analyses of genetic information and non-invasive phenotyping, the analyses of the underlying physiological mechanisms is lagging behind. The external phenotype is determined by the sum of the interaction of metabolic pathways and regulatory networks that is reflected in an internal, biochemical phenotype. These various scales of dynamic responses need to be considered and genotyping and external phenotyping must be linked to the physiology at the cellular and tissue level. A high-dimensional physiological phenotyping across scales is needed that integrates the characterization of the internal phenotype into high-throughput phenotyping of whole-plants and canopies. Thus complex traits can be broken down into components of physiological traits. Since the higher resolution of physiological phenotyping by wet chemistry is limited in throughput, non-invasive phenotyping needs to be validated and verified to be used as proxy for the underlying processes. Armed with this multi-dimensional phenomics approach, plant physiology, non-invasive phenotyping and functional genomics will complement each other, ultimately enabling the *in-silico* assessment of responses under defined environments with advanced crop physiology models. This will allow the establishment of physiological predictors also for complex traits to bridge the knowledge genotype - phenotypes gap for both basic research as well as applications in breeding and precision farming. A case study to combine non-invasive phenotyping in the automated, high-throughput phenotyping facility PhenoLab with the determination of enzyme activity signatures and phytohormone profiles to assess the infection of barley by biotrophic and necrotrophic fungi is presented.

Großkinsky, Svensgaard, Christensen & Roitsch (2015) Plant phenomics and the need for physiological phenotyping across scales to narrow the genotype-to-phenotype knowledge gap. JXB 66: 5429

Tackling the physiological phenotyping bottleneck with low-cost, enhanced-throughput gas exchange and ceptometry

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High throughput phenotyping platforms (HTPPs) are increasingly adopted in plant breeding research due to developments in sensor technology, unmanned aeronautics and computing infrastructure. Most of these platforms rely on indirect measurement techniques therefore some physiological traits may be inaccurately estimated whilst others cannot be estimated at all. Unfortunately, existing methods of directly measuring plant physiological traits, such as photosynthetic capacity (A_{max}), have low throughput and can be prohibitively expensive, creating a bottleneck in the breeding pipeline. We have addressed this issue by developing new low-cost enhanced-throughput phenotyping tools to directly measure physiological traits of wheat (*Triticum aestivum*). Our eight-chamber multiplexed gas exchange system, OCTOflux, can directly measure A_{max} with 5-10 times the throughput of conventional instruments, whilst our handmade ceptometers, PARbars, allow us to monitor the canopy light environment of many plots simultaneously and continuously across a diurnal cycle. By custom-building and optimizing these systems for throughput we have kept costs to a minimum, with OCTOflux costing roughly half that of commercially available single-chamber gas exchange systems and PARbars costing approximately 95% less than commercial ceptometers. We recently used these tools to identify variation in the distribution of A_{max} relative to light availability in 160 diverse wheat genotypes grown in the field. In a two-week measurement campaign we measured A_{max} in over 1300 leaves with OCTOflux and phenotyped the diurnal light environment of 418 plots using 68 PARbars. These tools could be readily modified for use with any plant functional type and also be useful in validating emerging HTPPs that rely on remotely sensed data to estimate photosynthetic parameters.

High-throughput versus manual phenotyping under repeated heat stress in wheat

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With climate change, heat stress and other abiotic stresses in wheat (*Triticum aestivum* L.) cultivation has become an important determinant of future food security. Using manual physiological phenotyping by chlorophyll fluorescence trait F_v/F_m, we had previously genetically mapped a QTL (in the F_2 generation) imparting heat tolerance by maintaining higher photosynthetic rate under heat stress. The source of the QTL was a cultivar originated from Afghanistan. In the present study, using its RIL (F_6) population homozygous for the QTL allele, we aimed to investigate the potential of automated high-throughput phenotyping platform versus traditional manual phenotyping in revealing the pleiotropic effects of the QTL under repeated heat stress occurring at vegetative and reproductive stages. Various physiological phenotyping was performed using a combination of high-throughput phenotyping (Lemnatech platform) and manual measurements for traits such as plant morphology and phenological stages, chlorophyll fluorescence, chlorophyll content, stomatal conductance, leaf reflectance, water use efficiency, thermal and RGB imaging, senescence, dry matter and grain yield. The results revealed that while most of the physiological traits showed a cumulative effect but some traits were differently affected by heat stress at vegetative stage and at reproductive stage. Interestingly, the favourable QTL allele showed a significant positive effect on traits such as plant dry matter, height, number of tillers, chlorophyll content and F_v/F_m while it was not linked to many of the traits measured in RILs, indicating a low pleiotropic effect. The obtained results will be discussed in relation to the effect of recurrent heat stress, the power of automatic high-throughput phenotyping versus manual phenotyping in revealing genotypic variation and the pleiotropic effects of the QTL allele on important physiological and harvest related traits. Overall, the study pinpoints whether the chlorophyll fluorescence as a genotypic selection criteria is valid for general plant performance and yield under heat stress.

SLEEP-MOVEMENT RHYTHMS IN PHASEOLUS LEAVES IN CONTROLLED LIGHT ENVIRONMENT WITH SIMULATED TWILIGHT PERIOD

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Plants subjected to daily cycles of light and darkness exhibit rhythmic behavior in association with these changes. The rhythms are generated internally and require an environmental signal to initiate their expression. Thus, plant leaf movements can be elicited by external stimuli including changing irradiance and/or light spectral quality. Under normal conditions transition to and from darkness is not abrupt and occurs through a gradually changing intensity and spectral quality of twilight; in higher latitudes these transition periods could be extremely long. It is very important to determine genotype-dependent threshold levels of plant sensitivity to light signals at dawn and dusk to predict genotype by environment interactions, *e.g.* in plant photoperiodic response. Progress in plant sensory physiology is closely linked to that in the new methodologies, particularly to further development of concepts and techniques bridging the gap between *in situ* and *ex situ* studies. Light-emitting diodes (LEDs) with their capacity for producing intense and easily tuned monochromatic light have opened a number of phenomena to investigate, including action spectra determination for both leaf-movement rhythm entrainment and time measurement in photoperiodic response. We studied stimuli that evoke leaf movement and the role of phytochromes as photoreceptors in rhythm entraining in nyctinastic plant *Phaseolus vulgaris* var. Sachsa bez volokna. Plants were grown in the specially devised light modules with tunable LEDs varying in the wavelength and spectral composition of the emitted light over wide ranges; civil twilight period of various mode (gradual changes in light intensity and spectral compositions) was simulated. Leaf-movement data have been obtained by automatic recording techniques involving an IR-based height-sensing unit. The circadian properties of the sleep-movement rhythms in *Phaseolus* individual primary leaves have been defined with more precision. Clear circadian rhythms were recorded with periods that match cycles in the environment superimposed on which was a short-period oscillation.

Using spectral signatures as a toolbox for assessing crop health

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The early detection of environmental stresses on crops is crucial for prompting immediate remedial action to be undertaken. One way to detect and measure crops performance (and stress) is through the use of spectral signatures. Reflected visible and near-infrared light from a leaf can be used to reveal information regarding chlorophyll content, water status, disease and biomass. Thermal imaging and chlorophyll fluorescence allow for direct measurements of stomatal conductance and photosynthetic performance. By focusing on the use of a combined technique approach, in which multiple spectral signatures can be employed simultaneously to measure numerous aspects of plant physiology remotely, the combination of these techniques can therefore be used to develop a 'stress catalogue', a reference of environmental stresses and health indicators and how they affect crop spectral signatures. The overall aim of this work is to allow for a non-invasive assessment of crop health in the field, and to develop a spectral toolbox to identify which stresses may be present and negatively affecting crop performance.
Using a combination of three key spectral techniques; chlorophyll fluorescence, spectral reflectance and thermography, the effect of water status and the onset of moderate water stress on the spectral properties of *Arabidopsis thaliana* plants was characterised. Decreases in water status resulted in a decrease in stomatal conductance as measured by thermography, but a minimal response to water status from chlorophyll fluorescence and spectral reflectance. The responses of these three techniques can therefore be used as a signature to identify lower water content in *Arabidopsis*. This work will form the foundation for future work, scaling up into crops such as wheat and barley and incorporating other environmental stressors, to fully develop a spectral toolbox for stress identification and crop health monitoring.

Wheat canopy characterization by determination of Leaf Angle Distribution

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The complex architecture of plant canopies influences light interception and is determined by different phenotypic parameters. One important factor is the leaf angle, which is difficult to measure under field conditions. Horizontal leaves intercept incoming light in the upper canopy layers, but they cause self-shading and reduce light availability in lower canopy layers. In contrast, erected leaves intercept less light and allow the light to penetrate through the canopy.
In order to determine leaf angle distributions of field-grown cereal crops, we established a new pipeline for stereo image processing and leaf surface modeling. The crucial part includes the approximation of leaf axis and leaf curvature by a second order polynomial. This step is essential to model proximodistal leaf twisting by a 'surface-twist-function', while leaf shape is estimated by a 'leaf-width-function'. The final leaf surface reconstruction allows precise calculation of leaf angle distributions.
An essential part of our work was the evaluation process. For this purpose, we developed an artificial plant model, which allows us to assemble crop plants with artificial leaves varying in size, leaf bending and proximodistal twisting. We reconstructed leaf zenith angle, leaf curvature and proximodistal twisting for different artificial leaves. For method evaluation ground truth data of leaf surface structure were obtained by structured light scans.
We found high accuracy for reconstructed leaf zenith angles of flat leaves. Determined leaf angle distributions from stereo images for bended and proximodistal twisted leaves were in concordance to reconstructions from structured light scans. The high accuracy in evaluation experiments indicate that our pipeline allows to estimate leaf angle distributions for wheat canopies in the field. Experiments were carried out on an image dataset of three wheat lines (Triticum L.) in different growth states and first results are shown.

Detecting Alterations in Starch Granule Morphology by high-throughput Screening Methods in a Barley Mutant Population

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Barley (*Hordeum vulgare* L.) is one of the major cereal crops worldwide and is used for feed and food purposes as well as a raw material for brewing beer. In particular, storage starch from barley seeds functions as the carbohydrate source for the yeast to produce alcohol. Starch is stored in discrete, semi-crystalline, water-insoluble granules, which differ in shape and size depending on the botanical origin. Barley shows a bimodal distribution of starch granules, where small, spherical shaped granules (ø 1 – 8 µm) account for the vast majority of the total starch granule number, but only for a small fraction of the starch weight. Accordingly, the large, lenticular granules (ø 8 – 25 µm) account just for a small amount of the total starch granule count but contribute most to the total starch weight.
While comprehensive knowledge about the chemical composition of starch and the enzymes involved in its synthesis is available, the explanation of how the bimodal distribution of granules is established is still lacking. To identify involved genes, a screening for granule characteristics is conducted in a population carrying induced variation using flow cytometry and high-throughput microscopy. Through these approaches, mutants exhibiting changes regarding the bimodal size distribution of starch granules can be identified. This will provide a basis for further studies on the genes underlying starch granule initiation and for crop quality improvement.

Phenotyping methodologies for anatomical changes of roots traits under impedance

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Soil compaction influences root architecture, morphology and anatomy. Plants change and adapt their root system at these different levels depending on the local environment. Root architecture is responsible for the spatial arrangement of roots in the soil profile. Localised adaptations of root morphology and cellular anatomy are important when roots encounter a local stress such as a compacted layer. Roots have been observed to radially expand when experiencing higher levels of impedance, but the impact on the cellular structure of the root tissues remains largely unknown. Anatomical changes as roots encounter soil compaction in both lab and field were studied by integrating different phenotyping techniques. X-ray Computed Tomography (CT) in combination with laser scanning confocal microscopy was used to study the ability of roots to penetrate through a hard layer in relation to changes in cellular responses within that layer. Additionally, a field study combining shovelomics, soil coring and Laser Ablation Tomography (LAT) demonstrates how high throughput can be achieved for the measurement of anatomical traits. In combination with image analysis, root morphological changes can be explained anatomically both on the level of an individual root axis, as well as in reference to the whole plant. In maize we observed that radial expansion is mainly due by the increase in cell size area in the cortex, while no extra cell layers were formed when growing through a layer. In the field we observed roots with certain traits were more capable of growing through compaction. This illustrates the importance of anatomical responses in roots encountering soil impedance. Phenotyping should thus not be restricted to architectural or morphological traits as for localised stresses, such as compaction, considering the root cellular traits could be equally important.

Genetic variability and evolution of Q gene in Aegilops and Triticum species

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Q gene is one of the major domestication gene of wheats that associated with spike shape, threshability and rachis fragility traits. The main role in regulation of thesetraits plays polymorphisms at two positions,located on 8 and 10 exonsof 5A chromosome. 5D homoeolog of *Q* gene encodes a functional protein, contributed to the suppression of the speltoid phenotype, whereas the 5B homoeolog is a pseudogene. The aim of this study was to investigate the genetic variability of *Q* gene in *Triticum* and *Aegilops* species with various spike morphology and establish the phylogenetic relationships between wheats and their ancestral species.
The combination of bioinformatical tools and molecular biology methods was used to determine the full-length sequences of *Q* genes from 10 wheat and *Aegilops* species with variable spike morphology. Phylogenetic analysis of the obtained sequences was performed by Maximum Likelihood method (ML) in the IQ-tree webserver.
In this study *Q* gene sequences from diploid (*T. monococcum*, *T. urartu* and *T. boeoticum*) and hexaploid (*T. macha*, *T. aestivum* ssp*. petropavlovskyi*, *T. spelta* ssp*. yunnanense*, *T. spelta* ssp. *tibetanum* and *T. vavilovii*) wheat species, *Ae. Tauschii* (syn. *Ae. squarrosa*) and *Ae. Speltoides* with variable spike morphology were obtained. The correlation between *Q* gene variability (a non-synonymous substitution in exon 8 and a synonymous substitution in exon 10) and spike shape, threshability and rachis fragility traits in di- and hexaploid wheat species was identified. Phylogenetic analysis allowed to trace the evolution pathway of *Q* gene in *Aegilops* and *Triticum* species.
The study was supported by the Russian Science Foundation (grant number: 16-16-10021).

Influence of light quality on development and photosynthetic activity of spinach (Spinacia oleracea L.) plants

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Plant development and physiology are strongly influenced by the light spectrum of the growth environment. The modulation of specific wavelengths affects a wide range of plant processes and also determine structural changes. The control light quality in the environment may promote several physiological, morphological and biochemical traits in the plant, such as gas exchanges, biomass production, pigment composition and antioxidant synthesis. In this work, we evaluate if appropriate light fertilisation protocols could improve *Spinacia oleracea* L. growth and quality regarding photosynthesis, biomass and bioactive compound production. Spinach seeds were germinated in a growth chamber and exposed to four different light quality treatments of 350 µmol photons m^{-2} s^{-1}: white light (WL), "pure" Red (R), Red-Blue (RB, 60% Red - 40% Blue), Red-Green-Cyan (RGC, 60% Red - 20% Green - 20% Cyan). Plants have been monitored until 100 days after sowing (DAS) and biometrical (leaf area, root and shoot biomass), physiological (gas exchanges and fluorescence emission) and biochemical (photosynthetic pigments, antioxidants, sugars and total protein content) traits were determined. Compared to WL and R treatments, the growth under RB induced the decrease of leaf area, a more compact plant size as well as higher photosynthetic and transpiration rates, likely due to the blue light stimulation on stomata opening. As additional valuable characteristics, the RB treatment determined an increase of carbohydrates and total chlorophylls as well as a reduction of phenolic compounds compared to the other treatments, increasing the palatability of leaves. The overall results confirm the light manipulation as a valuable tool to obtain favourable traits in plants.

PlotCut:
A high-throughput data extraction tool for UAS (drone) imaging used in phenotyping agricultural research plots and research fields

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Agricultural research plots in-situ can be imaged not only by stationary or tractor mounted imaging sensors, but also by unmanned aerial systems (UAS). Deploying the UAS and subsequently having the many hundreds of individual images made into one orthomosaic image is not the big hurdle anymore. Extracting data from the several hundred research plots (sometimes thousands when we're talking plant breeders), is now the most time consuming part of the process as outlined below:

> Plan flight ➔ Fly drone + take pictures ➔ Create orthomosaic ➔ **Extract pixel data from individual plots** ➔ Calculate relevant indices (e.g. Excess Green Index, NDVI) ➔ Deliver phenotyping research data to scientist and/or data to aid the plant breeder in decision making.

It used to be that the accuracy of the data extraction from the images relied solely on a human, visually based, marking off and extracting from the image snippet matching each research plot. This left much room for naming errors, and the repeatability would often suffer as well.

Now, perform these tasks upwards of fifteen times a season for just one research area, and it becomes apparent that a templating tool is needed. We are now building such a tool at the University of Copenhagen.

The tool we are developing is a high-throughput data extraction platform, named PlotCut. We do this together with several Nordic plant breeders. Their continuing input in the process - from one software version to the next - is essential for the long term success of the platform.

In its current status the platform calculates and outputs (to CSV files) - for each research plot in the images - the following:

> RGB and greyscale single-band values (i.e. "normal" photos as well as multispectral and thermal sensor outputs), relevant indices e.g. excess green index, NDVI. Crop coverage can also be calculated (only relevant in certain instances, depending on growth stage and the image resolution).

The tool also enables single-plot user annotation per instance (mission flown) in order to better document plot irregularities spotted while setting the extraction template.

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Method development, validation and application for root and shoot phenotyping at the Jülich Plant Phenotyping Center

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The Jülich Plant Phenotyping Centre (JPPC) focuses on the development and application of high-throughput, non-invasive phenotyping methods for shoot and root morphological and physiological traits. A multi-disciplinary team of plant biologists, physicists, engineers, image processing and data management specialists designs, develops and validates new image- and physics-based methods for quantitative plant traits analysis, and progresses these methods towards routine workflows. These include the collection, management and integration of metadata on plants and their environment. The plant species grown in the systems include a wide range of model and crop species for which research questions are being addressed on responses to abiotic stress related to climate change and the sustainable use of resources, including the improvement of water and nutrient use efficiency.
JPPC actively cooperates with both academic and industrial partners, and enables access to state-of-the-art phenotyping systems and experimental procedures. The JPPC infrastructure is currently available and in use in national and international research networks such as the http://www.dppn.de/dppn/EN/Home/home_node.html, and the https://eppn2020.plant-phenotyping.eu/. The access for selected users and collaborative projects includes the use of the infrastructure, and the logistic, technological and scientific support required to conduct the proposed experiment according to good phenotyping practices, and to answer the biological question under review in a most pertinent manner.

A dysfunctional allele at an E1-like locus is involved in flowering under long day condition in soybean.

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Soybean (*Glycine max*) is a short day plant. Its flowering is repressed under long day (LD) conditions. Cultivars adapted to high latitude areas have low photoperiod sensitivity. This trait has been known to be controlled by two different genotypes at maturity loci, *E1* (repressor of *FLOWERING LOCUS T* orthologues) or *E3* and *E4* (phytochrome A genes). An accession from the Russian Far East (R23) possesses lowered sensitivity, but possesses a sensitive genotype at the three loci (*e1-as*, *e3* and *E4*). The purpose of this study was to identify the molecular basis for the lowered sensitivity of this accession. Genetic analyses were carried out in F_2 and F_3 progeny of a cross between R23 and a near-isogenic line (NIL) of cv. Harosoy for a recessive *e3* allele (H*e3*), both of which share the same genotype at *E1*, *E3* and *E4*. The segregation pattern suggested an involvement of a single recessive gene for the lowered sensitivity. SSR markers analysis further indicated that the gene was linked with SSRs of Chromosome 4. Fine-mapping analysis delimited the gene into an 842kb-region in the pericentromeric region. According to a genome database (Phytozome v12.1/*Glycine max Wm82.a2. v1*), 6 genes including an *E1-like* gene (*E1Lb*) were annotated in the region. Sequencing analysis for the coding region of *E1Lb* detected a single-base deletion in R23, which caused a pre-mature stop codon to produce a truncated dysfunctional protein. Flowering times and expression profiles of *FT*s were further evaluated in NILs for functional *E1Lb* and dysfunctional *e1lb* alleles. Under LD conditions, the NIL for *e1lb* flowered earlier than the NIL for *E1Lb*, and the expression levels of both *FT2a* and *FT5a* were up-regulated in the former. Taken together, the results obtained in this study demonstrates that a novel allele at *E1Lb* is involved in the lowered photoperiod sensitivity in soybean.

Theme 2: Photosynthetic diversity

Different photosynthetic and morphological responses of three populations of Persea lingue, a native Chilean specie.

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Lingue *(Persea lingue*) is a native Chilean specie and while is not catalogued as threatened, its habitat has been subjected to severe fragmentation. This is the case of Cayumanque mountain (36°41´41´´S, 72°31´49´´W), located in central Chile, characterized by Mediterranean climate and ranked as high priority for conservation. However, despite the restoration efforts in place, the success of lingue establishment in Cayumanque is limited by severe drought and there is need to introduce individuals from neighboring populations, as Cayumanque is isolated and high rates of seedling deleterious and lethal mutations have been found. Thus, our objective was to characterize the morpho-physiological response of lingue from Cayumanque and two neighboring populations with coastal influence (Santa Juana: 37°19´4´´S, 72°59´29´´W; Nacimiento: 37°28´50´´S, 73°0´12´´W) used as plant material for restoration, under water restriction (WR). Parameters of chlorophyll fluorescence, photosynthesis, relative growth rate and leaf functional traits were measured on lingue during WR in nursery conditions. Results showed that different populations responded differently to WR. Thus, Cayumanque displayed morphological plastic responses to reduce water loss like a decrease in leaf area, increase in leaf mass area and in root mass ratio, while photosynthetic parameters were not affected by WR. Similar results were observed in Nacimiento, although leaf biomass and area increased under WR, which does not correlate with an efficient strategy to decrease the evaporating surface during drought. On the contrary, photosynthetic parameters of Santa Juana were severely affected by WR, consistent with a decrease in relative growth rate, explained by a decrease in net assimilation rate. In conclusion, Cayumanque population presented morpho-physiological responses highly consistent with Mediterranean climate, unlike other populations. This could be explained because, despite that Santa Juana and Nacimiento are the closest populations to Cayumanque, their climate is characterized by a coastal influence, lowering their suitability to be used for Cayumanque´s restoration.

Modulation of light spectrum affects the physiology of beet (Beta vulgaris L.) plants irradiated with heavy ions.

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Manipulation of the light spectrum during growth can induce changes in photosynthesis, biomass production and in the synthesis of metabolites that defend plants against abiotic stress.
In this study, beet (*Beta vulgaris* L.) plants were grown from seeds irradiated with 10 Gy Carbon (C) or Titanium (Ti) and from non-irradiated control seeds under three different light quality regimes: RGB (33:33:33 Red-Green-Blue), RB (66:33 Red-Blue) and white light (WL).
The impact of light quality and ionising radiation on plant growth and photosynthetic and antioxidant metabolism was monitored across plant development and until harvesting. Under WL, both plants from ions irradiated seeds (IS plants) showed a significant reduction of photosynthesis and stomatal conductance compared to non-irradiated controls. However, under RGB and RB light, photosynthesis of IS and control plants was similar. In IS plants, growth under RB stimulated total biomass, shoot and root elongation and anthocyanins production, compared to controls, while no difference was observed under RGB and WL. The RGB treatment induced higher levels of antioxidant and protein content in IS plants compared to control. A significant reduction of these parameters was observed under the other light regimes. In plants from C-irradiated seeds growth under RGB and RB determined a higher sugar production compared to WL, whereas in plants from Ti-irradiated seeds sugars decreased under RGB light. In conclusion, our study indicates that growth under light enriched with red and blue components of the spectrum can promote biosynthesis of valuable traits, such as antioxidants, proteins and sugars, that may help plants tolerate ionising radiations.

The role of diadinoxanthin de-epoxidation in thermal stabilization of diatom thylakoid membranes

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Although diatoms are the dominant organisms in cold habitats and provide about 40% of the marine primary production, there are only a few data on their acclimatization to rapid changes of ocean water temperature.
In eukaryotic photoautotrophic cells, the photosynthetic membranes belong to the structures which are most sensitive to temperature changes. In the present study we postulate a new mechanism which provides stabilization of photosynthetic membranes during temperature changes. This mechanism is based on the diadinoxanthin cycle in which diadinoxanthin is converted to diatoxanthin in a one-step de-epoxidation reaction.
In the present experiments de-epoxidation of diadinoxanthin was monitored in photosynthetic membranes of the model diatom species *Phaeodactylum tricornutum* using HPLC. Molecular dynamics of thylakoids were analyzed using EPR – spin label technique. The order parameter, S, calculated from the spectra of 5-SASL and 16-SASL provided information about molecular dynamics of the region close to the membrane surface and in its hydrophobic core, respectively. It was demonstrated, that S parameter measured for 16-SASL increased together with the increase of the diatoxanthin concentration in thylakoids indicating their rigidification. Such correlation was not observed in case of S parameter calculated from 5-SASL spectra. The ordering of head group lipid region was the strongest during intensive diatoxanthin production in the de-epoxidation reaction, and the weakest when the level of diatoxanthin was constant.
In summary, the dynamics of the de-epoxidation reaction and increase of the diatoxanthin concentration resulted in different responses of the hydrophobic region comparing to polar head groups of the membranes.
The obtained results show that diadinoxanthin de-epoxidation may plays an important role in the modulation and stabilization of diatom membranes during rapid temperature changes.

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Activity of Oxygen Evolving Complex Retains until Complete Inhibition of Photochemical Reaction in Spinach (Spinacia oleracea L.) Thylakoids

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This study was carried out to clarify the activity of oxygen evolving complex(OEC) following to temperature changes in spinach(*Spinacia oleracea* L.) thylakoids. Extracted thylakoids were treated at 15, 20, 25, 30, 35, 40 and 45°C for the desired period. At a temperature of 45°C, the thylakoid membrane lost all photochemical reactions within 6 minutes. On the other hand, at 40°C, activity was maintained for at least 50 minutes. In the temperature range of 15°C to 35°C, the thylakoid membrane was observed to maintain its activity throughout the experiment. Though the temperature was changed from 22°C to 35°C, the photochemical parameters of thylakoid membranes did not show any significant change. Under temperature conditions from 22°C to 30 °C, most of the photochemical parameters of thylakoid membranes increased slightly. It was observed that the chlorophyll content did not change at different temperature change conditions and electron transfer to PSII and PSI was maintained normally. From the spectral analysis we have found that there is a significant difference at 440nm and 680nm due to the temperature change of the thylakoid membrane. In conclusion, it was found that the activity of OEC was retained even though the photochemical parameters of the thylakoid membrane were considerably changed by extreme high temperature and their electron transfer activity was lost.
This study was supported by ”Study on ICT-based Smart Irrigation System for Plug Seedling Production”, RDA Agenda Project(PJ012783022018), The Republic of Korea.
Key words: Chlorophyll, Oxygen evolving complex, Temperature, Thylakoid

Inhibition of electron transfer by high concentration of glyphosate and exogenous shikimic acid in spinach (Spinacia oleracea L.) thylakoid

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As a secondary effect of Glyphosate, known as an inhibitor of EPSP-synthase, the inhibition of the electron transfer process in the thylakoid membrane was measured through JIP-test. The effect of aminomethylphosphonic acid (AMPA), known as a byproduct by glyphosate degradation, on photochemical reactions was also measured. The electron transfer rate from the maximum quantum yield (Fv/Fm) to the electron transfer from Q_A to Q_B (ET2o/RC) and electron flow until PSI acceptors(RE1o/RC) was drastically reduced by the high concentration of Glyphosate above 3 mM. In addition, PSII activity was extensively inhibited in thylakoid membranes treated with extremely high concentrations of AMPA(20mM). However, the low concentration of AMPA produced by glyphosate degradation, which is expected to be physiologically occurred in the stromal space, did not inhibit the photochemical reaction. At least in the concentration range of shikimic acid up to 5 mM, the photochemical reaction on the thylakoid membrane was not affected. Therefore, the inhibition of electron transfer by glyphosate was considered to be a secondary effect. Inhibition of EPSP-synthase by glyphosate has been shown to reduce the electron transfer process in the thylakoid membrane due to excessive shikimic acid accumulated in the chloroplast.
Key words: Glyphosate, Shikimic acid, aminomethylphosphonic acid (AMPA), EPSP-synthase, Thylakoid

High Throughput Photosynthesis Characterization of C3 Plants

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Single point measurements of leaf gas exchange provide basically only the parameters net photosynthesis, stomatal conductance, and instantaneous leaf water use efficiency. From analysis of assimilation rate vs. internal CO_2 concentrations (A vs. C_i) curves, four or five additional leaf parameters are obtained for plants with C_3 carbon metabolism, which allow estimation of photosynthesis over a range of conditions. However, determining A vs. C_i curves conventionally requires at least 20 minutes per leaf, compared with about 2 minutes for single point measurements, which greatly limits through-put. PP Systems has developed a method of linearly ramping CO_2 rapidly in their CIRAS-3 Portable Photosynthesis System, which provides a complete A vs. C_i curve in 5 minutes per leaf. Two initial steps are required: storing the changes in analyzer sensitivity with background CO_2, and collecting data from ramping of CO_2 with an empty chamber. These two steps need only be done once per day. The 5 minute total measurement period per leaf includes a 2 minute initial equilibration period followed by 3 minutes of ramping up of CO_2 concentration until the rate of change of A with CO_2 becomes small, i.e. until CO_2 becomes nearly saturating to A. With the CIRAS-3 system post-processing of the gas exchange data is very simple: the apparent ”A” of the empty chamber is subtracted from the ”A” value obtained with a leaf in the chamber at each time point of the CO_2 ramping period. This provides the actual A value at each time point, and the C_i is obtained from this actual A, stomatal conductance, and external CO_2 as in the conventional calculation of C_i. Because of the rapid change in CO_2, we have seen no significant change in stomatal conductance during the CO_2 ramps.

Molecular background of circadian clock-, light quality- and temperature dependent regulation of freezing tolerance in cereals

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Recently, more and more information accumulating that, apart from cold temperature, light – including its spectra – and circadian clock also have influence on cold acclimation process, both in dicot and monocot plant species. It has long been established that CBF (C-repeat binding factor) transcription factors play a key regulatory role in this processes. Accordingly, by lowering the red/far-red ratio in the illuminating spectra at 15°C, we were able to induce *CBF* gene expression and also to increase freezing tolerance in winter wheat (Cheyenne) and winter barley (Nure) genotypes. Based on gene expression data, a model was proposed illustrating that, in response to the increased ratio of far-red in the incident light, phytochrome A induces, while phytochrome B prevents the enhancement of freezing tolerance in cereals. To understand more of this complex acclimation process, we studied the expression patterns of the *CBF*s and the signal transducing pathways, including signal perception, the circadian clock and phospholipid signalling pathways, upstream of the *CBF* gene regulatory hub in barley. We found that, from among the *CBF*s, only the HvCBF4-phylogenetic subgroup genes showed circadian pattern. We found that, these genes were expressed in the late afternoon or early in the night. We also demonstrated that, the transcript accumulation of these *CBF* genes had appeared four hours earlier and more intensely under supplemental far-red illumination, which tendency was found in several gene expression patterns of signal transducing pathways too. Based on our results, we propose a model, which shows the complex regulatory network of freezing tolerance in cereals.
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A thermotolerant variant of Rubisco activase from a wild relative improves growth and seed yield in rice under heat stress

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There is an increase in frequency and severity of heat waves due to climate change and how crops can tolerate this increase in heat load is of major interest to the scientific and general community. It is well known that the photosynthesis protein Rubisco activase (Rca) is heat-labile and a main contributor to limited rates of photosynthesis at supra-optimal temperatures. We have recently established that a wild relative of rice, *Oryza australiensis*, has a variant of Rca which is more tolerant to higher temperatures when compared to domesticated rice. We have transformed this Rca variant from the wild relative into domestic rice (*Oryza sativa*) and observed enhanced growth and development when rice is exposed to heat stress throughout the vegetative life-cycle. This improvement culminated in a 2.5-fold relative increase in seed number for the transgenic line with highest recombinant Rca protein abundance. To our knowledge this is the first time an improvement in crop productivity during heat stress due to the thermostability of Rca has been established. We believe these findings can contribute to food security in a warmer future.

The role of leaf width and conductances to CO2 in determining water use efficiency among diverse C4 grasses

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C_4 plants achieve higher photosynthetic rates (A_n) and water use efficiency (iWUE) than C_3 plants, but it is unclear what constrain the concurrent optimization of A_n and iWUE in C_4 plants. We developed a new approach to quantify the relative diffusional and biochemical limitations of C_4 photosynthesis on A_n and iWUE among 24 phylogenetically and biochemically diverse C_4 grasses. iWUE increased with decreasing stomata conductance (g_s) and increasing mesophyll conductance (g_m, estimated by combining gas exchange with assay of PEPC activity), but not with increasing A_n. Leaf width correlated negatively with iWUE and g_m and positively with gs. Our analysis also showed that g_s and Rubisco activity strongly influenced iWUE while g_m exerted a small but significant influence on iWUE and A_n. In addition, bundle conductance exerted significant non-correlative influence on iWUE. NADP-ME grasses had lower g_m and leakiness of CO_2 from bundle sheath relative to NAD-ME species. We conclude that leaf width plays a major role in the variation of iWUE among C_4 grasses and may serve as a useful screening tool for breeding C_4 crops with higher water use efficiency.

Light dependent expression of LEA genes in rice (Oryza sativa) and Brachypodium distachyon roots

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As drought is one of the major abiotic stress factors limiting plant growth and grain yield, understanding the background of adaptation to water limitation became a hot issue of plant research and crop breeding. To reveal the molecular response of root to water limitation we performed a comprehensive oligo-chip experiment seeking for differently expressed genes in roots of upland and low-land rice cultivars under well-watered and drought conditions. We collected samples early in the morning, at noon and late in the afternoon to monitor whether gene expression changes in the roots follow daily fluctuation of water demand. Among differently expressed genes we found two LEA (Late Embryogenesis Abundant) genes (Os05g46480; Os03g62620) and an ABA/WDS-Water Deficit Stress Induced gene (Os04g34600) strongly upregulated by water deficit which activity correlated to the period of the day, indicating plausible circadian regulation of gene expression. Based on this we monitored the expression level of these genes by qPCR in higher resolution: we measured relative transcript levels in every four hours for two days in constant light and in constant darkness under well-watered and drought conditions, respectively. In the upland rice cultivar ”Sandora” transcripts of LEA genes and ABA/WDS accumulated periodically under drought condition. Homologues of rice genes showed the same phenomena in *Brachypodium distachyon*, the model plant of cereals. However, we observed that constant darkness led to dramatic decrease in gene expression both in rice and *Brachypodium* roots indicating that expression of these genes depends on light as well. Our results suggest that activation of selected genes in correlation to daily fluctuation of water-deficit might be conserved and supports the general concept of their preventive role in vegetative tissues under adverse conditions. Moreover, our findings indicate plausible circadian regulation of LEA gene expression which is yet a hidden part of LEA regulatory network.

Inhibition of PSII activity by polyamines during the etiolation process in spinach (Spinacia oleracea L.) leaf.

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The purpose of this study was to observe changes in the photochemical reaction of chloroplasts during the etiolation process. It was found that polyamine treatment prevented loss of chlorophyll and protein content, but the photosynthetic activity decreased during the etiolation process.
Nondestructive chlorophyll fluorescence analysis has demonstrated that polyamines can interfere with the electron transfer from Q_B protein of PSII to $PQ \rightleftharpoons PQH_2$. The PSII activity was retained for 72hr, but it decreased rapidly after 96 hr. The higher the concentration of polyamine, the worse PSII activity.
To confirm, the change of the electron transfer process was measured by mixing the polyamine with the spinach thylakoid membrane. Several parameters related to photochemical reactions, including PSII / RC, have changed. Certainly, the polyamine with multiple (+) charges such as spermine inhibited the activity of PSII during the etiolation process.
In conclusion, inhibition of chlorophyll degradation by polyamine wasn't involved in decrease of PSII activity. These results presumed to be due to the change in electro conductivity of thylakoid membrane surface by Polyamine, *i.e.* cyclic photophosphorylation was directly inhibited by positively charged thylakoid.

Stomata acclimation to low CO2 and low light in C3 and C4 grasses representing different photosynthetic subtypes

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Leaf-level water-use efficiency (WUE_i) is governed by CO_2 exchange from the atmosphere via photosynthesis and water vapour loss through transpiration. This gaseous exchange is facilitated by stomata, the microscopic pores located on the leaf surface comprising of two guard cells for most species and subsidiary cells in grasses. Grass stomata have evolved dynamic response mechanisms to fluctuating conditions thus allowing for relatively high WUE_i. However, there is no consensus evidence towards stomatal acclimation response to low CO_2 (LC) and low light (LL) conditions among C3 and C4 grasses. Thus, we investigated the stomatal responses of grasses with different photosynthetic subtypes (C3, C4 NADP-ME, C4 NAD-ME, and C4 PCK) to LC and LL conditions. Acclimation was assessed morphologically (stomata traits), physiologically (gas exchange parameters), and biophysically (stomata kinetics and ion fluxes) to better understand WUE_i. Results show that LC acclimation is characterized by an increase in stomatal conductance (g_s), decrease in intercellular CO_2 (C_i), net photosynthetic rate (A_{net}), and WUE_i but to a greater exacerbation among C3 species. LC acclimation also imparted an increase in stomata aperture (SA), open stomata density (OD), and reduced stomata size (SS). LL acclimation showed greater reduction in A_{net}, g_s, SA and OD regardless of photosynthetic subtype. Closing and opening half-response time ($t_{1/2}$) was unchanged among C4 acclimated under LC while C3 stomata closed and opened ~60% more slowly. On the other hand, LL stimulated 40% faster closure relative to control conditions in both C3 and C4 species. Furthermore, LC stimulated an elevated ion flux profile for K^+ and Ca^{2+} inward and outward rectifying channels in the guard cells especially in C3, while the opposite was observed under LL. These results indicate the coordination of stomata morphology, leaf biochemistry, and stomata biophysical traits provide critical factors in maintaining and optimising higher WUE_i among C4 grasses.

Heat acclimation of photosynthetic apparatus in different wheat genotypes

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Subjecting plants even with low basal thermotolerance to mild, non-lethal elevated temperatures may provide protection against a subsequent high temperature stress (heat-acclimation). Although the responses of plants to elevated temperatures have been widely studied, the exact mechanisms of heat acclimation are still poorly understood. The aim of present work was to evaluate of the effects of heat acclimation in young wheat plants with different origins. Various photosynthetic parameters were used to characterise the physiological responses of plants to high temperature stress with or without heat acclimation at 30 °C. The elevated acclimating temperature did not induce either stomatal closure or photoinhibition. Certain genotypes were able to induce transpiration at acclimating temperature and did not reduce net assimilation. Heat tolerant genotypes could also close stomata faster when they were exposed to severe high temperatures. Heat acclimation could also be detected in various chlorophyll-a fluorescence induction parameters; however, these were less genotype-dependent, and less reflected the differences between varieties. Heat sensitivity and heat acclimation indexes provided useful tool to differentiate the heat responses of the different wheat genotypes. Further studies are needed to establish whether the heat acclimation processes and the differences between the genotypes can also manifested in adult plants. This work was supported by GINOP-2.3.2-15-2016-00029.

Sulfur metabolism in C4 dicots and monocots

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While the uptake and accumulation of sulfate and reduced sulfur compounds is well characterized in C3 plants and dicots like Arabidopsis, less is known in C4 plants and monocots. Here, we describe our efforts to characterize sulfur metabolism in various C4 dicot and C4 monocot plants. First, in Flaveria, a dicot genus in the Asteraceae family having C3, C4, and C3-C4 intermediate species, ionomic and thiol profiling of roots and shoots in C3, C4, and C3-C4 intermediate species found significant differences in sulfur partitioning between the species. Results from reciprocal interspecies grafting further suggest that major differences in the demand for reduced sulfur compounds exist between C3 and C4 dicot species. Next, to evaluate sulfur metabolism in monocots, we performed a genome-wide association study by phenotyping a panel of 250 resequenced *Setaria viridis* lines for a number of sulfur phenotypes, including total leaf sulfur, total leaf sulfate, and leaf thiol levels. *Setaria viridis* is an emerging model C4 grass due to its rapid life cycle, short stature, high seed production, and rapidly expanding genetic and genomic resources. Its close phylogenetic relationship with economically important crops, including maize and sorghum, and its relatively small genome make Setaria an ideal system for rapidly discovering and characterizing agronomically important genes. Our preliminary results show significant natural variation in sulfur-related traits within Setaria. A comparison of our results in Flaveria and Setaria will be presented as well as key findings from the GWAS.

The first barley leaf as a model for developmental study of photosynthetic tissues: quantitative traits of mesophyll cells and chloroplasts

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A spatial gradient of mesophyll cells of different age, formed along the 7-day barley leaf blade from the base to the top allowed to evaluate quantitative traits of cells and chloroplasts during their development. The leaf was divided into 9 unequal zones by length from 0.3 cm near the base to 1.2 cm at the top. According to cell size and morphology zones I and II were determined as meristem, III-V as elongating, VI-IX as differentiated. Mesophyll cell volume changed from $4.34*10^3$ to $17*10^3$ μm^3 from I to IX. Cell shape also changed: in zones I and II, the cells had a regular shape, close to the isodiametric, and mitotic figures were observed. In III-V zones cells were cylindrical, the differentiated cells (zones VI-IX) often had a complex form, consisting of 2-5 lobes. The number of plastids increased from 18 in meristem cells to 50 in mature. The main growth of chloroplast number was fixed in I-IV zones. So the increase of chloroplast number occurred faster than cell growth. The chloroplast volume, on the contrary, increased significantly (from 16 to 122 μm^3) after the cells reached the final volume. So there is no synchronous growth of chloroplasts and cells in barley leaf. During cell development a significant increase in total plastid volume from 0.9% to 30% of cell volume was observed. The number of cells and chloroplasts per unit leaf area decreased from the base to the top mainly due to the increase in cell size. Photosynthetic and heterotrophic (dark) CO_2 assimilating rate depending on chlorophyll content, RUBISCO and PEPC-activities, consequently, were also different along the leaf blade. So the first barley leaf is a good model for monitoring the development of photosynthetic apparatus and its functioning.

Light intensity-dependent differences in metabolism during drought in wheat

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In order to determine the light intensity-dependent differences in the metabolism, 14-day old seedlings of two wheat (*Triticum aestivum* ssp. *aestivum* L.) varieties with different drought tolerance grown at 50 (low light; LL), 250 (normal light; NL) and 500 µmol/m^2/s (high light; HL) light intensity were subjected to drought. The water was withholded for 5 days which treatment was followed by one week recovery. The drought-tolerant Plainsman grown in LL or NL recovered better after stress as shown by its greater shoot fresh weight compared to the sensitive Cappelle Desprez variety. The amount of an important antioxidant, glutathione and its precursor, cysteine was 2-fold greater during the whole experiment in Plainsman compared to Cappelle Desprez. The concentration and ratio of reduced glutathione increased with increasing light intensity in both genotypes. .The concentration of several free amino acids (His, Val, Ile, Leu, Lys, Arg, Gln, Pro, Thr, Tyr, Phe) was ten-fold higher in Plainsman than in Cappelle Desprez after the drought stress at NL. In addition, the amount of these amino acids was also greater in both genotypes in HL at the end of the drought compared to the other sampling points. Expression of the genes related to the amino acid metabolism and redox system decreased in both genotypes during drought stress, and it increased during the recovery period to the values measured before the stress. The expression of the gene encoding ascorbate peroxidase was greater in Plainsman at the end of the stress compared to Cappelle Desprez at NL and HL. Changes in light intensity differentially affected the level of several transcripts and metabolites in the two genotypes which deviation may contribute to the difference in their drought tolerance.This work was supported by the National Research, Development and Innovation Office (grants ANN 117949, TéT_15-1-2016-0048 and TÉT_15-IN-1-2016-0028).

Alterations in chlorophyll index and phenolic compounds content under contrasting water supply conditions

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Determination of the optical activity and content of chlorophyll in leaves is one of the key techniques in measuring plant productivity. In addition, phenolic acids and flavonoids are plant secondary metabolites, which are intensively synthesized under drought. The experiment was carried out on five maize inbred lines differing in drought tolerance (B73, A632, L1, L2 and L3), with the aim to determine whether the tolerance and/or susceptibility to drought stress can be attributed to the level of phenolic compounds and chlorophyll in maize leaves. The inbreds were grown in two sets of field experiment: under well-watered and drought conditions, with two replications. At flowering, the chlorophyll index (Chl), the flavonoid index (FLAV) and the nitrogen balance index (NBI), which is based on the ratio between the mesophyll chlorophyll and epidermal flavone leaf contents were analyzed using a Dualex 4 Scientific device. This non-destructive quantification of chlorophyll was conducted on the middle, basal and apical position of the uppermost ear leaf on twenty plants per genotype. The same leaves were taken for quantification of six natural phenolic acids (protocatechuic, sinapic, *p*-coumaric, caffeic, ferulic, cinnamic) using HPLC with diode array detection (DAD). In drought susceptible lines, water deficit caused the decrease in NBI and Chl up to 33.8% and 21.7%, respectively, while increase in FLAV for approximately 18.1%. Opposite trend was noticed in drought tolerant lines (average increase of 30.6% for NBI, 13.3% for Chl and decrease of 15.5% for FLAV, respectively). Significant and positive correlation between Chl and grain yield was found ($r=0.880$, $p\leq0.05$), being more pronounced under drought. In all inbreds evaluated, only three phenolic acids were detected in both well-watered and drought-stressed plants fresh leaves. Under drought, significant increase of protocatechuic and *p*-coumaric acids (35.7% and 73.7%, respectively) were found in drought tolerant inbreds L2 and L3.

Metabolite responses of Arabidopsis thaliana rcd1 to paraquat reflect high tolerance to chloroplastic oxidative stress due to altered primary metabolism and enhanced antioxidant defense

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The ozone-sensitive *rcd1 (radical-induced cell death1)* mutant of *Arabidopsis thaliana* is deficient in the containment of programmed cell death and its many phenotypes include altered hormonal signalling, early senescence and high tolerance to paraquat. In a plant cell, paraquat interrupts photosynthetic electron transport chain inducing the generation of ROS and depletion of NADPH. To study the biochemical features and the mechanisms of paraquat tolerance in *rcd1* we performed untargeted metabolite profiling of Col-0 (wild type) and *rcd1* plants in light, after prolonged darkness and paraquat treatment. Primary and secondary metabolite profiles differed greatly between wild type and *rcd1* plants. The levels of several carbohydrates and amino acids as well as metabolites connected to nucleotide metabolism and senescence were high in *rcd1*. *Rcd1* accumulated 3-hydroxy-3-methylglutaric acid, a marker for plant senescence and a typical indicator of mitochondrial dysfunction in animal studies. Altered conversion of xanthine to urate and redox state of ascorbate and glutathione indicated redox imbalance in *rcd1*. The metabolite responses to extended darkness were mainly similar, but the responses to paraquat differed between the plant lines. Unlike wild type plants, *rcd1* did not show symptoms of oxidative stress in mitochondria and NADPH/NADH depletion in paraquat treatment. However, the accumulation of 2-isopropylmalate, an indicator of oxidative stress in chloroplast, suggested that paraquat reached its site of action in both lines. The levels of antioxidants (tocopherols, glutathione) were initially higher in *rcd1* than in wild type and their levels hardly changed in response to paraquat. This indicates that the available scavenging mechanisms in *rcd1* may be sufficient to avoid the negative effects of ROS under paraquat-induced oxidative stress.

The effect of moderate drought on the assimilation in the dependence to light at suboptimal temperature in monocotyledonous crop plants

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Acclimation, is of great importance in plant adaptation. Like other combinations of abiotic stress factors, the physiology of simultaneous cold and water deficiency and different illumination levels has not been investigated in detail. In order to dissect the acclimation effect of mild drought hypothesized at suboptimal temperature, the key processes of carbon and nitrogen assimilation were studied. We focused on the light utilization capability, net photosynthetic assimilation and the conversion of inorganic nitrogen to organic compounds.
Our results suggest that the assimilation of wheat and maize differently respond to mild drought in the dependence to light. The light harvesting and photosynthetic machinery was less damaged in the cold under moderate dehydration than well-watered state. Moderate drought has an acclimation effect in the C3 cereals investigated. Additionally, we found that durum wheat maintains light absorption/utilization in a better manner. As the temperature drops, the maintaining effect of mild water deprivation on C4 maize plants was more emphasized than in wheat. The CO_2 assimilation was also facilitated by moderate drought at suboptimal temperature in maize. Durum and bread wheat cultivars have different strategies for maintaining drought stress in the cold of the aspect of nitrogen assimilation. The nitrate reduction accelerated at low light both in durum and bread wheat, as well as in maize, independently to water supply. Low light has also defending effect on glutamine synthetase activity. In maize cultivars, glutamine synthetase showed low light sensitivity and maintained the activity around control level in the cold.
In summary, we suggest that moderate drought in the cold may not be regarded the same kind of dehydration as compared to the drought acts on plant at optimal temperature. In other words, under adverse temperature conditions, the potential acclimation effect of drought on plant life may be confirmed.

Background processes and the components of photoprotection and regeneration under rehydration in desiccation-tolerant and desiccation-sensitive bryophytes

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Although many of the light protection mechanisms of bryophytes are common with the higher plants, there are fundamental differences too. The mechanisms found in the algae ancestors that the vascular plants have lost during their evolution have been retained by the bryophytes. In vascular plants, NPQ is based on the activity of PSBS, while in *C. reinhardtii* green algae it requires LHCSR. So far, *P. patens* is the only described bryophyte in which both of these proteins are present and active in the induction of NPQ. Our investigations on desiccation-tolerant *Porella platyphylla* and desiccation-sensitive *Sphagnum angustifolium* were directed to detect photosynthetic activity, light protection and other regeneration mechanisms during varying degrees and duration of desiccation and rehydration with the use of violaxanthin cycle inhibitor, plastis and nuclear-encoded protein synthesis inhibitors. The regeneration of thylakoid function related photosynthetic processes in *P. platyphylla* 1 h after rehydration was extremely rapid and independent of the rehydration linked protein synthesis, while the total regeneration required protein synthesis. The bryophytes dried at more favourable conditions had a better light protection. Xanthophyll cycle has great importance during the regeneration in the light. Higher Z-dependent and lower ratio of DTT-insensitive NPQs were confirmed. The desiccation-tolerant bryophytes are characterized by the fact that they do not suffer from photooxidative damage due to the coexistence of Z-dependent and dehydration-induced thermal energy dissipation.

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Direct comparison of plasmodesmata structure and function indicates the mode of photoassimilate transport in the leaves of trees

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Forest trees cover around 30 percent of the planet's land area and account for 50 percent of plant productivity. Yet, key aspects of tree physiology remain poorly understood. Photoassimilates are transported from leaves to other parts of the plant that depend on their import for growth and development in the highly specialized cells of the phloem. While in herbaceous plants an active mechanism loads these photoassimilates into the leaf phloem, no active mechanism was found in trees, and it remains unclear what drives phloem loading. In this project, we aimed to test if photoassimilates could enter the phloem by passive diffusion.
Intercellular diffusion depends on the presence and permeability of plasmodesmata (PD), cell wall channels that link the cytosol of neighboring cells. Transmission electron microscopy was used to quantify PD structure and abundance at all cell-cell interfaces along the phloem loading pathway. To determine PD permeability, the live cell imaging technique fluorescence loss in photobleaching (FLIP) was used. Intact source leaves of four different tree species and several herbaceous species were analyzed.
For poplar, beech and chestnut trees, a high number and uniform distribution of PD at all interfaces of the phloem-loading pathway was detected. A high degree of cell coupling was confirmed by the functional tests. These results support the hypothesis of photoassimilate diffusion into the phloem in trees. Apple trees, however, showed lower PD abundance and lower cell coupling, indicating an optimization for active loading.
The results indicate that there are significant differences in the phloem loading mechanism between trees and herbs, and between different tree species. Furthermore, important insight on the structure-function relationship of PD could be gained as the study represents the first direct comparison of PD data from quantitative electron microscopy and live-cell microscopy.

External factors modifying acclimative leaf UV-B responses

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UV-B (280-315 nm) radiation is a regulator of developmental and metabolic responses. Model plants of the Nicotiana spp. maintain leaf photochemistry and give acclimative responses to supplemental UV-B in growth chambers. In our experiments 6.9 kJ/m^2/d biologically effective UV-B was applied for several days in combination with 175 µmol/m^2/s PAR. Acclimative responses included increased leaf flavonoid contents and higher antioxidant enzyme (especially peroxidase) activities to prevent oxidative damage (Czégény et al. 2016). The present work was aimed at studying the modifying effects of external factors, such as β-aminobutyric acid (BABA) and drought on these responses. BABA is a potential novel plant hormone, capable of inducing resistance against a variety of abiotic stresses (Cohen et al. 2016).
Soil application of 300 ppm BABA as a single factor had no effect on pigment content or leaf photochemistry but decreased leaf fresh weight. Plants exposed to both UV-B and BABA showed a marked decrease in non-photochemical quenching (NPQ), due to a rearrangement in regulated and non-regulated photochemical quenching yields.
Drought (limited watering) as a single factor increased leaf flavonoid content but had no significant effect on leaf photochemistry. An increase in both non-enzymatic total and ROS-specific antioxidant capacities, as well as higher peroxidase enzyme activity indicated, that tolerance was achieved by shifting the antioxidant-prooxidant balance towards protection. After a pre-treatment with UV-B, drought tolerance required a smaller increase in antioxidant capacities, especially in younger leaves that developed after the UV-B treatment. However, when drought was applied in combination with UV-B leaf responses indicated oxidative stress, due to an over-taxing of the antioxidant system.
Czégény Gy, Mátai A, Hideg É (2016) Plant Science, 248:57-63.
Cohen Y, Vaknin M, Mauch-Mani B (2016) Phytoparasitica, 44:513-538
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Defining optimal electron transfer partners for light-driven cytochrome P450 hydroxylations

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Plants produce many specialized metabolites with potential medicinal, fragrance and flavour uses. Cytochromes P450 occur frequently in natural product biosynthesis, but are difficult to express and require an NADPH-dependent reductase, which hampers pathway reconstitution. We previously demonstrated that plant P450s expressed in chloroplasts of tobacco plants and cyanobacteria will insert into the thylakoid membrane, and that photosynthesis supports P450 catalytic activity through the action of the small electron carrier ferredoxin. This study presents the characterization of selected electron transfer proteins as electron donors to the plant P450 CYP79A1. By fusing CYP79A1 with potential redox partners, grants the ability to obtain electrons for catalysis directly from photosystem I. These fusion enzymes sequester reducing power more effectively than their unfused counterparts. As a result, fusion alleviates the problem of competition for reduced ferredoxin by electron sinks coupled to endogenous metabolic pathways, and enables the construction of bespoke electron transfer chains serving pathways introduced through metabolic engineering in photosynthetic organisms. The fusion strategy reported enables control over partitioning of photosynthetic reducing power towards P450-dependent biosynthesis of important natural products, and has high potential in stable engineering of photosynthetic organisms for high-level light-driven cytochrome P450-dependent production of valuable natural products.

Special features of photosynthetic processes in some horticultural plants in vitro

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Considering the high sensitivity of photosystem-2 chlorophyll-protein complexes activity under varying *in vitro* culture conditions, we used fluorescence induction parameters to estimate the relative photosynthetic activity $(F_m-F_{st})/F_m$ in vegetative organs of some valuable horticultural plant cultivars. Morphological and histological features of assimilating organs were studied. The parameters of photosynthetic activity were measured using a portable fluorimeter LPT-1/CFU (fluorescence is excited at 470 nm, the spectral range of the photodetector sensitivity is 350-1100 nm, the time of the Kautsky curve output is 300 sec), which allows monitoring photosynthetic processes without violating the aseptic conditions. Plantlets of garden canna, chrysanthemum, clematis, essential-oil rose, lavender, lavandin, persimmon, common fig were studied under *in vitro* standard culture conditions and deposition. The assimilating tissues of the leaves were developed in different level. In garden canna, some chrysanthemum and persimmon cultivars leaf mesophyll was homogeneous, the chloroplasts were arranged diffusely. The leaves of essential oil rose, lavender, lavandin and fig plantlets had the differentiated palisade and spongy chlorenchyme as well as chloroplasts on the periphery along the cell wall. The photosynthetic activity, depending on the genotype and culture duration, ranged from 0.48 to 0.72 a.u. Photoinhibition in clematis, persimmons and lavender cultivars after more than 6 months culture was noted. Chlorophyll fluorescence induction were lower during *in vitro* storage: from 0.30 to 0.61 a.u. (in most cultivars – 0.38-0.42 a.u.). Plantlets had an elongated internodes in shoots (in essential oil rose and clematis), branching increase in shoots (fig, lavender, lavandin), reduced complexity of leaves (in essential oil rose the leaves were simple and oval instead of tripartite), leaf tissue with large intercellular spaces and stomatal apparatus. Positive correlation between the histological characteristics of palisade index and relative photosynthetic activity was revealed. This study was funded by the research grant N 14-50-00079 of the Russian Science Foundation.

Post-translational modifications (PTMs) modulate phosphoenolpyruvate carboxylase (PEPC) activity in response to abiotic stress in sorghum (Sorghum bicolor)

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Phosphoenolpyruvate carboxylase (PEPC) (EC 4.1.1.31) is a ubiquitous cytosolic enzyme that catalyses the irreversible *β*-carboxylation of PEP in the presence of HCO_3^- to yield oxaloacetate and Pi. PEPC plays a crucial role in C_4 and CAM photosynthesis, where it catalyses the initial fixation of atmospheric CO_2. PEPC also fulfils several important non-photosynthetic functions in all plants, including supporting carbon-nitrogen interactions, seed formation and germination, fruit ripening, guard cell metabolism, or root malate excretion among others. Due to its pivotal roles in plant metabolism, PEPC protein is tightly regulated by post-translational modifications (PTMs). We have studied PEPC PTMs both in photosynthetic and non-photosynthetic tissues using sorghum (*Sorghum bicolor*) as a model of study. In this specie, plant-type PEPC is phosphorylated in a conserved Ser residue producing an activation of the enzyme. Photosynthetic PEPC is inhibited by anionic phospholipids and presumably recruited to the membrane, in a manner independent from the phosphorylation state of the enzyme. Recently we have showed that PEPC from sorghum seeds, and in sorghum roots under ammonium toxicity, is monoubiquitinated and that this process is inhibitory. In addition, photosynthetic PEPC is S-nytrosilated and/or carbonylated under salt stress conditions. Carbonylation inactivates C_4 PEPC while nytrosilation has little impact on its activity but holds back carbonylation. Moreover, recent results suggest that photosynthetic PEPC can be Tyr-nitrated in a specific residue yielding an inhibited enzyme, and phosphorylated PEPC is protected from Tyr-nitration. The fact that most of these PTMs can coexist in the same protein, both with antagonist or synergist effects on PEPC activity/stability, highlights the fine tuning of this protein *in vivo* in response to a plethora of different environmental stresses.
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Bryopsis hypnoides and Codium tomentosum accumulate all-trans-neoxanthin and violaxanthin as a photoacclimation mechanism under high light

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During photoacclimation, plants and algae change the content of light-harvesting and photoprotective pigments and adjust the cellular ultrastructure to better cope to available light. The macroalgae *Bryopsis hypnoides* and *Codium tomentosum* lack a functional xanthophyll cycle, one of the most important photoprotective mechanisms against high light-induced damages. In this study, we present chlorophyll *a* fluorescence data and a detailed characterization of the pigment profiles and pattern of *B. hypnoides* and *C. tomentosum* cultured under different light intensities and under different stress conditions. We show an accumulation of the pigments all-*trans*-neoxanthin and violaxanthin under high light conditions. The accumulation does not occur during a short-term stress event. The concentration of the pigment remains stable and it does not participate to the recovery of the photosynthetic capabilities. Our results strongly suggest a role of these pigments in a long-term photoacclimation process and not in a short term photoprotective mechanism.

The relationship between antioxidant status and PSII activity with the transcript level of photoreceptors and some light signalling genes in Eutrema salsugineum callus exposed to different light quality

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The change in the spectral composition of light affects not only photosynthesis but also other processes associated with it, such as the biosynthesis of proteins and nucleic acids, formation of secondary metabolites, and antioxidant status of cells. Recent data showed that cryptochromes can participate in the generation of reactive oxygen species (ROS), including hydrogen peroxide. In particular, it has been shown that the ROS generated by blue light (BL) can act as intermediates in the transduction of the BL signal.
In view of the photoheterotrophic growth of the callus lines cultivated on nutrient media the trophic role of photosynthesis is obviously reduced, but this fact does not exclude the functioning of photoreceptors and the phytohormonal system. It this work we show the relationship between antioxidant status and PSII activity with the transcript level of photoreceptors and some light signalling gene in callus culture of *E. salsugineum* exposed to different light quality.
We believe that BL causes an increase in the H_2O_2 content in cells through the activation of plasma membrane NADH oxidase and/or possibly through the inactivation of the water-decomposing PSII complex. The increase in the activity of catalase and peroxidase under BL is associated with the activation of ROS formation, metabolic acceleration, senescence processes, and/or apoptosis. The maximum contents of H_2O_2, TBARS and a smaller number of living cells in BL are probably associated with a higher level of oxidative stress in the cells compared to red light. The obtained data indicate that in callus cells, despite the practically heterotrophic nutrition, primary photochemical processes are manifested, which may be important for the O_2 generation. The photoreceptors and light signalling components genes are expressed and the antioxidant enzyme activity is changed, which is important for maintaining antioxidant balance, the formation of chloroplasts, and the number of other metabolic processes.

The impact of light quality on cold stress tolerance of Arabidopsis thaliana

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Cold stress responses of plants are dependent on light intensity, as well as on light spectrum. In order to describe the effect of light quality during cold stress response and acclimation, we used *Arabidopsis thaliana* plants (wt) and mutants in blue light receptors cryptochromes (*cry1, cry2*) or in red light receptors phytochromes (*phyA, phyB*). Plants were exposed to normal (150 µmol/m^2/s) or low (20 µmol/m^2/s) light intensity at 5°C or 20°C. Samples were collected after 30 min, 6 h or 7 d. Mutant plants had similar phenotype as wt, only *phyB* had smaller blades with long petioles. Cold stress slowed down the growth of all genotypes regardless light intensity. Temperature drop to 5°C affected maximum quantum yield of photosystem II (QY_{max}) after 6 h (mainly in wt and *cry1*). Non-photochemical quenching (NPQ) was found the lowest in *phyB*. Low light caused decrease of QY_{max} after 7 d, most strongly in *cry2* and *phyA*. Combination of cold and low light significantly suppressed stress impact on QY_{max}, NPQ, as well as steady-state quantum yield. Membrane stability (MSI) was weakened after 30 min and 6 h under low light conditions (mainly in *cry2*, *phyA* and *phyB*). Cold stress lowered membrane stability in all tested genotypes in the long term. This effect was suppressed in combination with low light. Low light intensity in general stimulated lipid peroxidation (MDA) in all genotypes after 30 min and 6 h (except in *cry1*), while no effect was found in cold stress. Mutants *cry1* and *phyA* were more sensitive to frost than wt. Cold acclimation enhanced frost tolerance in all genotypes, but this effect was substantially diminished by low light. Our results indicate an involvement of particular photoreceptors in cold and low light responses.
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Thylakoid membrane plasticity is required for fine-tuning photosynthesis and plant fitness

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The thylakoid membrane system of higher-plant chloroplasts consists of interconnected subdomains of appressed and non-appressed membrane bilayers, known as grana and stroma lamellae, respectively. The shaping of the cylindrical grana stacks is mediated, in a dosage-dependent manner, by CURT1 protein complexes, which facilitate membrane curvature at the grana margins, the interface between grana and stroma lamellae [1]. Here, we show that CURT1-related alteration of thylakoid ultrastructure reduces photosynthetic efficiency by affecting regulatory mechanisms required for fine-tuning photosynthesis under variable, adverse light conditions and ultimately affects plant fitness [2]. Plants that lack CURT1 are impaired in adjusting grana diameter, which compromises the effectiveness of regulatory mechanisms known to optimize photosynthetic performance. We demonstrate that CURT1A suffices to induce thylakoid membrane curvature *in planta*, and thylakoid hyper-bending that is observed in CURT1A overexpressor lines. Further, our data suggest that CURT1 proteins are already present during the early stages of chloroplast differentiation and play a role in thylakoid biogenesis.
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How can the photosynthetic activity of desiccation tolerant plants with different strategies be regenerated after a few years of dehydration?

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Two subdivisions of desiccation tolerant (DT) plants, HDT (homoiochlorophyllous) and PDT (poikilochlorophyllous) plants apparently represent contrasting strategies to solve the same ecological problem at drought conditions, whereby HDT plants usually survive shorter drought periods of several days and weeks (up to a few months), whereas PDT plants can endure longer drought periods of 6 to 11 months. Species of HDT strategy well reflects the early regeneration ability after rehydration due to remaining their chlorophyll contents on dehydrating states. PDT plants need more time for activation because they must rebuild their dismantled chloroplasts structure.
Different DT species grown under similar ecological conditions have different and characteristic histo-physiological features. Physiological recovery scale and degree of these plants is useful for understanding plant interactions like colonization benefits under severe growth limitations and also plant-environment relationships which determine their production in their harsh ecosystems.
Significance of recovery and spending time in desiccated period is the basis of a successful surviving strategy because the carbon assimilation during hydrated periods must be higher than carbon costs with carbon losses during desiccated periods. Periods and effects can probably be changing due to global climate change.

The effect of light quality on yield, quality and morphology of four different Basil varieties (Ocimum basilicum).

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Total Controlled Environment Agriculture (TCEA, indoor farming) provides opportunities to produce food crops of consistent yield and quality at any time of the year and any global location through the application of a consistent crop growing environment. A key factor in the economic success of TCEA is the rapid optimisation of the light environment to maximise yield and quality whilst minimising capital costs and energy input. A range of horticultural LED lighting systems are presently available that differ in their spectral output and flexibility. Horticultural LED lighting fixtures typical provide light within the photosynthetically active region (PAR) of the spectrum (400-700 nm). However, next generation LED fittings offer greater flexibility with the capacity to independently control multiple wavelengths including those that are not direct harvested by the plant to drive photosynthesis. In the present study we examined the impact of far-red light (730 nm) at different intensities on the yield, morphology and quality of basil. We demonstrate that a shift in the growth spectrum from red to far-red can enhance crop fresh weight even when total light irradiance is not increased. This is achieved primarily through stem elongation promoted by water uptake. Crop quality, as estimated by leaf photosynthetic pigment content was not significantly altered by manipulation of the red:far-red ratio. On the contrary, the leaf content of specific secondary metabolites was significantly affected by the irradiance spectrum. Taken together our data indicate the need to optimise growth spectrum for plant biomass and quality. We additionally demonstrate the feasibility of using the light spectrum to manipulate quality parameters of fresh produce providing growers with an opportunity to respond to changing consumer demands.

Spekboom planting in degraded subtropical thicket improves soil properties and native species diversity

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In this study**,** we assessed whether *Portulacaria afra* (spekboom) planting improves both soil physico-chemical properties and native species diversity in the Saltaire Karroid Thicket. We collected topsoil samples from a spekboom planted site and compared them to an adjacent natural and degraded site and quantified gravimetric soil moisture, pH, soil resistivity, soil penetration, soil P, total C, total N, cations of K, Ca, Mg and Na and soil water repellency. We further conducted vegetation surveys in plots measuring 10 x 10 m. Results show significant ($P < 0.05$) increase in soil P, total C, total N, Ca and soil moisture in the natural and spekboom planted sites compared to the degraded site. Both soil compaction and water repellency were significantly ($P < 0.001$) high in the degraded site compared to the natural and spekboom planted sites. Species richness and diversity were significantly ($P < 0.05$) high in the natural and spekboom planted site compared to the degraded site. The presence of native trees and shrubs in the spekboom planted site points to a positive vegetation recovery trajectory. We conclude that the planting of spekboom in degraded areas improve some soil properties and native species diversity.

Synergistic binding of bHLH transcription factors to the promoter of the maize NADP-ME gene used in C4 photosynthesis is based on an ancient code found in the ancestral C3 state

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C_4 photosynthesis has evolved repeatedly from the ancestral C_3 state to generate a carbon concentrating mechanism that increases photosynthetic efficiency. This specialised form of photosynthesis is particularly common in the PACMAD clade of grasses, and is used by many of the world's most productive crops. The C_4 cycle is accomplished through cell-type specific accumulation of enzymes but *cis*-elements and transcription factors controlling C_4 photosynthesis remain largely unknown. Using the *NADP-Malic Enzyme* (*NADP-ME*) gene as a model we tested whether mechanisms impacting on transcription in C_4 plants evolved from ancestral components found in C_3 species. Two basic Helix-Loop-Helix (bHLH) transcription factors, ZmbHLH128 and ZmbHLH129, were shown to bind the C_4 *NADP-ME* promoter from maize. These proteins form heterodimers and ZmbHLH129 impairs *trans*-activation by ZmbHLH128. Electrophoretic mobility shift assays indicate that a pair of *cis*-elements separated by a seven base pair spacer synergistically bind either ZmbHLH128 or ZmbHLH129. This pair of *cis*-elements is found in both C_3 and C_4 Panicoid grass species of the PACMAD clade. Our analysis is consistent with this *cis*-element pair originating from a single motif present in the ancestral C_3 state. We conclude that C_4 photosynthesis has co-opted an ancient C_3 regulatory code built on G-box recognition by bHLH to regulate the *NADP-ME* gene [1]. More broadly, our findings also contribute to the understanding of gene regulatory networks controlling C_4 photosynthesis.
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Glyphosate-induced oxidative stress in non-target plants - a physiological and biochemical approach in tomato

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Nowadays, agriculture is heavily dependent on the application of agrochemicals, such as herbicides and fertilizers, to ensure crop's protection under adverse and unpredictable conditions. Among all, glyphosate (Gly) is the most used herbicide worldwide and its abundance in the environment is quickly increasing, posing a threat to non-target organisms, like non-resistant plants. In this perspective, this study aimed to understand the biochemical and physiological basis of Gly-induced stress in tomato (*Solanum* lycopersicum L.). For this, plants were grown for 28 days under different concentrations of Gly - 0, 10, 20 and 30 mg kg^{-1} soil. The exposure of plants to increasing concentrations of Gly resulted in a severe inhibition of growth (root and shoot elongation and fresh weight), accompanied by a decrease in photosynthetic pigments and relative RuBisCO contents, especially in the higher concentrations. Levels of both hydrogen peroxide and superoxide anion remained unchanged in shoots, but presented a significant increment in roots. Moreover, it was observed a decrease in lipid peroxidation in a concentration-dependent manner in shoots, though in roots only differences were found for the highest applied dose. The evaluation of the antioxidant system allowed to infer that Gly interfered with several metabolites and enzymes. Regarding the non-enzymatic component, increased levels of proline were found in shoots, along with a reduction in glutathione and reduced ascorbate. Regarding roots, both proline and glutathione were increased in response to 20 and 30 mg kg^{-1} Gly. In what concerns the enzymatic component, superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities were enhanced in both organs throughout treatments, excepting SOD in roots, whose activity was inhibited by the two highest Gly concentrations. Overall, data obtained in this study unequivocally indicate that soil contamination by Gly greatly impairs tomato growth and physiological performance, mainly by disrupting the oxidative redox homeostasis.

Studies on plant responses to LED quasimonochromatic light

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Recently developed high-brightness light-emitting diodes (LEDs) provide new opportunities for the plant lighting technologies. They can be used effectively both for basic research of plant photomorphogenesis and for the precise control of physiological processes in plants in industrial horticultural lighting technologies. Using combinatorial lighting technologies, *e.g.*, we can accelerate plant breeding process, improve food quality, and save energy. A large amount of data on growing various plants with LED lighting sources has been already accumulated. However, the question of optimal photosynthetic photon flux density (PPFD) reaching the crop, and best spectral composition of the light, which takes into account species biology and ontogenetic phase of plants, is still open. In our experiments, we studied application of LEDs with the narrow-bandwidth wavelength emissions for the control of basic physiological processes in several vegetable crops usually grown in plant factories. The effects of four PAR ranges were studied: ”blue” ($\Delta \lambda = 440 \div 475$ nm), ”green” ($\Delta \lambda = 500 \div 560$ nm), ”amber” ($\Delta \lambda = 560 \div 600$ nm), and ”red” ($\Delta \lambda = 630 \div 670$ nm). As the reference light source, white LED luminaires with Cct = 2500 and 5000^0K were used. Plant responses to several levels of PPFD were observed. The effects of main PAR ranges both on plant productivity and secondary metabolism target compounds were studied. Besides, changes in plant source-sink relations and developmental phase transitions were observed. Experimental data show that various LED systems with discrete wavelength ranges peaking at different spectral regions can be used effectively for plant growing, except amber light. For two plant species, lettuce and basil, ”rough” action spectra in response to different irradiance levels were obtained. Other plant responses to light spectral composition, besides biomass accumulation, were much more specific.

The alga that never read the literature - Fastest growing photodamage tolerant alga isolated from desert crust

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The unparalleled performance of *Chlorella ohadii* (Treves *et al*., 2016), clearly indicates that we lack essential information on the photosynthetic machinery and what sets the upper growth limits. When grown under optimal laboratory or controlled outdoor conditions, this alga, recently isolated from one of the harshest environments (a biological desert sand crust), exhibits the fastest growth rates ever reported for an alga, division times shorter than 2 h were recorded. The cultures perform very high photosynthetic rates and reach high cell densities ($1.3*10^9$ cells/mL). Unlike other photosynthetic organisms, *C. ohadii* productivity is unaffected by irradiances twice full sun light; and the level of protein D1, encoded by a single gene, is hardly affected. Rather than succumbing to photodamage *C. ohadii* undergoes major structural and compositional changes (including development of pyrenoids, 2-3 fold increase of the lipid and carbohydrate contents and a large rise in the thylakoids abundance), emphasizing the unique PSII functioning as well as highly efficient reductant utilization downstream of the photosynthetic reaction centers. *C. ohadii* may be used to clarify the processes that rate-limit growth and productivity of photosynthetic organisms. Based on these remarkable capabilities we were able to explore several novel and uncharacterized aspects of algal growth under extremely high illumination and temperature, and desiccation, which were so far too damaging for current model organisms. Growth of batch cultures under continuous high light (3000 µmol photons m^{-2} s^{-1}) combined with metabolome analyses revealed a highly coordinated metabolic switch, supporting growth to higher densities than those achieved if abolished, and regulated by specific signaling molecules (Treves et al., 2017). RNA-Seq revealed regulation of genes networks under changing light and trophic regimes, and provided novel insights on the mechanism underlying its exceptional photodamage resistance.

Treves et al., 2016 *New phytologist*
Treves et al., 2017 *Current Biology*

Screening of natural and man-made variability in Lolium under ambient and future atmospheric CO2 indicates that selection for increased biomass productivity is largely unrelated to variations in leaf photosynthetic rate.

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Perennial rye-grass (*Lolium perenne*) is the key component of the most productive pastures and one of the most important forage grasses worldwide. Agricultural grasslands in particular provide forage for the beef and dairy industries, thus an understanding of the processes driving grass productivity and how this is impacted by climate change is of particular importance for future food security. Our study investigated the performance of 38 annual, perennial and hybrid *Lolium* genotypes encompassing natural, semi-natural and cultivated accessions under ambient (400 ppm) and simulated future elevated CO_2 atmospheric conditions (800 ppm). Gas exchange determinations, elemental analyses and productivity measurements were carried out on 5 plants per genotype and treatment grown in four BDW40 growth chambers for 3 months. Under ambient conditions the cultivated genotypes displayed significantly higher photosynthetic rates, higher water use efficiencies and productivities compared with the semi-natural and wild genotypes. However, statistical analysis showed only a weak correlation between biomass productivity and leaf level photosynthetic and diffusional traits. The observed stimulation of photosynthesis under elevated CO_2 was more pronounced in the wild and semi-natural genotypes so that there were no significant differences in the leaf photosynthetic rates between any of the accessions at high CO_2 although the biomass productivity of the cultivated material was again higher. Estimates of whole plant photosynthesis indicated that variations in leaf area can explain ~65% of the variability in biomass productivity under both ambient and elevated CO_2. These results show that natural as well as breeding-related differences in leaf area and tillering, rather than alterations in leaf photosynthesis, are the major reasons for yield variations in Lolium and that this is unlikely to be modified by higher CO2 concentrations in the future.

Genome wide association analysis of photosynthesis efficiency in Arabidopsis

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Natural genetic variation in plant photosynthesis efficiency is not much investigated even though breeding for photosynthesis would be interesting to maintain increases in crop yields. One of the reasons this is not studied is the notorious difficulty in adequately phenotyping photosynthesis parameters for genetic research. We determined the light use efficiency of photosystem II electron transport (ΦPSII or Fq'/Fm') through chlorophyll fluorescence measurements, and in addition used near infrared reflection to measure projected leaf area. This system has been used to phenotype *Arabidopsis thaliana* recombinant inbred line populations as well as a HapMap population of around 350 genetically diverse accessions for genome wide association analysis. Plants were phenotyped at high throughput, several times per day, at optimal conditions and in response to cold treatment (5 °C) or upon a switch in irradiance from 100 to 550 µmol m^{-2} s^{-1}. The observed genotypic variation was used to identify quantitative trait loci (QTL) and candidate genes with allelic variation underlying those QTLs. Our work has shown that there is sufficient genetic variation for PSII efficiency in Arabidopsis amenable for gene identification, which suggests the same will be the case for crop species. Such would offer interesting opportunities for future crop photosynthesis, and subsequently yield, improvements.

Theme 3: Genome Based Breeding – New breeding technologies

Genomic Selection in Winter Barley: A comparison of One-Step and Two-Step Approach

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Recently genomic selection has demonstrated to be a promising tool for predicting important agronomic traits in plants. Several models have been developed for estimating accuracies through simulations and surprisingly no single method has emerged as a benchmark for genomic selection. The choice of model depends on a lot of factors, among which genetic architecture of the trait ranks high. In estimating prediction accuracies, a one-step and two-step approaches are used where the latter involves obtaining line-effects corrected for environmental factors from replicated plot data.
In this study, several prediction models were evaluated in a commercial barley breeding program consisting of 177 two-rowed elite winter barley lines grown at two different locations and 4997 SNP markers for five traits (protein content, specific seed weight, seed fractions weights >2.8mm, 2.8mm-2.5mm and 2.50mm-2.2mm diameter). The effect of model (Bayes-RR, Bayes A, Bayes B and Bayes C) and approach (one-step and two-step) on prediction accuracy and bias were assessed using a 10-fold cross validation. Overall, prediction accuracies were high for all traits ranging between 0.61 and 0.95. These high prediction accuracies could be due to the high relatedness of the lines used in this study. Models in the two-step approach produced higher accuracies and lower biases compared to the single-step approach. However within each approach, there was no difference in accuracy and bias in the models tested. The similar accuracies in all models under each approach indicate the quantitative nature of the traits where many but small loci may affect the trait and hence the assumption underlying the variable selection methods may not be beneficial. Predictions in both the one-step and two-step approaches were biased for all models; however the two-step approach where the lines were modelled as fixed effects in the first analysis, gave the lowest bias estimates.

Trans-species synthetic gene design allows resistance pyramiding and broad spectrum engineering of virus resistance in plants

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To infect plants, viruses heavily rely on their host's machinery. Plant genetic resistances based on host factors modifications are available among the natural variability and widely used for some but not all crops. While biotechnology can supply for this lack, new strategies should be developed to increase resistance spectrum and durability without impacting the plant development. Synthetic biology could offer this opportunity by developing functional synthetic alleles similar to the natural ones. The eukaryotic translation initiation factors 4E (eIF4E), that are associated with resistance to most single strand positive RNA viruses (including the major group of potyviruses), are the ideal targets to validate such approaches.
Natural variation in eIF4E from pea resistant accession was used to design a synthetic Arabidopsis thaliana eIF4E1 allele by the introduction of six amino acid changes. Complementation of loss-of-function *eif4e1* plants showed this new allele encodes a functional protein but still maintains the plant resistance to a potyvirus isolate that usually hijacks eIF4E1. Due to its biological functionality, this synthetic allele allows the pyramiding of resistances to potyviruses that use selectively the two major translation initiation factors, eIF4E1 or its isoform eIFiso4E at no developmental cost. Moreover, this combination extends the resistance spectrum to three potyvirus isolates toward which no efficient resistance existed so far, including two resistance-breaking isolates and to an unrelated virus belonging to the Luteoviridae family.
This study makes the proof-of-concept for the efficiency of synthetic biology combined with the knowledge brought by natural variation to generate trans-species virus resistance at no developmental cost for the plant. This has serious implication in breeding for the development, by recent genome editing techniques, of crops with broad-spectrum and high durability resistance.

References:
Bastet et al, 2017, Trends In Plants Sciences (DOI:https://doi.org/10.1016/j.tplants.2017.01.008)
Bastet et al, 2018, Plant Biotechnology Journal (DOI:https://doi.org/10.1111/pbi.12896)

Barley ovules as a genotype-independent starting material for targeted mutagenesis induced by CRISPR/Cas9

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Several studies have shown that the CRISPR/Cas9-tool can be used successfully to generate targeted mutations in barley. In these studies, the starting material was immature embryos isolated from the barley cultivar Golden Promise, which serves as the model cultivar in barley. Golden Promise is, however, genetically very different from elite barley cultivars used in current barley breeding programs and transfer of the targeted mutations to elite cultivars therefore require large numbers of backcross-generations. A less genotype-dependent transfer system for CRISPR/Cas is therefore highly desirable. We have previously established a genotype-independent transformation system in barley using the zygote within the ovule as starting material (Holme et al., 2006, Plant Cell Rep. 25, 1325-1335). In order to facilitate genotype independent genome editing in cereals, we recently have started using this system to induce targeted mutation by means of a CRISPR/Cas9 construct previously used for barley immature embryos (Holme et al., 2017, Plant Mol. Biol., 95, 111-121). This CRISPR/Cas9 construct shows a high mutation frequency of 44% at the targeted site when using immature embryos as starting material. Thus, this construct is ideal for the optimization of targeted mutagenesis in the ovule culture system. The first results indicates that ovules serves as a feasible tissue for CRISPR/Cas based modifications in barley. Current data will be presented at the meeting.

Genetic engineering of ?-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis, and its use in hybrid breeding

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γ-Aminobutyric acid (GABA) is a non-proteinogenic amino acid with health-promoting functions for human. Tomato (*Solanum lycopersicum* L.) is among the most widely cultivated and consumed vegetables in the world. Although tomato fruits have a relatively high GABA content compared with other crops, the levels must be further increased to effectively confer the health-promoting functions. Glutamate decarboxylase (GAD) is a key enzyme in GABA biosynthesis in tomato; it has a C-terminal autoinhibitory domain that regulates enzymatic function, and deletion of this domain increases GAD activity. The tomato genome has five GAD genes (*SlGAD1-5*), of which two (*SlGAD2* and *SlGAD3*) are expressed during tomato fruit development. To increase GABA content in tomato, we deleted the autoinhibitory domain of SlGAD2 and SlGAD3 using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) 9 technology. Introducing a stop codon immediately before the autoinhibitory domain increased GABA accumulation by 7 to 15 fold while having variable effects on plant and fruit size and yield. In addition, we evaluated the potential of the genome-edited tomato as a breeding material for producing high-GABA hybrid tomatoes. Hybrid lines were produced by crossing the genome-edited tomato with a pure line tomato cultivar, and were evaluated for GABA accumulation and other fruit traits. The hybrid lines showed high GABA accumulation in the fruits, which was sufficiently high for expecting health-promoting functions and had minimal effects on other fruit traits, suggesting that the high GABA is a dominant trait and that the genome-edited tomato would be useful as a parental line of hybrid cultivars. These results indicate that the genome editing technology is useful for the rapid breeding of high-GABA hybrid tomato cultivars.

Genetics of transpiration efficiency in a wheat Nested Association Mapping population

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In Australian wheat growing environments, a lack of water is typically the main cause of yield restriction. Increasing the efficiency with which plants are able to use available water has the potential to sustain or even boost yield in years with sub-optimal rainfall. To study the genetics of transpiration efficiency (biomass production per unit of water transpired) and underlying traits, a nested association mapping (NAM) population has been generated by crossing a reference parent adapted to eastern Australia with 10 donor lines which are adapted for drought-tolerance, with traits including transpiration efficiency. A high-throughput screening method for transpiration efficiency was developed and used to characterize NAM lines in well-watered conditions. The structure of the population allowed lines from both common and diverse backgrounds to be screened in the same population, increasing the statistical power to identify quantitative trait loci (QTL). QTL with significant marker effects for transpiration efficiency were identified in multiple genetic backgrounds and lysimeter experiments, where environmental factors varied between experiments. In addition, lines with superior TE and high yield were identified. Such lines and QTL will now be made available to breeding programs. We believe that combining an evolving NAM population with high-throughput phenotyping for adaptive traits provides a relevant superior framework to assist breeding for better adapted lines.

On the interface between transgenerational abiotic and biotic stress responses in crops

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Plants are under strong selection to respond adaptively to environmental stress, particularly when different stresses occur simultaneously or in rapid succession. Environmental pressure by abiotic and biotic stresses, as well as combinations thereof, influence the response and potentially induce resistance. Most stress responses are often studied in isolation and under controlled growth conditions. This leaves us with an ever-finer picture of single stress responses but little understanding of how additional stressors modify those responses.
Drought stress is used to study abiotic stress, and crown rust infection caused by *Puccinia coronata* is used to study biotic stress response in perennial ryegrass (*Lolium perenne*). The interaction between biotic and abiotic stresses is analysed by combining the two. Populations developed over two generations under stress conditions are used for transgenerational analysis of biotic and abiotic stress responses. Genetic material is isolated and sequenced, and this information is used for epigenetic profiling, transcriptome, and small RNA sequencing. The interaction between stress responses is characterized at the molecular level, focusing on regulatory mechanisms important for both pathways, and providing opportunities for developing broad-spectrum stress-tolerant crop plants. Furthermore, understanding how epigenetic mechanisms regulate biological processes and how they are inherited in plants would allow generation of stress-tolerant plants through techniques for precision epigenome engineering.
Keywords: crown rust, drought stress, transgenerational memory, epigenetic

A new approach to genotyping: Single Primer Enrichment Technology (SPET), an integrated system for both targeted and de novo genotyping

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Genotyping-by-sequencing (GBS) is a robust approach for enabling large scale, whole genome studies of genetic variation, such as genome wide association studies (GWAS).
In this study, two replicates of three *Zea mays* inbred lines (F7, HP301 and MO17) and six F1 crosses (A632 x B73, B73 x B96, B73 x F7, B73 x MO17, W153 x B73, W153 x HP301) were genotyped using the Single Primer Enrichment Technology (SPET).
This technique combines the high-throughput of classic GBS techniques, such as the RAD method, and the targeting of desired loci throughout a scalable probe design.
We designed 71,201 probes based on the Illumina MaizeSNP50 genotyping array, obtained from 529 lines of a MAGIC maize population and their inbred founder lines. Genotypes called by the SNP array were used as a gold standard to measure accuracy of SPET genotyping in 33,380 polymorphic target positions in the inbred lines and in the F1 crosses.
The accuracy of SPET was 95.5% at a coverage of 20x and was higher considering only positions targeted by two probes.
The reproducibility of SPET, obtained comparing the genotype calls for the two replicates, ranged from 98.5 to 99.5 %.
Similarly to accuracy, increasing the minimum required coverage led to an increase of reproducibility, reaching a plateau at about 20x. The accuracy of SPET genotyping was higher in homozygous positions with respect to heterozygous ones. Our results validated a high fraction of SNPs previously found using the SNP array.
SPET enabled the genotyping of 179,829 SNPs in the study subjects (compared to the approximately 50,000 included in the MaizeSNP50) and represented a cost effective alternative to SNP array for densely genotyping large number of samples, circumventing ascertainment bias.
Our experiment showed that SPET is a powerful tool for high-throughput, cost-effective genotyping, allowing both targeted and *de novo* genotyping.

Genetic diversity among IITA maize inbred lines and assessment of genetic gain for pro-vitamin A content using functional markers

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Maize (*Zea mays* L.) is a major staple cereal in Sub-Saharan Africa (SSA) and the preferred crop by peoples with varying socio-economic backgrounds. The enhancement of pro-vitamin A (PVA) content in maize grains through breeding using modern tools seems to be the most sustainable and affordable approach for the reduction of vitamin A deficiency in the SSA. We aimed to investigate the genetic structure and validate the effect of functional markers derived from carotenoid biosynthesis pathway genes (*crtRB1*, *lcyE* and *Zep1*) in maize inbred lines developed at International Institute of Tropical Agriculture (IITA), and finally, to assess the genetic gain for PVA through *marker assisted recurrent selection* (MARS). The material was evaluated in the experimental field of IITA, Ibadan. The individual and total carotenoid contents in maize grains were quantified by HPLC. SSR markers were employed to evaluate the inbred lines in terms of their diversity and genetic structure whereas SNP markers were used to assess the genetic diversity in a maize population (HGA) during MARS. The analysis enabled us to clearly distinguish all the inbred lines within five clusters, with favourable alleles for high PVA distributed across all the inbred clusters. The *crtRB1* derived functional markers exhibited highly significant association with PVA. The number of effective alleles and the observed heterozygosity decreased with selection cycles, but MARS caused desirable changes in the frequency of favourable allele for *crtRB1* resulting in the improvement of individual carotenoid levels up to 40% and PVA by 30% in the HGA population. In conclusion, the present genetically diverse inbred lines with high PVA content can be utilized to maximize PVA concentration in hybrids and cultivars. Furthermore, the genetic gain in PVA through MARS suggested rapid cycling strategies in maize for the development of PVA-biofortified maize.
Key-words: Diversity, genetic gain, maize, PVA and MARS

Unraveling the Cytosine-5 methylation machinery in Salix purpurea

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Salix (willow) L. species are one of the leading biomass crops in the northern hemisphere. Willows are ideal biomass feedstocks for bioenergy and biofuel applications given the ease of vegetative propagation and fast growth in short-rotation coppices.
For willow to attain its full potential as bioenergetic crop, it is crucial to understand the molecular mechanisms underlying wood formation and the modulation of cell wall composition. The study of epigenetic players in woody species is important to understand its phenotypic plasticity to a changing environment. Cytosine-5 methylation (Cy5Met) is a major and dynamic epigenetic DNA modification, encompassing multiple interacting cellular machineries, in complementary processes including de novo and maintenance DNA methylation and DNA demethylation.
Using *Salix purpurea* as plant model for bioenergetics crops and its genome availability, gene families involved in Cy5Met in *S. purpurea* were identified and characterized in terms of phylogenetic relatedness to other plant species. Seven DNA methyltransferases (*SpurMET1a, SpurMET1b, SpurMET1c, SpurCMT2, SpurCMT3, SpurDRM2 and SpurDNMT2*), divided in four clades, and three DNA demethylases (*SpurROS1, SpurDML1 and SpurDML2*), divided in two clades were identified. Furthermore, identified genes were characterized in terms of conserved domain and motif structure and chromosomic location. Finally, to understand the effect of DNA methylation on *S. purpurea* development, expression analysis of the identified genes in different tissues/organs and in artificially hypomethylated and control *in vitro* plantlets was performed. The results obtained in this study will provide a roadmap for future functional studies of Cy5Met gene families in Salicaceae and other woody species.
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Accelerating the domestication of a new oil crop through genomics application

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The importance of replacing fossil oils with a renewable source has been emphasized for many years. For this purpose vegetable oil is central, and meeting the growing demands and coping with the changing climate necessitate the development of more productive and resilient oilseed crops. Field cress *(Lepidium campestre),* a close relative of *Arabidopsis* has been targeted for domestication because it holds high agronomic potential as a biennial/perennial oilseed crop due to its many good characteristics of a high-yielding winter-hardy crop. Unlike other oilseed crops on the market, field cress can be highly productive in the northern parts of temperate regions. The domestication of field cress has progressed rapidly during the last decade and one of the potential end-uses for the oil is as raw material for the production of hydrated vegetable oil (HVO).
Recently, great efforts have been made to speed-up fast-track the domestication process through the use of genomics tools and resources by developing genomics based breeding techniques. A first draft of the whole genome sequence of field cress has been generated and was used as a reference genome for GBS analysis. The genome size of field cress was determined to be about 533 Mbp, and of this 219 Mbp was assembled. A genetic linkage map consisting of eight linkage groups containing 2331 SNP markers derived from 1044 contigs, and spanning 881 cM, was constructed based on the GBS data. In addition, 24 homologues of *Arabidopsis* genes regulating various desirable traits have been mapped. The effect of variation within these genes on the traits they regulate is being studied to develop genetic molecular markers for their use in marker assisted selection. These studies have shown a high level of collinearity between field cress linkage groups and *Arabidopsis* chromosomes providing an additional interesting insight into the evolution of *Brassicaceae* genomes.

A natural frameshift mutation in Campanula EIL2 correlates with ethylene insensitivity in flowers

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Ethylene plays a central role in development and senescence of climacteric flowers. In ornamental plant production, ethylene sensitive plants can be protected against negative effects of ethylene by application of chemical inhibitors. There is a crucial need for environmental safe alternatives. Ethylene signal transduction occurs via a characterized pathway by which Ethylene INsensitive 3/Ethylene Insensitive 3-Like (EIN3/EIL) transcription factors transmit the physiological response. In *Campanula,* we have identified a natural mutation in *Ethylene Insensitive Like 2* (*EIL2*). In *C. medium*, the *eil2* in both wild and domesticated species contains a frameshift mutation of 7 nucleotides which disrupts the putative DNA binding domain of the transcription factor. Flowers of *C. medium* show an increased ethylene insensitivity. EIL2 is a homolog to the *Arabidopsis thaliana* transcription factor EIN3, and our results support a pivotal role of *Campanula* EIL2 in the ethylene signal transduction pathway. Currently, we characterize ethylene signaling elements down-stream of EIL2 using transcriptome analyses by RNA sequencing. To obtain the full breeding potential of this mutation we will induce similar mutations using CRISPR-CAS9 in economically important *Campanula* species as well as in other plants. This may provide a useful tool to engineer ethylene insensitive flowers.

Tomato Bioresources in Japan based on cv. 'Micro-Tom' as model tomato cultivar

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Tomato (Solanum lycopersicum L.) is one of the most important vegetables in terms of production and economic value. And tomato is useful for studies of fleshy fruit biology and experimental genomic studies of other Solanaceae family including potato, eggplant, pepper, petunia and tobacco. Tomato fruits contain many functional metabolites including carotenoids, vitamin A, vitamin C, and GABA. In the future, bioresource collection and preservation will also become increasingly important from the viewpoint of Nagoya protocol and CBD. To accelerate breeding and functional genomics research of tomato, we have launched the tomato bioresource program since 2007 within the framework of the National BioResource Project (NBRP) in Japan (http://tomato.nbrp.jp/). The major purpose of the NBRP-tomato is to collect, preserve and provide tomato bioresources including major experimental lines, mutant lines, transgenic lines and cDNA collections in the genetic background of 'Micro-Tom-Japan' (TOMJPF00001). More than 18,000 mutant lines have been produced by EMS treatment and gamma-ray irradiation, and mutants with visible phenotypes have been isolated. All of the visible phenotyping data and other associated data in individual mutants were registered in the database 'TOMATOMA' (http://tomatoma.nbrp.jp/). We have measured metabolic components including amino acid compositions, carotenoid contents and Brix values in mutant fruits, and also these data have opened through TOMATOMA. These mutants with phenotypic and the metabolite information will help accelerate tomato fruit researches. As DNA resources, Micro-Tom full-length cDNA sequence and EST are available from database 'KaFTom (http://www.pgb.kazusa.or.jp/kaftom/) and EST database 'MiBASE' (http://www.pgb.kazusa.or.jp/mibase/), respectively. Information on genome structural annotations between Micro-Tom and Heinz 1706 is accessible through the genome browser in 'TOMATOMICS' (http://bioinf.mind.meiji.ac.jp/tomatomics/). Our comprehensive tomato resources will help to facilitate breeding and functional genomics research of tomato.

Overexpression of OsTF1L, a rice HD-Zip transcription factor, promotes lignin biosynthesis and stomatal closure that improves drought tolerance

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Drought stress seriously impacts on plant development and productivity. Improvement of drought tolerance without yield penalty is a great challenge in crop biotechnology. Here, we report that the rice (*Oryza sativa*) homeodomain-leucine zipper transcription factor gene, *OsTF1L* (*Oryza sativa transcription factor 1-like*), is a key regulator of drought tolerance mechanisms. Overexpression of the *OsTF1L* in rice significantly increased drought tolerance at the vegetative stages of growth and promoted both effective photosynthesis and a reduction in the water loss rate under drought conditions. Importantly, the *OsTF1L* overexpressing plants showed a higher drought tolerance at the reproductive stage of growth with a higher grain yield than non-transgenic controls under field-drought conditions. Genome-wide analysis of *OsTF1L* overexpression plants revealed up-regulation of drought-inducible, stomatal movement and lignin biosynthetic genes. Overexpression of *OsTF1L* promoted accumulation of lignin in shoots, whereas the RNAi lines showed opposite patterns of lignin accumulation. In addition, *OsTF1L* overexpression enhances stomatal closure under drought conditions resulted in drought tolerance. More importantly, OsTF1L directly bound to the promoters of lignin biosynthesis and drought-related genes involving *poxN*/*PRX38*, *Nodulin protein*, *DHHC4*, *CASPL5B1* and *AAA-type ATPase.* Collectively, our results provide a new insight into the role of *OsTF1L* in enhancing drought tolerance through lignin biosynthesis and stomatal closure in rice.

Development and management of tartary buckwheat genetic resources - an in silico approach

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Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) is an important crop not only for food value but also for medicinal value due to its high rutin and polyphenol compounds. In this study, we used high throughput next-generation sequencing (NGS) data in order to unravel the genetic resources for developing buckwheat as a highly functional food crop. Overall, 26 core resources of tartary buckwheat has been collected from six countries including China, India, and Nepal. A comparative genomic study has explored a large number of InDel (insertion/deletion) markers required for developing common platform. Bioinformatic analysis revealed 171,926 and 53,755 homo- and hetero-InDels, respectively. Among them, 50 *in silico* polymorphic InDels from 26 accessions were selected by gel electrophoresis, which were converted as barcode types by comparing amplicon polymorphisms with the reference sequence. In order to make user-friendly common platform for genotype, phenotype and chemotype resources, we incorporated genotypic data with that phenotype and chemotype (rutin content) data of 26 buckwheat accessions. As a user friendly system, the homology between the accessions can be visualized in both one (1D) and two dimension (2D) as blocks. Our platform could be not only used in genetic research and breeding programs but also used for efficient resource management system in buckwheat.

PRODUCTION OF WHEAT BARLEY TRANSLOCATION LINES USING THE 2C GAMETOCID SYSTEM AND PAIRING HOMOEOLOGOUS SYSTEM

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The intergeneric hybridization of common wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) makes it possible to transfer agronomically useful genes (favourable chemical composition, high content of dietary fiber/β-glucan, salt tolerance, etc.) from barley into the wheat genome. Intergeneric rearrangements can be detected using genomic *in situ* hybridization (GISH) molecular markers. Our main goal was to produce genetically stable translocations, which only carry DNA segments responsible for a useful agronomical trait.
Two different genetic systems were used: pairing homoeologous (*Ph*) system and the gametocidal system. The *Ph1* gene located on the long arm of chromosome 5B. *Ph1* prevents recombination between homoeologous chromosomes, when the *Ph1* gene absent from the wheat genome, homoeologous chromosomes are also able to recombine. The 'Asakaze'/'Manas' 7H disomic addition line was crossed with the CS *ph* mutant line in order to induce chromosome breakage and rearrangements. Out of 60 plants analysed, 9 carried wheat/barley monosomic centric fusions. In the F_3 generation, out of 120 analysed plants, 21 plants carried the centric fusion in stable, disomic form.
In nature, there are certain species carrying gametocidal (Gc) chromosomes, which after being incorporated into another genome will cause genetic rearrangements, such as translocations. Wheat/barley translocations were induced with the gametocidal system using the 2C gametocidal chromosome originated from *Aegilops cylindrica* L. (2n = 4x = 28; CCDD). The Asakaze/Manas 7H addition × *Ae. cylindrica* 2C line was backcrossed with the 7H wheat/barley addition line. Out of 176 BC_1F_2 seeds, 6 wheat-barley (7H) translocations were detected and translocations were transmitted to the next generation. In the F_3 generation, 3 plants carried the centric fusion in disomic form. We multiply these lines in order to analyse qualitative parameters and to determine the phenotypic description of the plants.
This research was supported by the WHEALBI EU FP7 (613556) project.

Dwarfism association mapping in F2 triticale population derived from [(Jana x Tempo) x Jana] x Aegilops juvenalis cross combination

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Lodging is one of the most serious problems affecting the cultivation of triticale. Most effective ways to limit this phenomenon involve the use of retardants or the introduction of genes reducing the plants height. The identification of new sources of resistance as well as development of molecular markers linked to these genes is therefore essential for triticale breeding programs.
The aim of the research was identification of DArTseq markers linked to new dwarfing gene found in triticale plants obtained from wide crosses and association mapping of this gene.
Research material consisted of F_2 plants obtained from crossing between dwarf and high triticale plants derived from [(Jana × Tempo) × Jana] × *Aegilops juvenalis* cross combination. The analysis was conducted on 186 hybrids plants and two parental forms. DNA was extracted from young leaves with the use of Qiagen DNeasy® Plant Mini kit, checked spectrophotometrically and electrophoretically and send to Diversity Array Technology, Canberra for genotyping. 10489 silicoDArT markers were used for the association mapping. The mapping was carried out using the TASSEL software. An attempt of determining their chromosomal location has been undertaken.
Acknowledgement
The results of the study were obtained within the framework of the project funded by Polish Ministry of Agriculture and Rural Development entitled ‘Development of new triticale genetic sources by wide crosses’.

Barley breeding: taking epigenetic processes into account.

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Epigenetic regulations are of paramount importance for almost every aspect of plant life including cell regulation, differentiation, and transposable elements control. By enabling the modification of traits without changing DNA sequence, the epigenome forms a vast source of phenotypic plasticity even if a minimal genetic variation is observed.
A total of 75 spring barley lines, grown in RadiMax facility designed to identify varieties with improved resource utilisation from deep soil layers, were exposed to a water-deficiency stress gradient. The population consisted of both breeding material and commercial varieties. Genome-wide DNA methylation was compared across lines and treatments. Bisulphite sequencing was followed by mapping, variant calling, single-nucleotide polymorphism and differential methylation analysis. The analysis allowed detection of context-specific DNA methylation at the single-base resolution, as well as analysis of the extent of methylation at each site.
Genome-wide methylation patterns and differentially methylated positions were identified. CG methylation accounted for a significant amount of methylation sites in gene bodies outnumbering CHG and CHH. Overall, methylation levels were significantly affected by the treatment applied, and numerous differentially methylated regions were identified, many of which were associated with differential expression of genes essential for abiotic stress response. Genome reconstruction allowed for the distinction of single-nucleotide polymorphisms with 7014 SNPs identified.
The results provide insights into the epigenetic status of sites in the barley genome and suggest its role in abiotic stress adaptation in the agriculturally important cereal. Understanding the nature of epigenome as a potent source of diversity for agronomical traits may support further strategies to incorporate epigenetics in crop breeding programs. The epigenetic status of sites in the genome can be considered as a phenotype, depending on DNA sequence, environmental effects, possible events in early development and inherited epimutations, and therefore could be treated as a selection criterion.

Improving anthocyanin content in carrot taproots

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Improving anthocyanin content in carrot taproots
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Anthocyanin, an important plant pigment, is responsible for most of the colors in plants. Anthocyanins are synthesized via the flavonoid pathway which also leads to the products including proanthocynidins and flavonols. The anthocyanin biosynthesis is regulated at the transcriptional level by a MYB-bHLH-WD40 (MBW) transcription factor (TF) complex, including the R2R3-MYB, basic Helix-Loop-Helix (bHLH) and WD40 classes. Carrot (*Daucus carota* L.) is one of the plant species that accumulates largest amounts of anthocyanin in the storage root where black or purple carrots are of particular interest because of their high anthocyanin content and extraordinary quality parameters as food ingredient. This study focus on investigating the mechanism of anthocyanin biosynthesis in carrot and screening the key MYB TFs involved in anthocyanin biosynthesis which will be used for the breeding of high anthocyanin content carrot cultivars by both traditional breeding and genetic engineering. Three carrot genetic stocks that differ in storage root color were compared by analysis of flavonoid biosynthesis pattern. The flavonoid biosynthesis pattern will be investigated in three aspects: metabolism (flavonoid compounds), flavonoid biosynthesis pattern (genes and enzymes), regulatory network in transcriptional level (MYB TFs). The candidate MYB transcription factors and biosynthesis genes were chosen from the analysis of the RNA-seq data and qPCR results, MYB-overexpression or MYB-RNAi transient carrot will be conducted to test whether these MYB candidates are related to anthocyanin biosynthesis. The transgenic plants should have high concentration of anthocyanin, thus confirming the function of the gene. The new plant breeding technology (CRISPR-Cas9) will be used to knock down the MYB repressors in order to increase the content of anthocyanin in carrot.

Targeting freezing tolerance in perennial ryegrass

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While enhanced freezing tolerance (FT) is aim of breeding programs at northern latitudes, perennial ryegrass (*Lolium perenne* L.) cultivars have moderate tolerance to freezing temperatures. This limits their persistence in northern regions due to poor overwintering. Genetic resources readily available from gene banks have vast collections of perennial ryegrass accessions, and most likely include accessions of enhanced freezing tolerance and better winter hardiness. A total of 150 gene bank accessions of perennial ryegrass were screened for freezing tolerance under artificial freezing conditions within the ongoing Private-Public Partnership on Pre-breeding in Perennial Ryegrass project. Our results demonstrate that the ploidy level of perennial ryegrass determines the tolerance to freezing temperatures, with diploid accessions being superior in FT to the tetraploid genotypes. The study also revealed that natural genetic diversity for FT is already well exploited in some of the existing cultivars, suggesting that novel combinations of FT alleles are needed for further improvement of this trait. A subset of 122 diploid accessions were genotyped by sequencing, resulting in 1.2 M Genome Wide Allele Frequency Fingerprints. Genome-wide association analysis highlighted eight significant markers with a bias towards genic/expressed genomic regions. A number of candidate genes previously associated with abiotic stress response in plants were located on marker-tagged genomic scaffolds, while some of the tagged yet uncharacterized genes once validated might clarify the complex genetic mechanisms underlying FT in perennial ryegrass.

Increasing secretory capacity and protein yield in barley grains

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At present, the production of recombinant proteins in cereal grains suffer from a low yield due to overloading of the secretory system. This results in accumulation of unfolded and misfolded proteins, inducing an ER-stress response and potentially stress-induced apoptosis. To alleviate the ER-stress, the unfolded protein response (UPR) is initiated to halt protein synthesis, degrade misfolded protein and increase production of chaperones involved in protein folding.
The purpose of our study is to increase the recombinant protein production in cereals, specifically in the grain, making it a more viable way of producing recombinant proteins on an industrial adequate scale. The first approach is modulation by overexpression or CRISPR/Cas9 based k/o of UPR genes and genes found to be highly expressed during induced ER-stress [1]. By measuring changed responses in ER-stress induced assays as well as measuring selected UPR genes expression during grain filling in barley, selected mutant lines is crossed with barley lines producing recombinant proteins in the grain. A second approach is using a storage protein deficient mutant with potentially more capacity for producing a recombinant protein during grain filling and cross this with the recombinant protein lines.
Eight genes have been stably knocked out with a high frequency of regenerated mutant plants showing CRISPR/Cas9-mediated indels, with +/- 1 bp being predominant. five of these eitght genes have been stably transformed and overexpressed in barley. At present, two k/o lines, one overexpressed line and the storage protein deficient line have been crossed with our recombinant protein lines.
[1] Barba-Espín, G., et al., *Gibberellic Acid-Induced Aleurone Layers Responding to Heat Shock or Tunicamycin Provide Insight into the N-Glycoproteome, Protein Secretion, and Endoplasmic Reticulum Stress.* Plant Physiology, 2014. **164**(2): p. 951-965.

Optimized CRISPR/Cas9 for Engineering Starch Quality in Potato Tubers

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Potato is the 4'th largest crop worldwide and Denmark has a long-standing track record as world leader in potato breeding and production. However, the moderate to low process-stability of current potato starches limits their functional potential. Furthermore, potato starch confers high glycaemia and excess consumption may cause obesity and diabetes.
In the present study we have established the CRISPR/Cas technique with the aim of improving process stability in potato varieties of industrial relevance. We targeted the amylose-synthesizing enzyme Granule Bound Starch Synthase (GBSS) and isolated novel endogenous potato U6 promotors for driving the expression of the CRISPR component in transformed potato protoplasts. Replacement to the commonly used Arabidopsis *At*U6 promotor with the endogenous U6 promoters resulted in more than 4-fold increase in mutation frequency. Regenerated plants were isolated and shown to have full allelic knock out of the GBSS gene.
The amylopectin synthesizing Starch Branching Enzyme (SBE) and the starch phosphorylator Glucan Water Dikinase (GWD) are additional key enzymes in starch biosynthesis which offer potential for engineering health-promoting resistant starch and tubers with low sugar content. Challenges and strategies for targeting these genes will be presented.

Increasing barley's arsenal against viruses

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Virus diseases are causing important yield losses in crops worldwide. In Europe, *Barley yellow dwarf virus* (BYDV), transmitted by aphids, and soil-borne *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV), transmitted by *Polymyxa graminis,* are of prime importance in this respect in barley. Chemical control of viral diseases only relies on the control of vectors, which is not desirable from an environmental point of view and with respect to soil-borne vectors not even possible. Thus, virus resistant cultivars are needed. We are investigating two resistance genes: *rym7,* a partial resistance locus to BaMMV, and *Ryd4Hb*, a dominant resistance gene against BYDV. In previous studies, they were allocated to chromosomes 1H and 3H, respectively (Yang et al., 2013; Scholz *et al.*, 2009). We are aiming at identifying these genes by forward genetics. After screening of 6000 F2 plants for recombination in the *rym7* interval, 232 segmental recombinant inbred lines (RILs) were selected, phenotyped, and genotyped by GBS. Linkage analysis revealed a 2 Mbp *rym*7 interval in which seven high confidence genes are annotated in the barley reference sequence. Ongoing work includes the exploitation of exome capture sequence data of both parents for an even finer mapping of *rym7* and sequencing of the candidate genes in the RILs. *Ryd4Hb* is a resistance gene derived from *Hordeum bulbosum*, a wild species representing the secondary gene pool of barley. Recombination in crosses between *H. vulgare* and *H. bulbosum* are scarce: after screening around 16000 F2 plants, we were able to identify less than 120 recombinant plants in a 13.3 Mbp interval. Recombinants offspring are currently undergoing phenotyping and finer genotyping, with the aim to identify candidate genes.

SNP identification and differential expression of drought stress related genes by RNAseq analysis of Danish spring barley

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In the future, crop plants will face more severe drought periods during the growing season. It is therefore necessary to identify plants that are resilient to drought stress, with high nutrient use efficiency. Roots play a key role in sustainable intensification of agriculture. The RadiMax facility at Copenhagen University (KU) provides a state of the art phenotyping platform for roots and nutrient uptake.
Leaf samples were collected from control and drought stressed plants grown in the RadiMax facility in 2017, and used to study the transcriptomic response to drought stress using RNAseq. The results from the RNAseq analysis of the barley pilot experiment shows that more than 800 genes were differentially expressed (DE) between drought and control treatments. We have furthermore identified single nucleotide polymorphisms (SNPs) in the DE genes, and investigate the effects of the haplotypes on expression levels and performance under drought stress. RNAseq results and the SNP markers will be used to develop prediction models for drought tolerance and nitrogen use efficiency. The project will provide new knowledge about the potential for drought adaptation in Danish breeding material and provide genetic markers and breeding values for the development of crops with improved drought tolerance.

Characterization of foliar diseases infecting quinoa (*Chenopodium quinoa*)

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Abstract

Quinoa (*Chenopodium quinoa*) is an ancient crop from the Andes of Bolivia, Peru and Ecuador and is increasingly cultivated outside its native range on all continents. Cultivation in new environments may lead to incidence of diseases previously not described for quinoa that may interfere with a commercially successful production of this crop. To test this hypothesis, we identified foliar pathogens at different stages of quinoa development to understand their epidemiology, host interactions and sources of resistance. We grew three cultivars (Vikinga, Titicaca and Puno) in an experimental plot in Tåstrup, Eastern Denmark. A first set of symptomatic leaves was collected from cv. Puno at the end of the growing season in late September 2017 to identify pathogens. A diversity of symptoms were observed that included small yellowish anamorph blots on the upper leaf surface; a pale chlorotic halo surrounded the yellow lesion which occasionally had slight pink colouring in the centre; and light brown spots, concentric rings and apical necrosis on the lower leaf surface. The symptomatic sections of leaf tissue were surface sterilized and plated onto potato dextrose. After 10 days, three main fungal groups could be differentiated by their morphological characteristics such us conidia shape, colour and number/occurrence of septae, colony diameter, reverse colour and formation of rings. Monoconidial isolations allowed to establish pure cultures of each fungus. For molecular identification we PCR amplified the intergenic transcribed spacer (ITS) and conducted BLAST searches for identification. Using this protocol we identified the following species: *Didymella chenopodii*, *Alternaria infectoria*/*methacromatica*, *Alternaria tenuissima* and *Epicocum nigrum.* To validate their identity, Koch postulates and pathogenicity tests are in progress. To our knowledge this is the first demonstration of *Alternaria infectoria, Alternaria tenuissima, Didymella chenopodii* and *Epicocum nigrum* causing lesions in quinoa foliage in Europe. Among the known fungal pathogens, downy mildew (*Peronospora variabilis*) is a serious constraint to quinoa production worldwide. The pathogen is seed transmitted, which is one of the reasons for its increasing prevalence. If the infection occurs during initial growth stages, susceptible crops could fail completely. Even in resistant cultivars, the loss may be 20-40%. Our results indicated the development of quinoa varieties for cultivation outside its native range need to include resistance breeding. We therefore are currently conducting a genome-wide association study of downy mildew-quinoa interactions with a large sample of Bolivian landraces with different levels of resistance against downy mildew.

Keywords: Quinoa, fungal plant pathogens, Bolivian landraces, biotrophic pathogen

Identification of foliar diseases infecting quinoa (Chenopodium quinoa), searching for resistance to downy mildew (Peronospora variabilis)

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Downy mildew (*Peronospora variabilis*) is a serious constraint to quinoa production worldwide. The pathogen is seed transmitted, which is among the reasons for its increased spread in quinoa cropping areas. If the infection occurs during the initial growth stages, susceptible cultivars could fail completely. In resistant cultivars, the loss may be 20-40%. If the disease attacks the panicle, the grain formation may be reduced. Despite the importance and wide dissemination of *P. variabilis*, knowledge about host specialization and sources of resistance in the host is missing.
Little is also known about other foliar pathogens infecting quinoa. Most symptoms are assumed to be caused by downy mildew. Careful inspections in the field have highlighted the urgent need of accurate diagnose, in order to make proper assessments of disease incidence and severity. Fungal pathogens belong to various genera such us: Ascohyta, Phoma, Cercospora, Clamidiosphora and Phytium have been reported sporadically. However, the gap between sporadic and consistent methodological identification needs to be closed.
The Plurinational State of Bolivia has funded '100 Scholarships for Scientific Sovereignty and Knowledge', among which is the current PhD project. The project aims to help improve our understanding of the host-pathogen interactions to contribute to find sustainable disease control options. It will include commercial quinoa cultivar characterisation for resistance to ***Peronospora variabilis***. The research also aims to identify other quinoa foliar pathogens at different development stages, and to follow their presence in the seeds. The information is being obtained from Danish quinoa cultivars but the diseases occur wherever quinoa grows, opening a research avenue of great potential around the world.

DILS MODEL - CROP SYSTEM FOR STUDYING SENESENCE & CELL DEATH RELATED MECHANISMS

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Our crop model to unravel early and late events of leaf senescence and deciphering time limit for dark to light transition for reversal of the senescence process is dark-induced leaf senescence (DILS) of barley. Non-invasive methods for quantifying photosynthetic efficiency and barley leaf nitrogen status established the time frame when DILS enters the irreversible phase. Chlorophyll fluorescence vitality index Rfd was determined as the earliest parameter that correlated well with the cessation of photosynthesis prior to the appearance of micro-autophagy symptoms, chromatin condensation, initiation of DNA degradation and several-fold increase in the endonuclease *BNUC1.* DILS was found characterized by the upregulation of processes that enable recycling of degraded metabolites, including increased NH_4^+ remobilization, gluconeogenesis, glycolysis, and partial upregulation of glyoxylate and tricarboxylate acid cycles. The most evident differences in gene medleys between DILS and developmental senescence were found to be hormone-activated signaling pathways, lipid and glutamine catabolic processes, RNA methylation and carbohydrate metabolic processes. Interestingly, the mega-autophagy symptoms were found to appear much later when disruption of organelles - nucleus and mitochondria - became evident. Further, during the latter stage PCD processes, namely, shrinking of the protoplast, tonoplast interruption and vacuole breakdown, chromatin condensation, DNA fragmentation and disintegration of the cell membrane were found to become prominent. Interestingly, reversal of DILS involved regaining photosynthesis, increased chlorophyll content, and reversal of Rfd, and took place irrespective of the activation of macro-autophagy-related (ATGs) genes. Delineated DILS system may contribute well to developing new strategies for genome- and phenotyping-based crop breeding.

Variation block-based genetic analysis of flowering time genes in soybean

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Flowering time is strongly influenced by climate and day-length adaptation in soybean [*Glycine max* (L.) Merrill]. In this study, we investigated soybean flowering time trait using genome-wide association study under highly three agroecological conditions. In total, 7,087 variation blocks (VB) were mined by analyzing whole genome sequencing data of 96 soybean genotypes. Phenotypic associations with flowering time and seed yield were calculated in the panel over the year 2016-2017. We identified 290 VB's associating with the onset of flowering among all soybean cultivars. Three clusters were inferred by STRUCTURE analysis, which is in good agreement with a neighbor-joining tree. In addition, soybean orthologs for a number of candidate genes for adaptation were detected, including soybean maturity locus *E1*. Further, backcross recombinant inbred lines (BC_2F_3, 'Hwangkeum' X 'Daepoong') exhibited significant variations in their onset of flowering, with a range of 18-20 days due to difference in *E1* locus. Hence, VB analysis of candidate regions suggested that selection of genes involved not only in flowering time but also in other trait may have high impact on diverse soybean cultivars. Furthermore, our study provides a valuable framework to improve the genetic resources of crop plants under changing environments.

Engineering GSTs at different scales: abiotic stress tolerance in transplastomic and transgenic tobacco for environmental resilience

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Tobacco (*Nicotiana tabacum* L.) represents a major economically important industrial crop cultivated in Greece and worldwide. This non-food crop is cultivated in a wide range of semi- to infertile, inclined land with partial or no irrigation in Greece, where other crops would not be productive. Plant glutathione S-transferases (GSTs) have been shown to modulate redox homeostasis by alterations in GSH content and redox state thus, conferring tolerance to a wide range of abiotic stresses including herbicide. To investigate the morphophysiological and molecular mechanisms and the role of GSTs in tobacco stress detoxification and other functional roles necessary for plant growth and development under abiotic and oxidative stresses, we used two different expression systems: i) overexpressing a *PvGST* gene from *Phaseolus vulgaris* and ii) engineering a *ZmGST* gene, directly into the chloroplasts. The T1 transplastomic tobacco lines were tested under *in vitro* drought (0, 100 and 200 mM mannitol) and salinity (0, 150 and 300 mM NaCl) and under *in vivo* herbicide stress (Diquat and Dimethenamid), whereas the transgenic tobacco lines were tested under combinations of control and drought conditions in ambient (23^{o} C) and high (38^{o} C) temperature. The effect of these stresses on the relative chlorophyll content and the maximum quantum efficiency of Photosystem II (*Fv*/*F*m) were assessed throughout the experiments and the morphological parameters were measured at harvest. Leaf samples for transcriptomics and metabolomics analyses were excised at 0, 3, 6, 12, 24, 48 and 72 hours and at harvest point and were analysed. The results presented herein would help to elucidate the role of GSTs in plant stress tolerance and the underlying mechanisms of the two genetic engineering approaches, towards environmental resilience of cultivated crops.

Molecular dissection of late flowering under a photoperiod-insensitive genetic background in soybean

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Reduced or lack of sensitivity to long daylengths is a key character for soybean, a short-day crop, to adapt to higher latitudinal environments. However, the photoperiod-insensitivity often results in a reduction of duration of vegetative growth and final yield. To overcome this limitation, we developed a photoperiod insensitive line (RIL16) with delayed flowering habit from the recombinant inbred population derived from a cross between a photoperiod-insensitive cultivar AGS292 and a late-flowering Thai cultivar K3. Expression analyses under SD and LD conditions revealed that the expression levels of *FLOWERING LOCUS T* (*FT*) orthologues, *FT2a* and *FT5a*, were lowered in RIL16 relative to AGS292, although the expression of *E1*, a soybean-specific suppressor for *FT*s, was inhibited in both conditions. A soybean orthologue of *TARGET OF EAT1* (*TOE1*), another suppressor of *FT*, showed an upregulated expression in RIL16, which appeared to reflect a lower expression of miR172a. Our data suggest that the delayed flowering of RIL16 most likely is controlled by genes involved in an age-dependent pathway in flowering. The QTL analysis based on 1,125 SNPs obtained from Restriction Site Associated DNA Sequencing revealed two major QTLs for flowering dates in Chromosome 16 and two minor QTLs in Chromosome 4, all of which accounted for 55% and 48% of the whole variations observed in natural daylength and artificially-induced long daylength conditions, respectively. The intervals of the major QTLs harbored *FT2a* and *FT5a*, respectively, on basis of annotated genes in the Williams 82 reference genome. Sequencing analysis further revealed a nonsynonymous mutation in *FT2a* and a SNP in the 3′ UTR region of *FT5a*. A further study may elucidate a detailed mechanism underlying the QTL for late flowering.

SeqSNP - Flexible and cost-effective targeted GBS for genomic selection and other high-throughput applications in plant and livestock breeding

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MAS (marker assisted selection) has contributed to genetic gains that are linked to simple traits and environmental adaptations for both plants and livestock. MAS uses markers in LD (linkage disequilibrium), that have associated phenotypic traits, with a relatively small number of SNP markers. Complex traits, however, often require larger numbers of associated markers to estimate breeding values. Genomic selection (GS) is a method that uses high densities of markers (>3K unique data points per sample), evenly distributed throughout a genome, to increase genotypic resolution and phenotypic association of complex traits, thus improving prediction accuracy for training populations to be used for commercial breeding programs.
For GS analysis to be of value, quick turnaround times that return data of high accuracy are essential Increasing allele number and concomitant increases in population sizes in the application of GS strains time, throughput and analysis resources. To remove these hurdles, LGC have developed SeqSNP, which tackles all of these problems with a complete sample to data service. Here we describe the utilisation of automation and Nugen's Allegro technology to genotype 5000 allele targets per sample in multiplex, with the ability to flexibly select markers for each project. We show a typical output using 96 tomato plants in a segregating population screened with 4744 alleles across the whole genome. We directly compare a 297 marker subset that was genotyped using KASP chemistry, and show a high concordance of genotyping calls between the two technologies and the influence of DNA concentration on the quality of output data obtained.

Uncovering the complex genetic base of leaf and stripe rust resistance in the Brazilian cultivar Toropi

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Leaf and stripe rust are important diseases of wheat world-wide and deployment of cultivars with genetic resistance is an effective and environmental friendly control method. The effective leaf and stripe rust resistance presented by the Brazilian cultivar 'Toropi', released in 1965, is an excellent choice for genetic resistance study, having previously unidentified sources of leaf and stripe rust adult plant resistance (APR). Resistance was investigated in a double-haploid population derived from 'Toropi_6.4' and the susceptible cv. 'Thatcher' (171 lines), which was phenotyped in Canada (2010 to 2017), in New Zealand (2011 and 2012) and in Kenya (2012), with a total of nine trials for leaf rust in four locations, and nine trials for stripe rust in five locations. The population was genotyping with the 90K iSelect array, 170 SSR and 120 KASP markers and provided a genetic map of 3302.2 cM. Significant quantitative trait loci (QTL) were identified in multiple environments on chromosomes 1B (LOD 4.47 to 18.61) and 2B (LOD 2.86 to 20.28) for leaf and stripe rust; 3B (LOD 2.86 to 20.28), 5A (LOD 3.06 to 17.26) and 5D (LOD 2.54 to 4.40) for leaf rust; and 5A (LOD 4.69 to 46.9) for stripe rust. All the resistance loci were contributed by the resistant parent Toropi_6.4. The durable resistance of Toropi apparently rests upon a combination of several genes. To our knowledge resistance for leaf and stripe rust has not been reported on chromosome 5A, potentially contributing novel resistance sources for leaf and stripe rust.

Theme 4: Seeds for the future

Conservation and use of the staple crops of sub-Saharan Africa

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The Genetic Resources Centre(GRC) of the International Institute of Tropical Africa (IITA), a CGIAR centre, has collections of many important staple crops for sub-Saharan Africa (SSA). These include cowpea, maize, soybean, cassava, yam and banana/plantian. Conservation of those crops typically propagated by seed is carried out through storage in cold and dry conditions. For the clonal crops(cassava, yam , banana/plantain) conservation at IITA is in the field, in vitro and most recently in a cryobank. Recent developments in the conservation of these crops will be described. Important elements include conservation, characterisation, indexing and cleaning, data management, quality assurance, safety duplication and distribution. the genebak operates withi the multilateral system and in particular Article 15 of the International Treaty of Plant Genetic Resources for Food and Agriculture(ITPGRFA). Aspects of the challenges associated with movement of germplasm under the ITPGRFA and Convention of Biological Diversity(CBD) will be discussed. Increasingly molecular tools are being applied for genebank management (eg identifying duplicates, trueness to type) and for enhancing use (eg links to pre breeding and breeding) and progress in this regard at IITA will be outlined. At the same time other methods to enance germpalsm use in breeding programmes are being developed eg trait based subsets.

Genetics and duplicate holdings of Brassica oleracea

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Around the world, there are more than 1500 genebanks storing plant genetic resources to be use in breeding and research. Each genebank tends to operate on its own and extensive duplication is a challenge. In Europe, a process of coordination has started, aiming at sharing responsibility and maintaining only unique accessions while allowing access to all according to the international treaty for plant genetic resources. Identifying duplicate holdings based on accession names and other passport data has been one approach, but same names do not always mean the same genetic material. In the past, and especially in vegetables, different selections or strains within same varieties were common and the naming practices of varieties was flexible. Here, we examined ten accession pairs/groups of cabbage (*Brassica oleracea* var. *capitata*) maintained in the Russian and Nordic genebanks. Both morphological characterization and SNP markers were included. Within five out of ten pair/groups, we detected clear genetic differences among the accessions and three of these were confirmed by significant differences in one or several important morphological traits. In one case, a white cabbage and a red cabbage had the same accession name. The study highlights the usefulness of morphological and/or genetic characterization in the duplication assessment process and subset of useful markers was suggested.

Low doses of ionising radiation and the modulation of light quality improve nutritional traits in soybean seedlings.

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Long-duration manned Space missions require the realization of artificial environments, similar to the ecosystems on Earth, called Bioregenerative Life Support Systems (BLSSs). In these self-sufficient closed habitats, higher plants would have a fundamental role in air regeneration and food production, reducing the demand for resource supply from Earth.
In this study, we exposed germinated seeds of soybean to different doses of X-rays (0.3, 10 and 20 Gy) and light quality regimes (W-White, R-Red and RB-Red-Blue light), to assess possible radiation-induced stimulation in the production of useful nutritional compounds in seedlings. Irradiated and control seedlings, grown under different light-quality treatments were analyzed in terms of growth (measuring stem, root elongation, fresh and dry weight), photosynthetic pigment production (chlorophylls, carotenoids and anthocyanins), antioxidants (total polyphenols), sugar and protein content, localization of phenolic compounds in the various organs. Our results indicated the occurrence of a significant interaction between light quality and ionizing radiation; in particular RB-light induced in irradiated seedlings a more compact ($P<0.05$) habit. The growth under R light determined an increment in stem elongation. The RB light treatment promoted in seedlings the highest photosynthetic pigment and total protein content, as well as the high expression of PSII antenna complex protein, particularly at doses of 0.3 and 10 Gy of X-rays. These specific doses, also enhanced ($P<0.05$) the total carbohydrates and polyphenols concentrations in seedlings compared to W and R light treatments and 20 Gy of X rays. The overall study suggests that it is possible to induce specific functional and nutritional traits in irradiated plants by manipulating light quality during plant growth. This result is particularly interesting as many functional compounds, assumed through the diet, could strengthen the astronaut's defense against the oxidative stress induced by the exposure for a long period to cosmic radiation.

Towards the selection of a safe feed from Vicia sativa seeds

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Plants in general, edible plants and legumes as common vetch (*Vicia sativa* L.) in particular synthesized a range of secondary metabolites as part of their protection against attack by herbivores, insects and pathogens. *Vicia sativa* seeds could be used as a valuable feed in animal diets as a source of highly digestible protein and minerals however it contain cyanogenic antinutritional factors (ANF's) identified as non proteinogenic amino acids and cyanogenic glucosides that reduce their palatability and it utilization as a feed stuff for monogastric animals. The main challenges nowadays in feed stuff production are to reduce feeding cost, improve products quality and diminish the impact of production on environment A number of methods have been tried to overcome the deleterious effect of such anti-nutritional factors is came at the head. The knowledge regards the challenge in feed stuff production and various ANF's present in *Vicia sativa* seeds represent the corner stone to control, minimize and reduce them in animal diets.
In this study we conducted a systematic screening and analytical chemistry analysis on the seeds of a collection of one hundred thirty three natural lines of *Vicia sativa* to identify cyanogenesis deficient lines of common vetch. Forty cyanoaminoacids deficient lines were identified using HPLC quantification method, and were subjected to cyanogenic glucoside quantification using LC-MS/MS.
Cyanogenic glucosides and cyanoaminoacids concentration varied widely in the forty lines.
Multivariate analysis PCA, heat map and hierarchical cluster analysis were performed and enable the selection of three lines with the lower content of cyano compounds and considered safe feed to be used in animal diets.
Finding from this work, gives insight to feed security program and sustainable agriculture, by improving the protein-rich legume crop common vetch as a feed stuff for livestock and consequently for protein rich animal feed crops.

Expression Studies of Insect Resistant Genes in Gossypium hirsutum

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--Lepidopteron insects fabricate substantial impact on cotton productivity globally. Despite augmentation in cultivated areas over years, production capacity of cotton per unit area still presents a very dismal picture in Pakistan. It is estimated that about 20-40% losses materialize annually due to different pests of cotton. Insecticides and pesticides not only pollute our environment but also escalate input costs for farmers. The current research activity is based on the hypotheses that lepidopetran insects can be controlled effectively by genetic manipulations through applicable biotechnological approach. This study exploited cotton variety VH-305 genetically for its characteristics to exhibit better germination index than other cultivars. Two *Bacillus thuringiensis* (Bt) genes Cry1Ac+Cry2A cloned in single expression vector under the influence of 35S promoter transformed in VH-305. Agrobacterium mediated transformation method was used in which mature embryos were injured by sharp blade at the shoot apex and infected with the *Agrobacterium tumefaciens* harboring transgene constructs. Transformation efficiency was calculated to be 2.05%. Putative transgenic plants were successfully acclimatized in pots and later shifted to green house under controlled environmental conditions. PCR amplification results by specific primer sequences confirmed the integration of transgene in the target genome. Protein estimation done through Bradford and enzyme linked immune sorbent assay (ELISA) witnessed the production of transgenic proteins. These insecticidal crystal proteins are very toxic to these insects and provoke their death. Insect Bioassay uncover that 90% larvae of *Heliothus armigera* feeding transgenic cotton leaves were dead while no mortality was observed in control feeding leaves from non-transgenic cotton. Transgenic plants also display better agronomic attributes, splendid photosynthesis and development than control.
Conclusively cotton yield losses due to lepidopetran insects can be limited effectively by transgenic varieties through introduction of foreign genes. It will not only curtail losses but also magnify farmer's output ultimately intensify economic growth.

DWARF14-LIKE2 (DLK2) is functionally divergent from its strigolactone-related paralogs, D14 and KAI2

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Strigolactones (SLs) are carotenoid-derived molecules bearing essential butenolide moieties. SLs control a wide range of plant developmental processes. Cumulative evidence support the idea that the DWARF14 (D14) functions as SL receptor and required for the perception of the SL signal. In Arabidopsis, two paralogs of D14 have been identified, KARRIKIN INSENSITIVE 2 (KAI2) and DWARF14-LIKE 2 (DLK2). D14 and KAI2 signalling pathways converge in MAX2 and employ similar apparatus to transduce the signal, the two proteins regulate separate physiological events. KAI2 stereospecifically perceives the non-natural (-)5DS SL signal, while D14 shows stereospecificity towards the natural (+)5DS SL. Very little is known about DWARF14-LIKE 2 (DLK2), the third member of the DWARF14 protein family, to which no physiological role has been assigned yet.
I*n vitro* data suggests, that DLK2 does not bind nor hydrolyze natural (+)5-deoxystrigol ((+)5DS), and weakly hydrolyzes non-natural strigolactone (-)5DS. Instead, it might be a receptor or hydrolysis enzyme for an unknown butenolide ligand. A detailed genetic analysis revealed that DLK2 does not affect SL responses and can regulate seedling photomorphogenesis. *DLK2* is upregulated in the dark dependent upon KAI2 and PIFs, indicating that DLK2 might function in light signaling pathways. Although DLK transcriptional regulation is mostly accomplished through MORE AXILLARY GROWTH2 (MAX2), DLK2 is not subject to GR24-induced degradation, suggesting that DLK2 acts independently of MAX2. We observed an unique spatio-temporal regulation of DLK2 expression in roots and identified potential protein interaction partners which could help us to uncover the proper function of DLK2. In conclusion, these data suggest that DLK2 represents a divergent member of the DWARF14 family. Whether DLK2 should be regarded as a key component in a separate butenolide signaling pathway, or its function is merely to regulate other SL/butenolide pathways through the sequestration of the signaling molecules is yet to be answered.

Effect of Spraying Brassinolide on Grain Filling and Qualitative Characteristics of Wheat Seed Cultivar Sirwan under End Season Drought Stress Conditions

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Drought stress due to its occurrence step is one of the factors affecting the yield and other traits of wheat. On the other hand, brassinolide hormone is important in terms of effecting some plant traits under drought stress. In order to investigate the effect of brassinolide in two stages of applying drought stress on the quality of produced seed wheat, an experiment was conducted as a split-plot factorial in RCBD with three replications over 2015 and 2016 at the research station of Education and Research Center of Agricultural and Natural Resources of Fars Province, Iran. Irrigation was carried out as the main plot at three levels of irrigation interruption from the flowering step up to the grain filling, irrigation interruption from the grain filling stage to the ripening the seed and full irrigation. The second factor was in six levels including factorial of concentrations of brassinolide (zero, 0.05 and 0.1 mg/L) and time of application (including spraying before the flowering step and before the grain filling step).The highest rate and the lowest filling period were observed in flowering stage and there was no significant difference between the maximum grain weight in the flowering stress and the grain filling stress. All of the qualitative traits of the seed were affected by drought stress and concentration of the hormone applied to the parent plant. The lowest germination percentage and vigor longitudinal index of seedling were observed at the flowering stage and no application of hormone. The highest seedling vigor index and the lowest electrolyte leakage from seed were obtained by using the 0.1 mg/L brassinolide. In general, the drought stress decreased the quality of seed wheat cultivar Sirvan at the grain filling step and the use of 1.0 mg/L brassinolide improved the effect of stress on the quality of seed wheat.

A barley aspartic-type proteinase reveals antifungal properties

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Fungal pathogens cause substantial losses of yield and quality in grains, both in the field and in storage. Proteinaceous inhibitors of microbial enzymes are central defensive component of plants against a wide range of pathogens, as they protect cellular structures from degradation and/or interfere with signal transduction pathways. Phytases are among the secreted fungal enzymes that enable the fungi to access phosphate predominantly stored as phytate in the grain. As described for bacteria, pathogenic fungi might use phytases as pathogenesis factor to colonize host tissues. Recently, we reported that barley crude protein extracts inactivate microbial phytases via an aspartic acid proteinase activity rather than a classical enzyme inhibition#_ENREF_19. Further *in vitro* analysis of recombinantly produced microbial phytase inhibitor exhibited significant antifungal properties.

Metacaspases, autophagy and cell death in stress-induced microspore embryogenesis

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Microspore embryogenesis is an important biotechnological tool in the search of new genetic variability and rapid production of doubled-haploid (complete homozygous) plants in crop breeding programs. In this system, the microspore is reprogramed, acquires totipotency and is induced toward embryogenesis in a specific stage of development by *in vitro* stress treatment. Nevertheless, after the inductive stress, the new developmental program is limited by the occurrence of microspore death, reducing system efficiency. In *Brassica napus*, microspore embryogenesis is induced by heat treatment at 32°C. Autophagy, a major catabolic process in eukaryotic cells, and metacaspases (MC), a cysteine-dependent proteases, are essential for cell death regulation in plants. Suppression of cell death would benefit potential application of stress-induced microspore embryogenesis for crop improvement. However, our understanding of cell-death pathways that operate in microspore cultures is still scarce.

In this work, we have analyzed the involvement of cell-death proteases and autophagy in the initiation and/or execution of cell death during microspore embryogenesis induction in *B. napus*. *In vivo* treatments with several autophagy and protease inhibitors were performed and their effects on cell death and embryogenesis induction efficiency analysed. Results revealed that cell death increased after microspore isolation and after stress treatment, accompanied with the formation of autophagic structures and induction of several ATG genes and proteins. Furthermore, preliminary assays in stress-treated microspores revealed increased MC activity in stress-treated microspores, which was inhibited by *in vitro* treatment with leupeptin but not with E64. *In vivo* treatments to inhibit autophagy or MC activity reduced cell death and increased embryogenesis induction efficiency. These findings suggest the involvement of metacaspases and autophagy in the initiation and/or execution of cell death during the induction of microspore embryogenesis in *B. napus*, opening a new way to improve its efficiency using chemical modulators of autophagy.

Asparagine slows down the breakdown of autophagic bodies in sugar-starved lupin embryo axes

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Autophagy is conservative process that occurs in yeast, animals and plants, and allows the degradation of entire organelles, macromolecules and protein complexes. One of the final stages of autophagy in plants is the decomposition of autophagic bodies inside vacuole. Degradation of these structures is so fast that their observation without using of inhibitors is impossible. Based on our results autophagy is significantly enhanced by sugar starvation in cells of embryo axes of lupin germinating seeds. However, feeding of sugar-starved embryo axes with asparagine causes clear inhibition of autophagic bodies degradation, and it allows analysing of their contents. At present we do not known what is the mechanism of asparagine action. We only known that asparagine inhibits lipolytic and proteolytic activity in sugar-starved embryo axes. To further explore of this topic we are going to perform proteomic and genomic analyses. Moreover, asparagine inhibits degradation of storage lipid in sugar-starved embryo axes. We observed also inside non-degraded autophagic bodies structures which can be identifies as peroxisomes. Thus we formulated hypothesis that pexophagy occurs in sugar-starved lupin embryo axes.
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Combating Hidden Hunger: Zinc Localization and Speciation in Barley Grains

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Background
Hidden hunger, also known as mineral malnutrition, affects approximately one in three people globally. Herein, zinc deficiency leads, amongst others, to growth retardation and immune dysfunctions. Through a greater understanding of zinc accumulation mechanisms in plants, breeding programs can help to increase concentrations and bioavailability in staple crops. We therefore investigated zinc concentrations, localization and chemical binding environment in the model crop barley (*Hordeum vulgare* L.) during grain development.
Materials and Methods
A high zinc accumulating barley cultivar, 'Giza 126', was grown in soil in the glasshouse. Whole ears were harvested 7, 15, 27 days after pollination (dap) and at maturity. Zinc concentrations were measured using ICP-OES. For tissue specific analysis, µ-Proton-Induced-X-Ray-Emission (µPIXE) measurements were performed on transversely cut grains at the position of the embryo. Additionally, endosperm tissue was freeze-dried, homogenized and extracted with 100 mM ammonium acetate for speciation analysis by SEC-ICP-MS.
Results
Zinc concentrations in whole ears ranged between 57.0 ± 8.0 mg kg^{-1} DW and 78.5 ± 11.7 mg kg^{-1} DW. Between 7 and 27 dap, concentrations remained stable and climaxed at maturity. Accordingly, µ-PIXE analysis revealed greatest zinc concentrations at maturity in almost all tissues. Three zinc species were detected by SEC-ICP-MS, which did not co-elute with sulphur or phosphorus. The solubility of zinc species decreased during ripening from 45% at 7 dap to 7% at maturity.
Conclusion
Greatest zinc concentrations were found in mature barley grains of 'Giza 126'. These included endosperm tissue, which accounts for about 70-80% of the grain's total zinc and is therefore especially nutritionally important. In soluble form, zinc does not seem to be bound to phosphorus, indicating other molecular species than phytate are important. However, zinc was found to be increasingly bound to water-insoluble species during grain ripening, why further characterization of the non-soluble fraction is in progress.

Affinity capture and MS identification of barley (Hordeum vulgare L.) and wheat (Triticum aestivum L.) grain proteinaceous inhibitors of bacterial subtilisin and GH11 xylanase

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Microbial proteases and xylanases are commonly added as feed additive for improving the digestibility of cereal grain proteins and lower the viscosity in the gastrointestinal tract. Unfortunately, the effect of feed protease/xylanase additions varies considerably between different barley/wheat cultivars. To study the inhibitor effect on the individual feed hydrolases it is necessary to identify the inhibitors at protein level. In the current study, Ronozyme® ProAct, a recombinant subtilisin-like serine proteases and Biofeed L., a fungal GH11 xylanases have been immobilized in a solid support (NHS-activated Sepharose® 4 Fast Flow) and used as bait to affinity capture the endogenous inhibitors using barley cv Golden promise and wheat cv Chinese spring grain albumins. NHS-Sepharose immobilized baits were mixed with albumins fractions of barley and wheat respectively. After several NaCl washes, the last including detergent (Rapigest, Waters), the captured inhibitors were eluted either by 1 M HCl or identified and quantified by label free LC-MS/MS. Both barley and wheat Ronozyme® ProAct inhibitors were found to be bound to the bait-resin (strong inhibitors) or eluted by 1M HCl (medium-strong inhibitors).

Alterations in growth of maize seedlings influenced by mixed tetraoxanes

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Application of natural or synthetic substances could increases maize vigor, particularly of seeds with poor viability. The aim of this experiment was to examine the influence of five mixed tetraoxanes (T1–T5) on germination and early growth (seven-day old seedlings) of maize inbred line, from the lot with high germination ability (>90%-G1) and low germination ability (<50%-G2). After soaking in tetraoxanes solution (10^{-6} and 10^{-9} M) for 24h, at room temperature, the seeds were germinated under controlled laboratory conditions on filter paper (BP, 20⇔30°C, ISTA Rules). Results show diverse effects of applied treatments. The significant increase in germination of 21.3% (10^{-9} M) for G2, as well as in seedlings roots and shoot fresh biomass for both lots, were observed in T1 treatment. The highest increase of seedlings root and shoot dry matter (8.79% and 8.08% for G1, as well as 9.52% and 8.99% for G2, respectively) was obtained by T4 treatment. For G1, increased seedlings root to shoot ratio for fresh matter, was achieved with T4 (10^{-9} M), while for G2, T3 brought the highest values of the ratio for both fresh and dry matter. For G1, increased seedlings root to shot ratio for dry matter was achieved under T4 (10^{-9} M). All applied treatments increased hydrolysis and biosynthesis. The highest hydrolysis values for G1was achieved by T2 (0.1640 g) and for G2 by T1 treatment (0.1187 g). The highest values of biosynthesis were achieved under T4 for both G1 and G2 (0.0723 and 0.0426 g, respectively). For G2, interdependence between germination and seedlings root and shoot fresh matter, and between hydrolysis and biosynthesis, implied a significant and negative correlation between germination rate and root fresh matter. Moreover, significant increase in germination rate for G2 was followed by increase in seedlings root and shoot fresh matter, hydrolysis and biosynthesis.

Chinese Yam - a tuber crop with high potential as a functional food in Europe

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Yams (*Dioscorea spp.*) are monocotyledonous plants that produce edible and nutritious tubers that constitute an important staple food for millions of people in tropical and sub-tropical countries. The Chinese Yam (*Dioscorea opposita*) is a yam species that can grow in temperate climates like central Europe and could be cultivated here to enrich our daily diet. The underground tubers, as well as the small aerial tubers (bulbils), are rich in starch and minerals and contain healthy ingredients that can be applied for treatment of diabetes and hypertension as well as regulation of blood cholesterol levels. Therefore, Chinese yam tubers have a high potential as ”functional food”. However, cultivation is difficult because the heavy underground tubers reach up to 1.5 meters deep into the ground and have a club like shape with the tuber being thin at the top and getting thicker at the base. This shape makes harvest a challenge as tubers cannot be pulled out but have to be dug out carefully. Consequently, commercial yam cultivation in Europe is scarce. Our research focuses on the molecular mechanisms of this unique growth behavior of Chinese yam tubers characterized by a strong gravitropic response. Based on marker-assisted breeding we want to identify cultivars suitable for successful and economically attractive cultivation and therefore promote future yam cultivation in Europe.

Effect of SbPPC3 silencing in sorghum (Sorghum bicolor) plants

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Phosphoenolpyruvate carboxylase (PEPC) (EC 4.1.1.31) is a key cytosolic enzyme in carbon and nitrogen metabolism, displaying pivotal roles in photosynthesis, respiration, aminoacid synthesis, and during seeds development and germination. PEPC in sorghum (*Sorghum bicolor*) is composed of a multigenic family formed by six different isoforms (Sb*PPC1-6*). SbPPC1 is the photosynthetic PEPC, SbPPC2-5 anaplerotic proteins, and SbPPC6 the bacterial-type isoform. Among all these proteins, we focused on SbPPC3 for this study. SbPPC3 protein is located mainly in roots and seeds, being the most abundant PEPC protein found in these tissues. By the interfering RNA technique (RNAi), we silenced the PEPC3 isoform in sorghum and analyzed the plant and seeds phenotype. Modified plants showed a lower growth rate, smaller shoots and roots, as well as a delay in the flowering time, compared to the wild-type. In addition, transformed plants produced smaller panicles with lower number and weight of seeds. Seeds from modified plants showed different starch and aminoacid levels. Finally, the metabolic profile of these plants and seeds was analyzed by GC- and LC-MS, both under control or salt-stress conditions. Altogether, the results obtained suggest that SbPPC3 protein has an important role in vegetative growth and seed composition, as well as in the plants responses to salt.
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Exploring the Hydration-Dehydration-Rehydration (H-D-R) cycle in Medicago truncatula seeds to disclose novel hallmarks of seed priming.

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Seed quality is a priority in the agricultural context which demands high standard seeds to meet the ever increasing global challenges. Improving seed vigor to gain rapid/uniform seedling emergence/establishment, is a daunting task. Priming is regarded as a valid treatment to enhance seed quality. One of the many priming techniques consists in controlled seed hydration (H) to trigger the metabolic processes activated during the early phase of germination. Controlled seed hydration boosts the pre-germinative metabolism and enhances the antioxidant and DNA repair responses. Following a defined temporal hydration, seeds are dehydrated (D), the 'dry-back' step, stored, and subjected to rehydration (R) or post-priming imbibition. Novel approaches that will elucidate the molecular bases of the seed response are required to optimize these techniques.
The H-D-R cycle, used in the present study to pinpoint seed quality indicators, has specific effects on the seed metabolism. Hydration activates the pre-germinative metabolism, associated with *de-novo* synthesis of nucleic acid and proteins, ATP production, accumulation of sterols and phospholipids, activation of antioxidant and DNA repair mechanisms. Investigation on the seed DNA repair mechanism and antioxidant activity has revealed their role as hallmarks of seed quality.
Dehydration has an opposite effect since water removal results in deleterious effects on cell components. To prevent ROS-induced DNA damage during dehydration, metabolic activity is reduced and ROS scavengers are accumulated. Rehydration is the crucial step of the H-D-R cycle, as germination and biomass parameters are the endpoints to understand the effectiveness of priming.
Our working system for investigating the H-D-R cycle has been established using the model legume *Medicago truncatula*. Phenotyping of the germination response and evaluation of biometric parameters in seeds subjected to H-D-R revealed interesting information relative to the most appropriate timepoint to end hydration as well as seedling establishment timing. Molecular analyses are currently in progress.

The effect of drought stress on Norway spruce somatic embryo development

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Somatic embryogenesis is a developmental process where a plant somatic cell can dedifferentiate to a totipotent embryonic stem cell that has the ability to give rise to an embryo under appropriate conditions. Desiccation is the final phase of embryonic development and appears to be important in the transition from embryogenic phase to germination. The objective of the presented study was to follow morphological and selected biochemical characteristics of somatic embryos induced by various air humidity during desiccation of Norway spruce somatic embryos. The fully developed embryos were desiccated in three different levels of air humidity (90%, 95%, 100%) for 10 days, the rest of desiccation (another 10 days) took place in 100% air humidity. We described changes of chitinases and beta – 1,3, glucanases activities as well as abscisic acid and malondialdehyde content. Concurrently we observed the expression of two beta – 1,3, glucanase and three chitinase genes and selected ATG (autophagy related) genes.
Low humidity in desiccation reduced activity of monitored enzymes, which was to some extend restored after embryo rehydration at the end of desiccation according to drought stress level. Transcriptional levels of studied genes followed the same pattern, their expression was diminished after water stress treatment. Rehydration of embryos started massive expression of some of them. Morphology of desiccated embryos was comparable in all three variant of relative air humidity. However, germination of somatic embryos cultivated in 90% of air humidity was affected negatively.

Selective modification of the wheat seed microbiota affects hydrogen peroxide production in wheat seedlings

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Seed performance underpins agricultural productivity and food security. Despite the emerging number of reports on the seed microbiome, the contribution of bacterial endophytes to seed germination and vigour remains unclear. Here, we investigated seed-microbe interactions in wheat (*Triticum aestivum*). Seed surface sterilisation with a ”hot steam” treatment significantly reduced the number of microbes, accelerated germination, and decreased rates of hydrogen peroxide (H_2O_2) production, compared to non-surface-sterilised seeds (control). Considering H_2O_2, a key signalling compound in biotic stress response, we tested whether seed inoculation with single bacterial strains was associated to elevated levels of H_2O_2 production by seedlings. The microbiota of dry seeds was dominated by *Gammaproteobacteria*, in the families of *Pseudomonadaceae* and *Enterobacteriaceae*, as revealed by 16S rRNA gene sequencing. Typical for wheat seeds, the genus *Pantoea*, which is known to induce systemic acquired resistance, abounded. Restriction fragment length polymorphism and sequencing of the intergenic spacer region, combined with sequencing of the *gyrase B* gene, identified two *Pantoea* species (spp. *agglomerans* and *eucalypti*). Notably, inoculating surface-sterilised seeds with *P. agglomerans* and *eucalypti* stimulated seedlings to increase and decrease H_2O_2 production, respectively, 48 h after the onset of imbibition. An H_2O_2 burst was also measured in seedlings exposed to pure cultures of *P. agglomerans* and *eucalypti*. We then hypothesised that the differential responses of seedlings to distinct *Pantoea* spp. related to endophytic colonisation and distinct species lifestyles. Therefore, 48 h after the onset of imbibition, endophytic and epiphytic isolates from seedlings were sequenced to assess whether selective inoculations of bacterial isolates in dry surface-sterilised seeds alter the microbiota composition, and whether this affected wheat physiology, during seed germination and seedling growth. Our overall aim is to improve the understanding of plant-bacterial interactions in seeds and seedlings, and decipher how the seed microbiota can be optimised to enhance seed germination and vigour.

Plant genetic resources stored in the genebank of IHAR Radzików, Poland: current status and perspectives

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Over 70 000 accessions of plants, belonging to the following groups: cereals, grasses, large seed legumes, small seed legumes, oil plants, industrial plants, vegetables, and medicinal plants, are currently stored in National Centre for Plant Genetic Resources. The seeds are kept in temperature controlled chambers. There are three long-term storage chambers where the seeds are stored at a temperature of -18 °C and five medium-term storage chambers where the temperature is 0 °C. In recent years, the genebank infrastructure has been modernized. Currently, the introduction of a bioinformatic management system is planned. Qualitative and quantitative analysis of the distribution and use of genetic resources collected in the National Centre for Plant Genetic Resources was carried out. During the last 10 years there were 297 unique recipients identified, whom 11.323 accessions samples were distributed, out of 456 taxa. The largest share was made by researchers. The next largest groups were individual recipients and breeding. These three groups placed a total of 85% of the recipients and acquired 92% of the sample. Other groups, i.e. education, genebanks, botanic gardens and others, represented a combined 15% of the recipients and took 8% of the sample. The number of individual consumers is increasing, which may help to inhibit genetic erosion but it increases the exploitation of resources in the genebank.

Analysis of molecular role of transporters and nutrient utilization genes in rice (Oryza sativa L.) during arsenic - silicon exposure

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Arsenic (As), as nonessential and carcinogenic metalloid has become a major limiting factor for the production of crop plants. Rice (*Oryza sativa* L.) paddies contaminated by As is a staple crop over half the world population, and its consumption creates severe health problems. Due to higher availability of As in anaerobic flooded soil, rice efficiently accumulates more As than other cereal crops. Presence of As in soil/water effectively reduces the nutrient content in rice plant, results in impaired growth and development of the crop. Therefore, selecting rice varieties tolerant to As stress or able to cope up As stress along with other metals is important for the sustainable production of rice. Silicon (Si) attracted substantial attention because of its positive effect on plants during abiotic stress, including arsenic (As) stress. We here report that priming rice seeds with As and Si together, helped the plant to sustain for a longer period. We examined Si induced tolerance in rice seedlings at short (7 d) and long (15 d) exposure periods under $As^{(III)}$ and Si treatments since their germinating stage. Results showed that the expression of *OsLsi1, OsLsi2* and *OsLsi6* transporters was more in $As^{(III)}$+Si treatment as compared to control and Si treatment, but lower than $As^{(III)}$ alone treatment. Expression was maximum in shoot and root at 15 d over 7 d under both $As^{(III)}$ and $As^{(III)}$+Si treatment, which ultimately leads to decreased accumulation of As in the presence of Si. Morphological characters and macro- micronutrient contents also improved with Si, and differentially regulated 12 key genes (*NR, NiR, AMT, NR, GS, GOGAT, PT*, *PHT1, PHT2*, *APase, KAT1* and *HAK10)* related with NPK transport and utilization. Results highlight that Si plays a decisive role in growth recovery of As-stressed rice.
Keywords: Arsenic, Silicon, transporters, nutrients, *Oryza sativa* L.

Built-in Pathway to the Biosynthesis of Capsicum Red Color Pigment in Rice Endosperm

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Capsanthin is the main constituents responsible for the red color of ripe fruits in pepper (*Capsicum* spp.). With a range from a deep red to a pale pinkish-yellow depending on the concentration, capsanthin has been the most sought-after natural ingredient as colorant for diverse industry of dairy products, beverages, cosmetics and foods. Over property of capsanthin to assign red shade, activity to reduce risk related to obesity has been recently reported in addition to various those such as antioxidation, anticancer, and anti-inflammatory effects although it is non-provitamin A carotenoid. Nevertheless, no case for capsanthin biogenesis has been yet reported as genetically engineered plants. To allow the *de novo* biosynthesis of capsanthin in rice, a *Capsicum* capsanthin-capsorubin synthase gene (*CaCcs*) of Korean pepper (cv. Nockwang) was overexpressed under the control of seed-specific promoter, yielding *Ccs* rice line. As male parents with another three rice lines showing golden color in endosperm, two β-carotene-enriched rice using *PAC* (*CaPsy-2A-PaCrtI*) gene and it synthetic *stPAC* (*stPsy-2A-stCrtI*) gene and one zeaxanthin enriched rice using *B-PAC* (*CaBch* with *CaPsy-2A-PaCrtI*) gene, a *Ccs* rice exhibiting white endosperm color due to lacks of carotenoids, was conventionally interbred as the female parent. As results, *PAC* x *Ccs*, *stPAC* x *Ccs* and *B-PAC* x *Ccs* lines showed a change in seed color and carotenoid composition. Total levels of carotenoids strongly correlated with those of male parental lines to provide carotenoid precursors but orange-red color intensity was dependent on the highest levels of capsanthin in *B-PAC* x *Ccs*, despite the highest total carotenoid levels in *stPAC* x *Ccs*. It suggested that the conversion step from b-carotene to zeaxanthin by BCH might be necessary for the production of capsanthin in rice endosperms. Collectively, a combination of genetic engineering and conventional breeding is effective for multi-step metabolic flux engineering and biochemical pathway extension.

The Role of Different Physical Dormancy rate on Seed Longevity in Vicia tetrasperma (L.) Schreb

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The objective of this study was to track the changes in the longevity of *Vicia tetrasperma* (L.) Schreb. Seeds after storage at different temperatures and equilibrium relative humidity (ERH). *V. tetrasperma* (L.) seeds with different germination rates of 0, 50, and 100 percent were artificially aged at a temperature of 20 or 50 ℃ . For storage, seed moisture contents were adjusted from 6.6 to 16.2 percent. It has been observed how the longevity of the seed changes over the designated storage period. The seed coats were scratched and thereafter carried out for 3 weeks after the dormant break. The final survival rate was assessed by seed clush test after germination test.
Using Ellis and Roberts's seed longevity equation, the lifespan of each treatment was predicted. Estimated viability period was well fitted to their equation in a one step and two step approach. *V. tetrasperma* (L.) seeds showed characteristics of orthodox species. *V. tetrasperma* (L.) seeds were found to have different equilibrium times and velocities due to differences in dormancy rates. Compared to seeds with 0% and 50% physical dormancy rate, seeds with a physical dormancy rate of 100% were found to be able to be equilibrated at an ERH lower than 6% to 10%. In conclusion, we could predict the lifetime change of the physically dormant seeds.

Nano-priming with MWCNTs: a new technique for improving plant germination characteristics under drought stress

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Iran has mean annual rainfall of 250 mm and experiences many problems with the establishment of plants in urban green space and botanical gardens. In this country, there are some provinces, such as Semnan, face more serious difficulties e.g. very low precipitation (130 mm annually), soil salinity, and limited water storage for irrigation. This is despite the fact that creating urban green spaces and botanical garden (especially in Semnan University with the research approach) is necessarily demanded. The use of nanotechnology in plant sciences issue has been rapidly expanding and in the future it can be help us to solve the problems of planting in harsh environmental conditions. In current research, the effects of different dosages of multiwalled carbon nanotubes (MWCNTs) on seed germination of myrtle have been investigated. After nanopriming and drying under room temperature, the seeds were placed into a germinator under 20 ˚C constant temperature and 65% relative humidity with a photoperiodic regime of 16 h light/ 8 h dark at 1000 lux fluorescent light. MWCNTs improved seed germination, root and stem lengths and also plantlet fresh weight. Based on the results of this study, nanopriming with MWCNTs should be considered for enhancing seed germination and initial plant growth in nursery, afforestation, and gardening projects.

Analysis of salt-tolerant mutants in M2 generation of maize inbred lines mutagenized by Ethyl Methane Sulfonate

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The EMS(Ethyl Methane Sulfonate) induced mutants was used to measure the quantification including disease resistance, abiotic stress, and yielding ability. The two maize inbred lines were treated with 0.3%, 0.5%, 0.7%, and 0.9% EMS respectively for 8 hours. The results of phenotyping analysis of mutagenized maize population under each treatment condition that had not obtained the M_2 generation seeds from condition treated with 0.7% and 0.9% EMS(v/v) for 8 hours. Otherwise, over the course of eight hours, 0.5 % EMS(v/v)- treated conditions showed a variation in expression, but we were able to obtained the M_2 generation seed that was mutated to analyze the concentration of salt. A total of 1041 independent M_2 familiar of EMS-induced maize inbred mutants have been investigated for salt tolerance. We selected salt-tolerant maize inbred lines from mutants populations treated with 0.7% NaCl in a greenhouse for three weeks. The salt-tolerant mutation was identified in the M_2 mutant populations. We generated whole-genome sequencing data to the two maize inbred mutants for gene variations in the enhanced salt-tolerant population. We expect the results to have a significant effect on the genetic modification of mutant maize inbred lines and comparative genetics.

Structure of plasmodesmata and symplasmic communication during embryogenesis among Crassulaceae

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In the embryogenesis of many angiosperms it is typical for the early embryo to differentiate into two parts, the embryo proper and the suspensor, a rapidly-developing, short-lived organ which degenerates before the formation of the mature seed. Crassulaceae is one of the families in which the suspensor develops haustorial branches that invade the micropyle and adjacent tissues. In this work we showed the ultrastructural aspect of suspensor development in selected species of five genera of Crassulaceae: *Aeonium*, *Aichryson*, *Echeveria*, *Monanthes* and *Sedum*. We focused our attention on the plasmodesmata (PD). There are changes in the structure of the PD in the suspensor in the above-mentioned genera. In *Sedum*, two types of PD were found; simple and branched with a dome of an electron-dense material depending on a pattern of development suspensor. However, the remaining genera were found the PD with electron-dense material. The studies presented in this work also concern an analysis of symplasmic tracers movement between different seed compartments on the example of *Sedum acre*. The symplasmic communication between *Sedum* seed compartments and the embryo and within the embryo change during the development. An unusual electron-dense dome associated with plasmodesmata on the border between the basal cell/chalazal suspensor cells and the basal cell/the endosperm has been described. One of the key questions is what is the chemical nature of a dome of electron–dense material. The structure of these compound of PD was examined using an antibodies against callose, plasmodesmata-located proteins (PDLP1) and reticulon proteins (RTNLB3 and RTNLB6).

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Identification and Functional Characterization of Rice F-bZIP Transcription Factors

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Zinc is an essential micronutrient for all organisms, which plays key structural and regulatory roles in many proteins. However, Zn-deficiency is widespread in agricultural soils and this adversely affects the yield and nutritional quality of crop plants. As a result, billions of people suffer from Zn malnutrition leading to serious mental and physical health defects. Since plants are at the base of the food chain, Zn biofortification of crops is a high priority in the fight against malnutrition.
It is, therefore, important to understand the processes involved in the Zn uptake by the roots from the soil, and its transport and distribution within the plant. This is done by uptake transporters, which include members of the ZIP (Zrt-Irt-like Protein) family, efflux transporters such as the HMAs (Heavy Metal ATPases), and chelators such as NA (nicotianamine).
Many of these transporters and chelators are induced transcriptionally under Zn-deficiency. That is, plants respond to Zn-deficiency by upregulating the expression of these transporters and chelators thereby enhancing their capacity for Zn uptake and distribution. The Arabidopsis bZIP19 and bZIP23, members of the group-F bZIP transcription factors family (thus F-bZIPs), were identified as key regulators of these genes involved in the response to Zn-deficiency.
While understanding the mechanisms of how Zn modulates the activity of these transcription factors in Arabidopsis remains the central goal of my PhD project, I am also involved in a translational approach where we look into orthologues of these F-bZIPs in other agricultural crops. We have recently shown that F-bZIPs and their function is likely conserved across land plants. In my presentation, I will show the role of Rice F-bZIPs in regulating Zn-deficiency responses with gene expression, protein localization, elemental profile and phenotypic data, which overall support the functional conservation of plant F-bZIPs.

The Highs and Lows of Wheat Phytase Activity

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Wheat and its closest relatives stand out among grains by having substantial mature grain phytase activity (MGPA). The trait is conferred by the *PAPhy_a* genes, which exist in one locus on each subgenome (i.e. two or three loci)[1]. We have identified a higher expressed "HighPhy" as well as silent *PAPhy_a* alleles[2]. This allow us to expand the MGPA variation far beyond that of elite cultivars. Microbial phytase is an important feed additive used to increase the bioavailability of phosphorous and cationic micronutrients. The HighPhy wheat can increase the phytase activity in feed above the standard dose of microbial phytase. A feeding experiment showed that the HighPhy wheat provides even higher performance improvements than the control with microbial phytase[3]. The discovery of additional HighPhy alleles is an attractive prospect since it would allow stacking of multiple HighPhy alleles and also introduction of the trait in durum wheat. Genetic variation is available among wild relatives[4] but it is difficult to discover alleles with moderately higher or lower expression when the scoring is done in a background of one or two other homeologous loci. We are therefore developing low phytase diagnostic lines in tetra- and hexaploid wheat by stacking deleterious *PAPhy_a* mutations. We expect that these lines may not only aid further improvements of MGPA in wheat but also help solve an evolutionary conundrum; what is the adaptive benefit, if any, of high MGPA? Bear in mind that most grains, including maize and rice, are able to mobilize phosphorus during germination with *de novo* synthesized phytase only.

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Biochemical changes induced in Brassica napus L. seeds after long-storage and accelerated aging

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Oilseed rape is one of the most productive vegetable oil crops dominating at the global market. Therefore, a complete understanding of the changes in metabolism of seeds, accompanying their storage is a pre-requisite for safety and sustainable oil production. However, evaluation of seed longevity in course of natural aging is time-consuming. Therefore, to address this question, seeds are usually incubated under high relative humidity and temperature, i.e. subjected to so-called accelerated aging (AA). Although this model provides reliable and reproducible results, it is still unclear how close the changes occurring in seeds during AA resemble the alterations accompanying long-term storage (LS). Therefore, a LS procedure was carried out at 18°C and 5% water seed content during four and seven years. An AA experiment was performed at 40°C and 10% water seed content for one and seven days. Accordingly, seed germination rates were 91% decreased after four years of storage and one day of AA, whereas a 46% reduction was observed after nine years of storage and seven days of AA. Interestingly, these changes were accompanied with an electrolyte leakage, which was two-fold higher in AA than in LS experiment. Although LS did not affect the contents of reduced (GSH) and oxidized (GSSG) glutathione, AA induced a significant alteration in a GSH/GSSG ratio. To explain this difference in oxidative status, primary metabolome of aged seeds was addressed by gas chromatography-mass spectrometry (GC-MS). A comprehensive metabolite profiling revealed a significant difference between two seed ageing models, indicating sugars as the most strongly regulated metabolites. Thus, the changes, accompanying a long-term and accelerated ageing of seeds targeted different metabolic pathways. This work was performed by using the equipment of the Research park of St.Petersburg State University Center for Molecular and Cell Technologies and supported by grant no. 16-16-00026 from the Russian Science Foundation.

Activation of auxin biosynthesis by trehalose 6-phosphate is required for normal seed filling in pea (Pisum sativum)

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Seeds determine the reproductive capacity of plants and are vital to their existence. To ensure seed survival and nourishment of seedling growth upon germination, the embryo enters the maturation phase, which encompasses the accumulation of reserve compounds and the acquisition of desiccation tolerance. Trehalose 6-phosphate (T6P), which functions as a signal for sugar availability in plants, is believed to regulate storage processes in seeds, since disruption of T6P synthesis in *Arabidopsis thaliana* causes embryo abortion at the onset of the seed filling phase. To investigate the role of T6P during seed development, we modulated the T6P content in pea embryos by ectopic expression of the T6P synthase (*OtsA*) or T6P phosphatase (*OtsB*) genes from *E. coli*. We show that T6P promotes cotyledon growth and starch accumulation in maturing seeds, and that this requires transcriptional induction of auxin biosynthesis. Thus, our data indicate that T6P integrates auxin levels with sugar availability to facilitate seed filling in pea.

The potential environmental impact of transgenic Brassicaceae in Russia

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In Russia GM crops are not approved for commercial cultivation yet. However, the knowledge about hybridization processes in actual environment is necessary for the future regulation and environmental monitoring. *Brassicaceae* are the most common plants in Russia, represented by a great diversity of species. We performed transformation of popular domestic plants *B. napus*, *B. rapa*, *B. juncea*, *Eruca sativa* and *Sinapis alba* with empty 2301 pCAMBIA vector in order to study gene flow from these species. Hybrids were identified after selection on kanamycin, PCR and then RAPD analysis. All experiments were carried out in separate plots within the city.
Amongst the seeds of *B. rapa*, 20 were hybrids with *B. napus*, 5 were hybrids with *S. alba* and 2 were hybrids with *E. sativa*. Amongst the seeds of *B. juncea*, 9 were hybrids with *B. napus*. Amongst the seeds of *E. sativa*, 1 was a hybrid with *B. rapa*. Most of them had reduced fertility, however, several *B. napus* x *B. rapa* and *B. napus* x *B. juncea* hybrids produced as many viable seeds, as parental plants. No hybridization was observed in other combinations.
In *Brassicaceae* hybridization plays a key role in formation of species. Moreover, different genotypes of both GM-crops and their wild and domestic relatives, the location of transgene in a crop genome as well as environmental conditions can affect the hybridization rates. That is why it should be studied carefully to avoid mistakes. It's unlikely that gene flow can be fully prevented. So we assume that transgenic *Brassicaceae* plants should have visually detectable selective trait that will help to find and eliminate the hybrids.

Functional divergence of DOG1 and DOG1-LIKE4 in the seed maturation program

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The *DELAY OF GERMINATION1* (*DOG1*)-*LIKE* (*DOGL*) family proteins are plant-specific proteins of unknown biochemical function. RNA-seq analysis using the chemically inducible lines for *nine-cis-epoxycarotenoid dioxygenase 6* (*NCED6*), an ABA biosynthesis gene, identified *DOGL4* as an outstanding ABA-induced gene. While *DOGL1*, *DOGL2* and *DOGL3* were not expressed substantially in developing seeds, *DOGL4* was expressed together with *DOG1*, a major regulator of seed dormancy, during seed maturation. DOGL4 shared only limited homology in amino acid sequence to DOG1. The reduced dormancy and longevity phenotypes observed in the *dog1* seeds were not observed in the *dog1-like4* mutants, suggesting that these two genes have limited functional overlap in terms of seed germination control. Induction of *DOGL4* in imbibed seeds at the testa rupture stage that is immediately before radicle protrusion induced 70 genes typical of seed maturation, suggesting that *DOGL4* is capable of re-introducing at least a major fraction of the seed maturation program in germinating seeds. The specific sets of genes induced by *DOGL4* included sugar-, peptide- and lipid transporters, which are important for resource allocation in seeds, and seed reserves, such as *albumins*, *cruciferins* and *oleosins*. These results suggest that *DOGL4* is a master regulator of seed reserve accumulation. *DOGL4* also induced seed desiccation-associated genes, such as *LATE EMBRYOGENESIS ABUNDANT* and *HEAT SHOCK PROTEIN*, which are also regulated by *DOG1*. However, the majority of *DOGL4*-induced genes, including seed reserves, were not affected by the *dog1* mutation, suggesting that DOG1 and DOGL4 have distinct roles in the seed maturation program, in addition to their overlapping function in seed desiccation. The DOG1 family proteins may have diverged over the course of evolution into independent regulators of seed maturation, each of which has the specific and redundant roles in seeds.

Translational Seed Biology - proofs of concepts to control seed germination and reserve accumulation

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Our understanding of seeds has been advanced significantly through Arabidopsis research. The mechanisms underlying how seeds develop and make a decision for germination or dormancy have been largely unfolded, which now offers great potential for applications to food and energy crops. This poster presents and discusses examples of proofs of concepts obtained from basic research on seed maturation and germination, which could be directly translated into crop species. In the course of seed dormancy research, the induction of *nine-cis-epoxycarotenoid dioxygenase 6* (*NCED6*), an ABA biosynthesis gene, was demonstrated to be sufficient to suppress germination, demonstrating the importance of continuous ABA biosynthesis and the robustness of the rate-limiting hormone metabolism enzyme in seed germination control. Seed-specific overexpresson of the sorghum *NCED* using the wheat Early Methionine promoter caused spontaneous hyperdormancy through positive-feedback regulation, which does not require chemical induction. This result opened a possibility of technology development for the prevention of precocious germination, such as preharvest sprouting in cereal crops. This proof of concept is now being translated into wheat and barley in international collaborations. Further basic research using the *NCED*-inducible lines and RNA-seq identified novel ABA-regulated genes, including *DOGL4* (*DOG1-LIKE4*), a homolog of *DELAY OF GERMNATON1* that is a master regulator of seed dormancy. *DOGL4* does not seem to be heavily involved in seed dormancy but rather turned out to be a major regulator of seed reserve accumulation. The potential of *DOGL4* to increase seed storage proteins is now being tested in soybean. The *DOGL4* analysis also contributed to our understanding of diverged functions of the DOG1 family proteins in basic research. The two cases of studies demonstrated the usefulness of basic research in Arabidopsis for translational biology and also the synergy between basic and translational research.

Exploring the natural variation of specialized seed metabolites of *Arabidopsis thaliana* to detect biosynthetic and regulatory genes using GWAS

Thomas Naake, Molecular Plant Physiology in Potsdam-Golm

Specialized metabolites are synthesized by plants to facilitate ecological or environmental interactions. Plants often accumulate these metabolites in response to stress, suggesting that these metabolites convey evolutionary benefits. Natural variation in plant species is a valuable resource to link genetic variation with differences in metabolite levels. We will employ natural variation of *Arabidopsis thaliana* accessions to identify genes governing metabolic traits using the statistical framework of genome wide association studies. In this project, seed specialized metabolites of *A. thaliana* accessions were determined using metabolic profiling by untargeted ultra-pressure liquid chromatography followed by mass spectrometry targeting known and unknown polar seed metabolites. To this point, the validity of the pipeline was checked by mapping known flavonoids and glucosinolates to reported loci: the flavonoid kaempferol 3,7-dirhamnoside mapped to a region containing *At5G07990*, encoding cytochrome P450 75B1 (TT7), a protein controlling the quercetin/kaempferol metabolite ratio; aliphatic methylsulfinylalkyl and methylthioalkyl glucosinolates exhibited strong differences between accessions and mapped to the known *GS-ELONG* locus on chromosome 5 containing *methylthioalkyl malate synthase* (*MAM1*) and *MAM3*, and to the *GS-ALK* and *GS-OHP* locus on chromosome 4 *containing alkenyl hydroxyalkyl producing 2* (*AOP2*) and *3* (*AOP3*), respectively. After confirmation of the validity of our approach, the pipeline was extended to known and unknown metabolites focusing especially on putative sinapoyl containing glucosinolates, and will be followed by functional characterization of candidate genes in future studies.

Bridging Basic and Applied Research in Seed Biology

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Seed pre-germinative metabolism involves several physiological processes that globally ensure a successful seedling establishment and prevent damage to genome and cellular structures. Seed vigor and quality are fundamental for plant propagation and crop production and their improvement represents a priority for seed companies and plant biotechnologist (Waterworth *et al*. 2016, *Proceeding of the National Academy of Sciences U.S.A.*, 113, 9647-9652; Pagano *et al*. 2017, *Frontiers in Plant Science* 8, 1972).
A multilevel approach is necessary to elucidate the complex network that determines seed vigor. This work, through phenotypic and high-throughput analyses, explores the effects of genotoxic stress on seed metabolome. Seeds of the model legume *Medicago truncatula* have been imbibed in presence of sodium butyrate (NaB), a short-chain fatty acid that causes genotoxic stress. The biometric evaluation of its effects revealed delayed and decreased germination rates, along with impaired seedling growth. Single Cell Gel Electrophoresis showed increased DNA damage in NaB-treated samples, while quantitative Real-Time PCR indicated a significant up-regulation antioxidant and DNA repair genes. Finally, a non-targeted metabolomic profiling highlighted a chemically and functionally heterogeneous subset of metabolites that are differentially accumulated in NaB-treated seeds as putative molecular signatures of the seed repair response.

Genome wide analysis of W-box element and its variants in Arabidopsis thaliana under various abiotic stresses reveals spacing of 3-4 bp to be prevalent between two TGAC motifs

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Cis-regulatory elements play a major role in regulating the spatial and temporal gene expression at the transcriptional level. Thus, to design synthetic promoters leading to stress-specific induction of a transgene, the study of cis-regulatory elements is of great importance. The present work focuses on cis-regulatory element, W-box having TGAC as core motif which serves as the binding site for the members of WRKY transcription factor family. The aim of the present research is to study the frequency of occurrence of TGAC(N)TGAC motif for varying spacer lengths (ranging from 0 to 30 base pairs) separating the two motifs across the genome of *Arabidopsis thaliana* in order to determine biological and functional significance of relevant conserved sequences. Further, microarray data was analyzed to identify for the role of TGAC motif in abiotic stresses namely salinity, osmolarity and heat responsive pathways. TGAC motif was seen to be involved mainly with genes down regulated by heat and salinity stress. This is the first report to best of our knowledge where TGAC has been shown to downregulate heat and salinity stress. We believe that this analysis of w-box motif will be beneficial for designing synthetic plant promoters with defined regulatory elements to modulate gene expression under specific stress conditions.

Deciphering nitric oxide function during seed germination and early seedling development

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Nitric oxide (NO) is a key gasotransmitter involved in plenty of physiological processes during plant growth and with important implications during seed germination and root development processes (1, 2). We have demonstrated that NO acts at early stages of development through a complex signalling network that includes the cellular redox level, mainly by the posttranslational modification of specific proteins by *S*-nitrosylation, leading to the crosstalk with other plant growth regulators (i.e. ABA and auxins) using common molecular players (3, 4). Our current research aims to decipher the role of NO through the regulation of bZIP transcription factors involved in completion of seed maturation to promote seedling establishment (5).

Additionally, we uncovered a regulatory role for NO on primary root growth in *Arabidopsis* at the cellular and genetic levels (1). We found that NO-deficient mutant roots display small root meristems with abnormal divisions and have decreased meristematic cell production. We further show that *PROHIBITIN3* and *NO-ASSOCIATED1* jointly confine WOX5 expression to the quiescent centre, suggesting that NO could play an important role in regulating stem cell decisions, as has been reported in mammals. New findings about NO-related key regulators in stem cell niche associated processes will be presented.

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Comparative analysis of the photochemical activity, plastid differentiation and gene expression in relation to the maturation of Pisum sativum L. seeds with yellow and green cotyledons

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Developing embryos of some angiosperms have photochemically active chloroplasts and are able to produce assimilates, further converted in reserve biopolymers. At the late steps of embryogenesis, seeds undergo degradation of chlorophylls (Chls), the transformation of chloroplast in storage plastids, and enter the dormancy period. However, in many crops, the process of Chls degradation remains incomplete, and residual Chls are present in mature seeds. It results in loss of seed quality and represents an essential challenge for modern agriculture. On the other hand, Chls does not degrade during seed maturation in some species. Then the mature seeds preserve their green color. A classic example is green pea seeds. Its color is determined by a mutation in the genes STAY-GREEN (SGR) encoding proteins involved in Chls catabolism or disassembly of Chls-protein complexes, making Chls available for cleavage. Using a PAM-fluorometric analysis of Chl *a* fluorescence, we demonstrate similar kinetics of photochemical activity in developing yellow and green embryos. The kinetics decline rapidly at the late cotyledon stage, which is accompanied by the decrease of the seed water content. Plastids of subepidermal and parenchymal cells transform into amyloplasts with one or two large starch grains enclosed in plastid envelope. Plastids of epidermal cells with poorly developed thylakoid system and possibly with small lipid droplets transform into structures resembling leucoplasts. Chls in green cotyledons of mature seeds is localized in plastids of subepidermal and parenchymal cells with residues of thylakoid membranes. We also address the expression of genes encoding the enzymes of Chl degradation. The expression of the gene which encode pheophorbide *a* oxygenase differ most significantly. This work was performed by using the equipment of the Research Resource Centre for Molecular and Cell Technologies of Saint-Petersburg State University and supported by grant no. 16-16-00026 from the Russian Science Foundation.

Analysis of Yield and Yield Components of Maize Hybrids in Response to Low Nitrogen

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A field experiment was conducted during 2015 and 2016 rainy season at Tudun Wada, Kano and Shika, Zaria in the Northern Guinea Savanna of Nigeria in order to study the effect of low nitrogen on the yield and yield components of hybrid-maize. The experiment consisted of two nitrogen levels 0 and 120 N kg ha^{-1} as main plot and 8 drought–tolerant maize hybrids (M0826-7, M0926-8, M1026-10, M1026-13, M1124-4, M1124-10, M1227-12 and M1227-14) and 2 controls as sub-plot laid out in a randomized split plot design and replicated three times. Yield and yield components of hybrids were significantly affected by low nitrogen at both locations. Based on these results, application of nitrogen significantly increased the grain yield and yield components of maize-hybrids. However newer hybrids were more tolerant to nitrogen stress and out-yielded the older hybrids. Therefore the recently released maize-hybrids were more adapted to abiotic stresses.
Key words: low nitrogen, maize-hybrids, yield and yield components.

Modification of flour proteome by nitrogen topdressing timing revealed through iTRAQ proteomics

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Both the quality and yield of wheat are largely influenced by nitrogen fertilization. In the present study, field experiments were conducted where wheat plants were topdressed with nitrogen fertilizer at the emergence of the top fifth (TL5), top third (TL3) and top first leaf (TL1). A proteomic study of all the proteins of common wheat flour based on isobaric tag for relative and absolute quantitation (iTRAQ) was first time carried out to obtain a better understanding of the functions of these proteins as well as their responses to N topdressing timing. Collectively, 591 proteins classified into 17 molecular functions were identified. Gluten proteins, particularly gliadins and HMW-GS, increased with delaying N topdressing, which were confirmed by DEPs revealed by iTRAQ. In TL1 vs TL3, the up-regulation of GSP indicated a modification of grain hardness, and the down-regulation of globulin 3, beta-amylase and alpha-amylase inhibitor suggested an alteration in the allergic property. Besides, the combination of iTRAQ and qPCR contributed to the understanding the mechanism of gluten proteins variation in different treatments. Lastly, the identification of gliadin protein (Q94G97) which showed the largest response to N topdressing timing provided a target for the breeding program.iTRAQ-based proteome analysis revealed a precise distinction between different N topdressing timing and provided new perspectives for the application of N regime.

Theme 5: Niche crops

Exploiting variation in Plantago seed polysaccharides for food and human health applications

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Members of the *Plantago* genus are myxospermous, meaning their seeds produce a thick polysaccharide-rich gel called mucilage upon imbibition in water. *Plantago ovata* is the dominant species within the genus to be grown commercially, where its mucilage is used as a thickener and stabiliser in the food industry and, when milled off the seed in a dry form, as the dietary fibre supplement for human health called psyllium. Current agronomic constraints have prevented a stable supply of high-quality *P. ovata* seed for industrial use, which has highlighted the importance of investigating the potential suitability of its near relatives. Therefore, this study aims to investigate the natural variation in the composition and properties of polysaccharides derived from seeds of 14 diverse *Plantago* species, including some native to Australia. To do this, a novel extraction pipeline was developed and used to isolate three distinct fractions from the seed mucilage. Glycan-directed immunolabelling confirmed an abundance of heteroxylan accompanied by minor amounts of pectin in the mucilage, while monosaccharide analysis and oligosaccharide mapping were used to demonstrate intra- and interspecific chemical differences between these polysaccharides. Within each species, an easily isolated fraction obtained through extraction with cold-water was found to be rich in pectin-associated monosaccharides while subsequent hot water- and mechanically-extracted fractions were diminished in pectin but enriched in heteroxylan, with increasing rates of heteroxylan substitution. The level of heteroxylan and the rate of substitution also varies between species. These combined factors may contribute to the relative ease of extraction and may influence the functional properties in their end-uses in food technology and human health.

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Effect of temperature and light quality on regulation and levels of adaptogenic compounds in Rhodiola rosea

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Rhodiola rosea is a perennial flowering plant with a long history as a medicine plant. The plant contain a range of bioactive compounds including salidroside, rosavin, rosarian and rosin. Some of the compounds are characterized as adaptogens, meaning they can increase the body's resistance to various stressors. An increased demand for better pharmaceuticals has stimulated the development of new methods for agricultural as well as in vitro cultivation of medicinal plants.
A new technology, called rhizosecretion of biologically active chemicals, can provide a continuous supply of biologically active compounds over the lifetime of plants. The plants will then be grown under controlled conditions. In order to increase the production of bioactive compounds in *Rhodiola rosea* under these conditions it is therefore hypothesized that the biosynthesis can be upregulated by growing it under specific temperature and light quality treatments. An experiment with different light and temperature regimes was established for optimal accumulation of biologically active compounds. Four different clones of *Rhodiola rosea* were grown under three different light conditions (red, blue and white) combined with two different temperatures (9 and 18 °C) for three weeks. The gene expression of Tyrosine decarboxylase (TyrDC), found to have a key role in the biosynthesis of salidroside, were investigated. In addition, the content of various bioactive compounds were quantified before and after treatment. The results indicate that use if high producing clones is most important for high production and that there is a short-term upregulation during blue light treatment. During the three-week treatment, there was no significant effect of the temperature treatments.

Unlocking the genetic potential of an underutilized legume species, bambara groundnut (Vigna subterranea L.) for a changing climate

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More food and greater nutritional content will be needed to feed a growing world population, with reduced use of fertilizers and agro-chemicals, under the impact of climate change. However, over-dependence on too few crop species could limit our options of how best to respond to food and nutritional security threats. An alternative approach is to revisit the many minor and neglected crops from around the world. Bambara groundnut (BGN) is an African native legume that bears a rich food, nutritional and cultural history for the poor resource-base farmers in sub-Saharan Africa. Although rich in protein, able to fix nitrogen, drought tolerant and with reasonably good disease resistance, BGN is an example of a tropical legume species that is underutilised.
To meet the challenges of achieving food, nutritional and environmental security, there is an urgent need for research to unlock the genetic potential of these important crops. A key component of plant adaptation is the way that reproductive development is influenced by environmental factors. Photoperiod is one of the main factors influencing this development, as it does for flowering date in many crops. However, for Bambara groundnut, the strongest effect is on pod-filling. At the University of Nottingham, through work in climate-controlled glasshouses, we have identified genotypes that are less sensitive to the influence of daylength on pod development. We have also developed new genetic combinations through controlled crossing, alongside tools for efficient molecular breeding and detailed genomic analysis. Here we will present progress so far to develop material specifically adapted to different photoperiod requirements as a way to stabilise yields and improve uptake of this underutilised crop. Many other potential underutilised crops face similar challenges and the lessons learned in BGN could have important implications for the development of new crops to help to achieve global food and nutritional security.

Field evaluation of nine cultivars of Calendula officinalis, a novel oil crop for pharmaceutical and industrial applications

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Calendula officinalis is an annual or short-lived perennial herb native to southern Europe, cultivated at commercial scale for the flower and seed production. Oil extracts from the flowers are used in pharmaceutical and cosmetic applications because of their antibacterial, antiviral, anti-inflammatory, anti-tumor and antioxidant properties. Furthermore, the carotenoids can be used as colorant in food or textiles. The seeds are a source of the conjugated C18:3 fatty acid calendulic acid and can serve as organic compound in several industrial products such as paints, coatings and adhesives. We have tested *C. officinalis* as a dual purpose crop, cultivated for the flowers and for the seeds. This would potentially result in a higher profitability for the farmer and increase the sustainability of the production system.
The flower and seed production of a set of nine cultivars were tested in a field trial in Melle, Belgium. During cultivation, four consecutive flower harvests at time intervals of two weeks were performed, followed by a final seed harvest in September. The overall flower dry matter yield varied between 0.47 and 1.57 ton DM/ha. A yield decline over time was observed for most of the cultivars except Lemon Beauty which did not show any yield decline even after the fourth flower pick. Seed yield varied between 0.8 and 3.53 ton/ha. The flowers were analyzed for carotenoid content and flavonoid content. Clear differences were observed between cultivars in biochemical composition. Carotenoid concentration in flowers varied between 0.5 and 2.0 mg/g DM; polyphenol concentration varied between 470 and 650 µmol /g DM. There was no trend observed over time on biochemical composition. Our data revealed significant differences among the nine cultivars tested regarding flower and seed yield as well for biochemical composition.

Grain amaranths: Developing an ancient crop for modern agricultural systems

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The diversification of crops is an important component of food security for a crowded and warming planet. Modern breeding methods contribute to the improvement of minor crops to increase their competitiveness against major crops that dominate current agriculture. Grain amaranths are ancient crops that were domesticated from weedy amaranths in Central and South America. They have the potential to be crops of future because of their C4 photosynthesis and grains of high nutritional value. Starting from the analysis of domestication and genetic diversity, we initiated a program to adapt amaranth to modern agricultural production systems. A population genomic analysis of grain amaranth species *Amaranthus hypochondriacus*, *A. cruentus* and *A. caudatus* and their wild ancestors shows that they were domesticated independently in three different regions of Central and South America from genetically differentiated populations of the weedy *A. hybridus*. On average, genetic distances between the three domesticates are larger than to the wild ancestors indicating domestication-related selection and local adaptation. Crosses between three grain amaranths result in strong heterosis, which can be exploited for plant breeding. We used whole genome resequencing to model the domestication history of grain amaranths and to identify domestication genes by analysing footprints of selection. QTL mapping and bulk segregant analysis of traits like plant height, seed size and seed color shows that few major genes determine these traits. Our phenotypic and genomic analyses also support the hypothesis that amaranth is an incompletely domesticated species and we propose that this is due to genetic constraints and continued gene flow with their sympatric wild ancestors. A strong genetic influence on traits of agronomic interest, a moderate genome size of 500 Mb, and a short generation times under controlled conditions allow a genomics-based speed breeding strategy to develop grain amaranths into a modern and commercially viable crop.

Unraveling the transcriptome of rooibos (Aspalathus linearis)

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Rooibos (*Aspalathus linearis*) is an endemic South African plant, which is widely used for making herbal tea and cosmetics. It has a wide range of health promoting properties ranging from anti-carcinogenic and anti-inflammatory properties to ”anti-ageing” benefits. These health benefits are mainly associated with the plants diverse range of polyphenols with potent antioxidant activity.
Currently, the species *A. linearis* combines a group of phenotypically diverse biotypes. These growth forms have been categorized according to the plant’s fire survival strategies (seeders or re-sprouters), vegetative and reproductive morphology, and flavonoid composition.
Aspalathin, a C-glucosyl dihydrochalcone, is the main polyphenol found in rooibos and is unique to rooibos. This polyphenol may be involved in the plants unique health promoting properties.
The genetic background of rooibos in general, and the biosynthetic pathways of the medicinally active flavonoids in particular are unknown.
The transcriptomes of seven diverse rooibos ecotypes, selected based on their unique polyphenolic profiles were selected for RNA sequencing and their transcriptomes were assembled *de novo.* The assembled transfrags were annotated computationally using standard functional analyses procedures (e.g. BLAST, pfam). The transcriptomes were compared to each other to identify candidate genes, and functional annotations were used to shortlist gene candidates that may be involved in polyphenol biosynthesis.
This knowledge may promote targeted plant selection and breeding, as well as the development of in-vitro gene expression systems for the commercialization of selected compounds.

Floral induction in short-day and long-day Chenopodium ficifolium, a close relative of Chenopodium quinoa

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Chenopodium ficifolium is a diploid species closely related to the donor of the subgenome B of tetraploid quinoa, an important crop of South America. Most *C. ficifolium* accessions are long-day plants, which accelerate flowering under long photoperiod, but a short-day accession was also discovered in a remote area of China. We estimated the expression patterns of the genes from the *FLOWERING LOCUS T*/*TERMINAL FLOWER1* (*FT*/*TFL1*) family in long-day and short-day *C. ficifolium* plants under the contrasting photoperiods. The *CfFTL1* gene, a homolog of the floral activators *BvFT2* in sugar beet and *CrFTL1* in *Chenopodium rubrum*, was rhythmically expressed with peaks at night. It reached much higher expression under short days in both the short-day and long-day *C. ficifolium* genotypes. These results are surprising, because they are consistent with the function of the *CfFTL1* gene as the activator of flowering in short-day *C. ficifolium*, but contradict this function in long-day *C. ficifolium*. As we have not found any other *FTL1* paralog in the transcriptome of *C. ficifolium*, we conclude that another gene acts as the floral inducer in long-day *C. ficifolium.* The floral inhibitor in sugar beet *BvFT1*, has two homologs in *C. ficifolium.* One of them is substantially deleted and the second shows very low expression in *C. ficifolium*, which precludes its function in floral induction. We speculate that *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*), may mediate floral induction in long-day *C. ficifolium.*

Rhodiola rosea - growth and production - effects of abiotic and biotic factors

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***Rhodiola rosea* - growth and production – effects of abiotic and biotic factors**

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Rhodiola rosea is a well-known herbal medicinal plant, valued for highly active secondary metabolites. It is growing wild in most parts of Norway and mountainous areas in a number of countries. Some of the most important metabolites are believed to be salidroside, cinnamyl alcohol, glycosides (rosine, rosavine, rosarine), flavonoids (rhodionin, rhodiosin, rhodiolin) and terpenes (Galambosi 1999). In Norway, germplasm collections of *R. rosea* are maintained by NIBIO; at Apelsvoll in southern Norway, consisting of 97 different clones. The ranges in content of secondary metabolites in the collection are for rosavin 2.90-85.95 mg g-1, salidroside 0.03-12.85 mg g-1, rosin 0.08-4.75 mg g-1, tyrosol 0.04-2.15 mg g-1 and cinnamyl alcohol 0.02-1.18 mg g-1. Clones selected from the collection has throughout been studied for different aspects affecting plant growth and production of secondary metabolites. We have looked into cultivation requirements of the plant like water requirement, effects of nutrient levels (N and K) and soil types. Postharvest treatment from washing, cutting, drying and differences in the plant parts. Finally we will in this presentation also present results on requirements for dormancy release and the clonal differences and also how use of primers may affect production of secondary metabolites.

Theme 6: Plant Microbiome

TaWRKYrA-Transcription Factor Plays a Central Role in Tolerance of Winter Wheat to Aphid Infestation under Different Nitrogen Conditions

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Limiting nitrogen input in wheat increases aphid tolerance, but reduces yield. Although direct damage caused by phloem-feeding aphids such as *Sitobion avenae* is minimal, their propensity to vector numerous viruses means they are one of the most damaging pests of wheat. Changing agricultural conditions are leading to insect pests becoming a more serious threat to sustainable crop production and therefore understanding the molecular basis of endogenous tolerance to aphid infestation will help mitigate shortfalls in global crop yield. WRKY transcription factors that regulate gene expression play important roles in the response to biotic and abiotic stresses. Previous work showed *TaWRKYrA*, a novel wheat gene which showed consistent, large changes in gene expression under aphid and nitrogen stress. To establish a role for WRKYrA in the stress response TILLING lines with mutations to the *WRKYrA* gene grown under different nitrogen concentration and were also infested with aphids. Aphid fecundity on one mutant line showed no difference between high and low nitrogen levels, suggesting potentially that WRKYrA plays a role in the link between nitrogen stress and aphid tolerance in wild type plants. Protein-DNA interaction assays were used to show binding of the Wild Type WRKYrA protein to W-box elements (*TaPR1-1* promoter and synthetic) and that DNA binding is disrupted by the mutation found in the TILLING line. The regulation of PR1-1 gene expression is key to the activation of plant defence response, the present work demonstrates that TaWRKYrA may regulate this important response through binding to the W-box element in the promoter. Furthermore, the low nitrogen conditions may prime the defence of wheat against insect attack as a result of cross-talk via a regulatory network of WRKY transcriptions factors. These results provide new knowledge and valuable resources that should be useful in the effort to produce crops with reduced nutrient input.

Ethylene induced plant stress tolerance by Enterobacter sp. SA187 is mediated by 2-keto-4-methylthiobutyric acid production

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In a world with a rapidly growing population, clear-cut solutions should be found to increase crop yield in order to provide enough food for everyone. However, abiotic stresses, such as salinity or drought, are among the most limiting factors to achieve this goal. One solution might come from the soil or more precisely from studying the plant interacting microbiota communities. Indeed rhizophere and endosphere microbial communities are distinct from the surrounding soil, and these specific communities are highly contributing to the health and growth of plants, by increasing mineral availability or plant resistance towards pathogens for example.
Here we show that a beneficial plant microbe induces plant tolerance to multiple stresses. From a strain collection isolated from the desert plant *Indigofera argentea*, we could identify the desert endophytic bacterium *Enterobacter sp. SA187*. SA187 increases crop yield of wheat, barley and alfalafa in field tests while it induces *Arabidopsis thaliana* tolerance to drought, salinity and heat stress *in vitro*. We show that SA187 acts by inducing the plant ethylene signaling pathway. Interestingly, although SA187 does not produce ethylene as such, association of SA187 with plants induces the production of bacterial 2-keto-4-"methylthiobutyric acid (KMBA) that is converted into ethylene by the host.
Our findings demonstrate a novel beneficial dialogue between microbe and plant at a molecular level that induces plant stress tolerance. Taken together, this study shows how a microbe of a given plant microbiota community can help the host plant to enhance tolerance to abiotic stress thereby increasing yield and biomass.

Three-way interaction in oilseed rape

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Plants in natural conditions have to interact with other inhabitants of their environment forming both mutualistic and parasitic interactions. Besides beneficial microorganisms, plants combat a wide range of phytopathogens and pests. Numerous data have been published on individual plant-microbe or plant-arthropod interactions; however, the investigations dealing with combined interactions are less frequent. Our preceded research has been devoted to the interaction of oilseed rape with an economically important fungal pathogen *Leptosphaeria maculans*. We clearly showed that resistance to this pathogen is mediated by salicylic acid signaling in combination with ethylene. This was confirmed both by expression study of SA-associated and ET-associated genes, hormone quantification in infected tissues, as well as pharmacological experiments. We ascribe this unusual cooperation of SA and ET signaling to the hemibiotrophic nature of *L.* maculans. Our present project is focused on the interaction of the three counterparts: plant (oilseed rape) – microorganism (*L. maculans*) – arthropod (*Plutella xylostella*), forming a complicated dynamic network of relations in which each can modulate plant resistance or susceptibility to the others. In addition, resistance inducer of natural origin efficient against *L. maculans* will be involved in the study. The suggested research should reveal, whether the preinoculation of oilseed rape with *L. maculans* influences following insect infestation, how does insect infestation change the susceptibility/resistance of oilseed rape to *L. maculans*, what is the impact of induced resistance against *L. maculans* on susceptibility to insect, and which signalling pathways participate in defence responses of oilseed rape during *L. maculans* infection and insect infestation.
The work was supported by a grant from Ministry of Education, Youth and Sports of the Czech Republic INTER-COST ELTC17013 and is solved in a framework of COST Action FA1405.

The apple fruit microbiome: influence of orchard management, variety and storage time

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Microbial spoilage in commercial apple storage facilities can lead to food loss of up to 30% during the storage period. While some of the causal pathogens such as *Penicillium* spp. and *Botrytis* spp. are well characterized, others such as *Neofabraea* spp. are less known due to difficulties in culturing. Metagenomics allows the screening of apples for the abundance and dynamics of various pathogens or the microbiome as a whole in a culture independent way. Here we aimed to characterize the microbiome on the apple fruit and to elucidate the influence of the apple variety, orchard management practices and the dynamics of the microbial composition during the entire storage period. The results allow for description of infection levels of different pathogens at harvest and, to some extent, the prediction of post storage symptom development. Additionally, information about the community composition allows for identification of the main factors driving the composition of the microbiome, the change in diversity during the storage period and the identification of beneficial microorganisms that may eventually be applied as biocontrol agents in the future. The diversity of the microbiome was shown to increase during the storage period while variables such as variety and orchard management showed a significant effect on the abundance of various storage pathogens. The results can be applied in the development and improvement of infection models, educate breeders on how the host genotype interacts with the microbial community and inform researches on how microbial communities change over time. Therefore metagenomic sequencing may provide a valuable tool to inform practitioners and researchers on disease risks and prevent post-harvest losses in the near future.

Barley response to Phytophthora from a proteomics perspective

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Genus *Phytophthora* represents a world-wide spread pathogen with more than hundred recognized species and Its devastating effect on plants has a serious economic and ecological impact. Recent studies found that *P. palmivora*, a well-known plague of tropical plants, can infect a non-natural host barley (*Hordeum vulgare*). This presents an interesting opportunity to employ a model crop plant and characterize molecular changes induced by *P. palmivora.* Barley seeds were surface-sterilized, germinated on a filter paper and transferred onto a liquid medium containing *P. palmivora* and components suitable for elicitins production. After 24 hours, barley roots were collected and analyzed by a gel-free LC-MS and GC-MS for proteomics and metabolomics, respectively. The presence of *P. palmivora* in treated samples was confirmed via a targeted SRM-based analysis of its proteins (even though there were no visible symptoms on seedlings phenotype) and PCA analyses of proteome/metabolome profiles clearly separated control plants from those incubated in the presence of *Phytophthora*. In total, we identified over 1,400 barley protein families and more than 140 showed a significant change in response to *P. palmivora* in three biological replicates. These candidates include proteins associated with the initial infection event, including jasmonate-responsive factors and mediators of ROS signalling. Further, alterations in energetic metabolism correlate with the patterns found in metabolome profiling.

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Role and mechanisms of cell wall remodeling in Populus root cells for the establishment of ectomycorrhizal symbiosis

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Ectomycorrhizal (ECM) fungi invade the apoplastic space around and in between root epidermis and cortex cells to establish a symbiotic interaction and exchange nutrients with tree roots. This requires an extensive cell wall modification in both the plant and fungal partner (1), including the loosening or degradation of the middle lamella that connects adjacent root cells. The mechanisms behind this cell wall alteration, from cell adhesion and physical barrier towards an effective nutrient exchange passage, are largely unknown. The middle lamella is rich in pectin, a polysaccharide with a sticking consequence on cell loosening and stiffening properties that needs to be modified for the plant to accommodate the fungus in between of its root cells. ECM fungi have a reduced set of cell wall degrading enzymes in their genome compared to their saprotrophic ancestors (2) and thus, the contribution of the fungal partner to cell wall modification needs investigation. Using a model *Laccaria bicolor*/ poplar *in vitro* interaction system, we investigate pectin modification in ECM and the contribution of the fungal partner to this process. Our strategy is to characterize the ECM cell wall based on pectin de-methyl-esterification patterns and to link the observations to the key genetic regulators namely the pectin methyl-esterases (PMEs), the first enzymes acting on pectin (3). Our result from immuno-localization of pectin epitopes suggests that pectin de-methyl-esterification is an important event of cell wall remodeling during ECM development. The data is supported by further investigation that transcripts and protein activity of PMEs are increasing during ECM formation. An early and high induction of a *L. bicolor* PME suggests that *L. bicolor* contributes to the pectin de-methyl-esterification process. *L. bicolor* transgenic lines with altered level PME levels are under construction. Examining their capacity to form ECM will reveal the importance of fungal PMEs for ECM formation.

An Investigation of the Bioactivity of Cyanobacterial Exometabolites in Plant Stress Tolerance

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Cyanobacteria are part of the plant growth-promoting rhizobacteria consortia that promote plant growth, while also acting as putative protectors against biotic and abiotic stress in plants. The ability of cyanobacteria to alleviate plant stress is attributed to a diverse range of exometabolites. This project investigates the bioactivity of *Nostoc* spp. exometabolites in essential Irish crops (winter and spring varieties of barley and wheat) in response to applied salinity and heat stress. Previous work observed that the extracellular medium of *Nostoc muscorum* - termed conditioned medium (CM) - inhibits stress-induced programmed cell death (PCD) in *Arabidopsis thaliana* root hairs. PCD is a genetically controlled and conserved cell death mechanism in the plant life cycle; plant cells often undergo PCD to mitigate abiotic and biotic stress. This project uses a novel *in vivo* Root Hair Assay (RHA) to enumerate the stress response of plants; results are expressed as levels of cell viability, and levels of total cell death (PCD and necrosis). Additionally, the RHA is used to identify the bioactive compound(s) in *Nostoc* CM and stress-tolerant cereal varieties. To this end, seedlings are pre-treated with *Nostoc* CM fractions, abiotic or biotic stress applied, and ensuing cell viability and death levels enumerated using the RHA. Further characterisation of *Nostoc* CM bioactivity is obtained by measuring plant biochemical stress marker activity and using HPLC/GC to identify compounds of interest. A hydroponics system streamlines the handling of seedlings and stress treatments and allows the high-throughput analysis of many crop varieties simultaneously. Preliminary results indicate proline is one compound that mitigates stress in cereals. The project findings have potential to identify stress-mitigating *Nostoc* spp. exometabolites and develop an early, inter-species method for identifying crop varieties exhibiting enhanced stress tolerance.

A smart method for monitoring root-rhizosphere activity in field-grown plants

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The root electrical capacitance method is based on correlation between root system size and its electrical capacitance (C_R), measured between a ground electrode embedded in the soil and a plant electrode attached to the stem-base. The technique is suitable for assessing root growth and root–rhizosphere activity *in situ*, but soil water content (SWC) strongly influences the measurement results, usually precluding the field application. We aimed to adapt the method for field monitoring by evaluating the effect of SWC on root capacitance to ensure the comparability of C_R detected at different SWC. First a pot experiment was conducted with soybean to establish C_R–SWC functions for the field soil. Ontogenetic changes in root activity were monitored under field conditions by simultaneously measuring C_R and SWC around the roots. The C_R values were normalized using SWC data and experimental C_R–SWC function to obtain C_R*, the comparable indicator of root activity. Leguminous soybean is a typical host for root-associated microorganisms, including arbuscular mycorrhizal fungi (AMF), which can influence plant development and water relations. Therefore, the effect of AMF inoculation on the C_R* and biomass of field-grown soybean was investigated. The pot trial showed an exponential increase in C_R with SWC. The root activity (C_R*) of field-grown soybean continuously increased until flowering, steadily but slowly declined during pod filling, then sharply decreased during seed ripening. This was consistent with data obtained with other methods. AMF inoculation resulted in significantly higher C_R* during the late vegetative and early flowering stages, when destructive sampling concurrently showed higher shoot biomass. The results demonstrated that the root capacitance method could be useful for time course studies on root–rhizosphere activity under field conditions, and for detecting the functional aspects of plant–microbiome interactions. Consequently, it is potentially beneficial for plant physiology and agricultural research.

GRAPE BERRY AND BOTRYTIS CINEREA PARAMETERS DURING NOBLE ROT OF TOKAJ'S GRAPE SAMPLES

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Botrytized (”aszú”) wines are produced from ripe grape berries subjected to a unique interaction with the filamentous fungus *Botrytis cinerea*. This special interaction between the berries and *Botrytis* is called noble rot. During noble rot the berries gradually dehydrate and shrivel and the increasing concentration of cellular constituents results in an inhibition of fungal growth. The process of noble rot takes several days and the berries go through four distinct phenotypic stages.
We have developed a high-throughput technique to assess the ratio of living and dead plant cells in the berries and some crucial physical parameters for each phenotypic stage. The level of *Botrytis cinerea* biomass was also simultaneously evaluated by ELISA. Our results enable us to gain insight into the complex relationship between the grape berries and the fungus during noble rot.
This research was supported by a grant of the Economic Development and Innovation Operational Programme (GINOP 2.3.2-15-2016-00061).

NF-Y transcription factors complex: identity and function during defence

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Transcriptional reprogramming plays a significant role in the defense of plants against pathogen infection. This defense response involves a complex and highly sophisticated regulatory circuitry in which transcription factors (TFs) play a key role in determining the specificity of the response to different pathogens. Some TFs fine-tune gene expression by binding to their promoter as individual protein, however combinatorial regulation can also occur by variations in the composition of multi-subunit TFs. The eukaryotic NF-Y is a highly conserved heterotrimeric TFs composed by three subunits, NF-YA, NF-YB, NF-YC, which directly bind CCAAT boxes in target gene promoters and act as positive or negative regulators of transcription. In Arabidopsis, a multi-gene family encodes each subunit of the complex, having 10 NF-YA, 10 NF-YB and 10 NF-YC which can hypothetically combine in 1000 unique complexes and are differentially expressed during plant stress responses. To investigate functional NF-Y complexes a putative trimer formed by NF-YA2, NF-YB2 and NF-YC2 subunits was identified, based on a combination of gene expression levels and knock-out mutants availability. We have shown that NF-YA2, NF-YB2 and NF-YC2 mutants displays significantly altered susceptibility to *Botrytis cinerea* suggesting a key role in the defence response. Furthermore, BiFC assays in *N. benthamiana* revealed that NF-YB2 and NF-YC2 are able to heterodimerise in planta, and this interaction was confirmed in Arabidopsis expressing tagged NF-YC2 and NF-YB2. We are now investigating whether this NF-YB2/C2 dimer binds the NF-YA2 subunit, and if so, the regulatory function of the resulting NF-Y trimer during defence against *B. cinerea*.

Volatile emissions from Streptomyces species have both positive and negative effects on Arabidopsis; a study of hormone homeostasis and growth

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Common *Streptomyces* bacteria are integral organisms for a healthy soil biome. The organisms within are interconnected through co-evolution. Previous studies have shown that emissions of volatiles from these bacteria initiate a broad range of plant responses. In this study we show a clear effect of bacterial lipid volatiles on plant growth. The volatiles emitted by *Streptomyces coelicolor* will at high concentrations strongly inhibit plant growth, however, below the inhibitory threshold, *S. coelicolor* volatiles cause *Arabidopsis* seedlings to increase in biomass through the expansion of both roots and shoots. RNAseq transcript analysis identified significant induction of auxin response genes. Interestingly, this is accomplished without induction of classic auxin response genes (ARFs/IAAs). Instead, we have identified unidirectional induction of a family of F-box genes which has been shown to suppress cytokinin responses thereby altering auxin/cytokinin homeostasis. Plant mutational studies verified these responses and demonstrated that both cytokinin and auxin networks needed to be intact for growth increase to occur. Furthermore, we have identified a compound emitted by *S. coelicolor* that when applied to *Arabidopsis* seedlings can account for most of the plant volatile responses observed. Our current understanding spans large relatively uncharacterized gene families, cell wall loosening and hormonal homeostasis.

Defence-related metabolic reprogramming in Sorghum bicolor in response to Burkholderia andropogonis infection

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Metabolic alterations of sorghum cultivars responding to *Burkholderia andropogonis*, the causal agent of bacterial leaf stripe disease, were investigated. Plants were spray-inoculated with diluted bacterial suspensions at the 4-leaves growth stage and the infection monitored over time: 0, 3, 5, 7 and 9 d.p.i.. Non-infected plants were used as negative controls. Intracellular metabolites were extracted with 80% methanol-water. The extracts were analysed on an UHPLC system coupled to high-definition mass spectrometry and the acquired data were processed and analysed. Chemometric models indicated time- and cultivar-related metabolic changes that reflect defence responses to the infection. Metabolic pathway and correlation-based network analyses revealed a functional metabolic web, containing defence-related molecular cues to counterattack the pathogen's invasion. Components of this network are altered-metabolites from a range of interconnected metabolic pathways with the phenylpropanoid/flavonoid pathways being the central hub of the web. One of the key features of this altered metabolism was the accumulation of an array of phenolic compounds, particularly apigenin and related derivatives and conjugates that exhibit antimicrobial properties to halt pathogen proliferation. Interestingly, the pathway to the 3-deoxyanthocynidin phytoalexins, apigeninidin and luteolinidin, was not activated. Moreover, chemometric analysis indicated the di- and tri-hydroxy-octadecenoic acids as biomarkers in the global defense response. Unravelling key characteristics of the biochemical mechanism underlying the sorghum–*B. andropogonis* interactions provided valuable insights with potential applications in crop protection. Furthermore, the study contributes to ongoing efforts towards a comprehensive understanding of the regulation of plant metabolism under biotic stress.

Differential expression of stress related genes in Hypericum perforatum cells challenged with Agrobacterium tumefaciens

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St Johns wort (*Hypericum perforatum* L.) is an important medicinal plant well known for its use in the treatment for mild to moderate depression. This herb is home for several classes of unique secondary metabolites. In spite of the recent advances in the understanding of biosynthetic pathways of *H. perforatum*, genetic engineering remain difficult mainly due to its recalcitrance against *Agrobacterium tumefaciens*- mediated transformation. Previously we have shown that *H. perforatum* recalcitrance against *A. tumefaciens* is due to the activation of plant defense response upon co-cultivation. In the present study, genes participating in *H. perforatum* plant defense against *A. tumefaciens* are analyzed. The time-course expression levels of phenolic oxidative coupling protein (*HYP1*), leucin rich repeats (*LRR*), 1-deoxy-D-xylulose-5-phosphate synthase (*DXS*), stomatin/ prohibitin/ flotillin/ HflK/C (*SPFH*), xylanase inhibitor I (*TAX1*), phenylalanine ammonia-lyase (*PAL*), chalcone synthase (*CHS*) and benzophenone synthase (*BPS*) in *H. perforatum* cells after *A. tumefaciens* treatment were tested. Semi quantitative RT-PCR analysis of total RNA isolated from the cells after different time points of *A. tumefaciens* inoculation revealed the upregulation of *LRR, TAX1, SPFH* and *HYP1* gene expression up to 24 h, whereas the *DXS* gene expression was upregulated for the initial 12 h and declined thereafter. Although the expression of *PAL* and *BPS* genes encoding key enzymes of the phenylpropanoid pathway were upregulated, *CHS* was downregulated after treatment.

The Christmas tree rhizosphere microbial community

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*Abies nordmanniana*is a major Christmas tree species in Europe, but production is hampered by prolonged growth and early root development is considered important for plant development by the growers. The rhizosphere represents a diverse source of plant growth promoting bacteria that may influence *A. nordmanniana* root development; however, the *A. nordmanniana* rhizosphere community remains unknown. The aims of this study were to characterize bacterial communities associated with roots of *A. nordmanniana* at nursery stage and to isolate rhizosphere bacteria able to regulate plant growth and development. The composition of the bacterial communities from bulk soil and rhizosphere of *A. nordmanniana* at different sampling sites was compared by 16S rRNA gene sequencing. There were clear differences in community composition between rhizosphere and bulk soil, and a significant effect of the sampling site on both rhizosphere and bulk soil communities. *Proteobacteria, Actinobacteria*, *Acidobacteria*, and *Bacteriodetes* dominated in the rhizosphere independently of the site. The same phyla dominated in bulk soil, but the relative abundance of *Acidobacteria* was higher while that of *Proteobacteria* was lower than in rhizosphere. Among bacterial strains isolated from the rhizosphere, we identified strains able to improve seed germination as well as increase root development and branching when seeds were bioprimed with bacterial suspensions. A total of 22 bacterial isolates were tested, seed germination percentage improved up to 40% when the seeds were bioprimed with strain 040, furthermore, a bigger development of secondary roots and root hair was also observed. Other strains such as 037 and 050 also improved seed germination, both up to 35%. The capability of these bacterial isolates to produce phytohormones was evaluated, these isolates shown capability to produce auxins (IAA) in pure culture. These results suggest that plant growth promoting bacteria is associated with the rhizosphere of *A. normanniana* and could enhance their growth.

Understanding the Rhizosheath: Opportunities for Manipulating the Soil Root Interface

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Temperate cereals produce rhizosheaths, which are discrete structure at the root-soil interface consisting of soil particles, root hairs, microorganisms and biological mucilage. The trait was first noted on desert species over 100 years ago and thought to be limited to grass species in the Poales order until recently, when its presence was demonstrated in many orders of flowering plants [Brown et al. (2017) Plant Soil 418:115–128]. Rhizosheath weight can be screened easily and rapidly and has been shown to be related to the ability of plants to tolerate abiotic stresses.
We have demonstrated genotypic variation in this trait in a range of crop species, specifically barley. A range of QTLs and candidate genes associated with rhizosheath formation were identified using a population of elite genotypes [George et al. (2014) New Phytologist 203: 195–205]. We will present a validation of these associations in other populations of barley including recombinant chromosome substitution lines and a population of landrace barleys from the highlands and islands of Scotland.

We have also investigated the role of root hair length and mucilage production on rhizosheath formation. We have generated novel insight into the physical conditions at the root soil interface using high resolution synchrotron X-ray tomography [Koebernick et al. (2017) New Phytologist 216: 124–135] and recently performed experiments which will link modifications in the root-soil interface to changes in the microbiome.
Understanding the biophysical nature of the rhizosheath is the first step to engineering the root soil interface and improving our ability to manipulate the function of the rhizosphere. Breeding cereal genotypes for beneficial rhizosheath characteristics is achievable and we have identified potential to do this in many other crop species. Enhancing this trait could contribute to agricultural sustainability in future environments where nutrient availability and water relations may be compromised.

Isolation and characterization of endophytes from Vitis vinifera

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Endophytes are microorganisms that colonize the plant's internal tissues and can have beneficial growth promoting effects for the host plant. Endophytic bacteria and microscopic fungi have also been isolated from grapevine (*Vitis vinifera*), however, the complete structure as well as the role of endophytes in grapevine have not been thoroughly studied. The aim of this work was to isolate endophytes from leaves and green shoots from 4 grapevine varieties. Identification of isolated bacteria was performed by MADI-TOF mass spectrometry. Most of the isolated bacterial endophytes were from the genus *Pseudomas*, *Bacillus*, *Kocuria*, *Pantotea*, *Rhodococcus* and *Micrococcus*. The isolated microorganisms have been tested for host plant properties (production of antioxidants and siderophores, or minerals phosphate solubilization and nitrogen fixation). The isolated microbes have proved to have beneficial effects on the plant such as growth promotion or resistance to biotic and abiotic stress.

Plant responses as the behavior switchers of the phytopathogenic bacterium Pectobacterium atrosepticum inside the host body

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Plant soft rots caused by *Pectobacterium* species are among the most devastating plant diseases. It is recognized that *Pectobacterium* species colonize plants because of the pathogen's "brute force" expressed in hyperproduction of the plant cell wall degrading enzymes. Herewith, the responses of plants were not considered as the factors determining the strategy of host colonization by pectobacteria. By comprehensive analysis of plant-*Pectobacterium atrosepticum* (*Pba*) pathosystem formation, we have demonstrated that the pathogen-induced plant reactions drive the microbial behavior at both latent and acute stages of infection.
The latent stage is associated with colonization by *Pba* of the primary xylem vessels, in which bacterial emboli are formed (specific "multicellular" biofilm-like structures). The assemblage of these structures is preceded by a specific plant response related to the primary vessel wall modification that leads to the release of one of the pectic polysaccharides – rhamnogalacturonan (RG) – into the vessel lumen. This polymer forms an extracellular matrix necessary for the association of separate microbial cells in a holistic structure. Plant cell wall proteins involved in RG release into the lumen were predicted based on RNA-Seq analysis. Additionally, the reconstruction of the vessel cell walls during the infection was shown to be associated with the accumulation of reactive oxygen species that presumably mediate plant susceptible response.
By comparing *Pba* transcriptomes at asymptomatic and symptomatic stages of host colonization, coronafacic acid (cfa)-related genes were predicted to switch pathogen's behavior in plants. The analysis of cfa-devective *Pba* mutant has confirmed that cfa is involved in up-regulation of plant jasmonate-mediated pathway, which is a prerequisite for a transition of *Pba* to the necrotrophic stage of interaction with plants. Taken together, we have revealed various plant responses that determine stealth and brute force modes of action of *Pba*. This study was supported by RSF (15-14-10022) and grant MK-2191.2017.4.

Does tree phenology drive the ectomycorrhizal fungal composition - a case study on silver fir (Abies alba Mill.)

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Interactions between aboveground and belowground components of terrestrial ecosystems are highly important in driving ecosystem processes. Processes included in above-belowground communication require very efficient co-ordination of resource allocation and signalling between above- and belowground plant parts. We collected 24 soil samples with fine root from naturally regenerated silver fir plot. Four different groups were selected based on phenological data of silver fir trees (project LIFEGENMON). For identification of root associated fungi, ITS2 region of fungal rRNA gene was amplified and sequenced on Illumina MiSeq platform. For diversity estimation, Shannon-Wiener and Simpson indexes were used. Differences in fungal community were tested using PERMANOVA on Bray-Curtis distances. Analysed data showed statistically significant differences in fungal communities of silver fir between groups of early and late flushing saplings. Also, alpha diversity indexes differed between mentioned groups, as there was an indication on higher OTUs diversity in fungal community of silver fir of phenologically early young trees. Other groups have showed no statistically significant differences in fungal communities, although there were some differences in alpha diversity as well as in fungal composition between early flushing adult trees compared to late flushing adult trees. There is an increasing body of literature, showing that fungal communities impact on plant growth and vigour, as they influence the quality, direction and flow of energy and nutrients between plants and fungi. Moreover, many of the observed plant growth responses may be also regulated in part by alterations in fungi endogenous phytohormone levels. However, further researches are needed for better understanding of possible fungal impact on plant host physiological and phenological processes and vice versa.

RNA-Seq analysis of Pectobacterium atrosepticum-infected plants revealed potential susceptibility/resistance-related traits of the host

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Plant soft rot is a pectobacteria-caused disease that greatly impacts crop production worldwide. Pectobacteria are usually considered as brute force pathogens that degrade plant cell walls. Nevertheless, these phytopathogens may presumably apply much more sophisticated strategies during interaction with the host, since the genomes of pectobacteria contain genes that encode effector proteins as well as phytotoxins – the weapons of ”stealth” biotrophic pathogens that normally induce specific plant susceptible responses in order to colonize macroorganisms. Unfortunately, plant responses to pectobacteria including those related to susceptibility/resistance to these pathogens remain unknown.
Here we compared the transcriptional profiles of mock and *Pectobacterium atrosepticum* (*Pba*)-infected plants in order to describe general picture of the host physiology in the course of soft rot development. RNA-seq revealed over 8 thousand genes expressed differentially (DEGs) in infected and control plants. Using various databases and software (KEGG, BioCyc, MapMan) as well as custom scripts, DEGs were classified into functional categories. Automatic classification was additionally complemented by manual gene categorization in order to interpret transcriptomic data in terms of general physiology.
Gene categories related to some hormonal systems (jasmonic acid- (JA) and ethylene-mediated), cell wall modification, secondary metabolism, respiration, protein and starch degradation were up-regulated during the infection. In turn, processes that control cell cycle, plant development, auxin- and gibberellin-mediated responses, photosynthesis were repressed after pathogen invasion. Gene expression changes of several functional categories are discussed in terms of susceptibility/resistance of the plants. For example, the induction of JA-related genes represents plant susceptible response, since 1) the coronafacic acid-deficient mutant unable to induce JA-pathway is avirulent, and 2) pre-infectional treatment of plants with JA did not reduce lesion development caused by *Pba*.
This study was supported by Russian Scientific Foundation #15-14-10022.

Attack on potatoes - the armoury of Colletotrichum coccodes

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Colletotrichum coccodes influences plant health in many ways[1] such as leaf[2] and stem health, tuber development, crop yield[3] and shelf life[4] of potatoes. Additionally, the phytopathogen causes anthracnose symptoms on many different host species, mainly Solanaceae. The plant pathogen´s armoury includes morphological adaptations (e.g. appressoria for intrusion)[56] as well as biochemical alignments (e.g. cellulases and pectic enzymes)[7] to facilitate infection and colonisation of host plants. We investigated morphological elements by different microscopic methods (inter alia SEM and TEM). Extracellular cellulases and pectic enzymes were determined by culture method and plate assays (spot test). Identification of the respective enzymes will be done with MALDI and further biochemical methods.

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Rhizobacteria from Irish Potato Soils and Their Contribution to Plant Growth-Promotion and Genetic Transformation in Solanum tuberosum cv. 'Golden Wonder' via Volatile Organic Compounds

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Isolation of rhizobacteria from potato soils across the Southern coast of Ireland in both organic and conventional cropping systems yielded 120 bacterial isolates. Of these 120 isolates, 50 were selected for identification via 16S rDNA sequencing and further biochemical analysis to determine their plant growth-promoting activity. Isolates from genera commonly known to promote plant growth such as *Bacillus*, *Pseudomonas* and *Serratia* were screened for the production of hydrogen cyanide, ammonia, siderophores and phosphate solubilisation activity. Particular attention was payed to the ability of these isolates to produce volatile organic compounds (VOC's) which can contribute to plant growth-promotion via alterations in plant gene expression related to defence and growth, and/or by directly inhibiting the growth of phytopathogens such as *Rhizoctonia solani*. Solid phase micro-extraction GC/MS analysis has revealed that a number of well-reported VOC's such as 2, 3-butanediol, 2-nonanone and 2, 5-dimethylpyrazine are also present in PGPR isolates from this study. Six isolates known to efficiently inhibit growth of *R. solani* were selected to determine their VOC-effect on the growth of *Solanum tuberosum* cv. 'Golden Wonder' in a passively-ventilated growth system. A number of pure synthetic VOC's produced by these PGPR will also be tested for their activity on growth promotion. Preliminary data suggests that plant exposure to these VOC's may also induce an increase in the efficiency of transient genetic transformation by *Agrobacterium tumefaciens.* Whole-transcriptome analysis will be employed to potentially identify any transcriptomic modulation of gene expression which may have an effect on growth and/or transient genetic transformation. With a burgeoning worldwide population reaching ~9 billion by 2050, the plant microbiome holds great potential as a sustainable solution to the growing demand for food.

Rice WRKY11 enhances pathogen defense and drought tolerance

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Plants are frequently subjected to abiotic and biotic stresses, and WRKY proteins play a pivotal role in the response to such stress. *Os*WRKY11 is induced by pathogens, drought, and heat, suggesting a function in biotic and abiotic stress responses. This study identified *Os*WRKY11, a member of WRKY group IIc. It is a transcriptional activator that localized to the nucleus. Ectopic expression of *OsWRKY11* resulted in enhanced resistance to a bacterial pathogen, *Xanthomonas oryzae* pv. *oryzae*; resistance was compromised in transgenic lines under-expressing *OsWRKY11*. Ectopic expression of *OsWRKY11* resulted in constitutive expression of defense-associated genes, whereas knock-down (kd) of *OsWRKY11* reduced expression of defense-associated genes during pathogen attack, suggesting that *OsWRKY11* activates defense responses. *Os*WRKY11 bound directly to the promoter of *chitinase 2*, a gene associated with defense, and activated its transcription. In addition, ectopic expression of *OsWRKY11* enhanced tolerance to drought stress and induced constitutive expression of drought-responsive genes. Induction of drought-responsive genes was compromised in *OsWRKY11*-kd plants. *Os*WRKY11 also bound directly to the promoter of a drought-responsive gene, *Rab21*, activating its transcription. In addition, *Os*WRKY11 protein levels were controlled by the ubiquitin-proteasome system. *Os*WRKY11 integrates plant responses to pathogens and abiotic stresses by positively modulating the expression of biotic and abiotic stress-related genes.

Development of mlo resistance in wheat against powdery mildew

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Powdery mildew is a severe disease in wheat, usually controlled by fungicides. In barley, durable *mlo* resistance exists, based on non-functionality of the *Mlo* gene. In wheat, *mlo* resistance requires that all of the three *Mlo* homoeologs are mutated. As a model to analyse the effects of mutagenesis in the *Mlo* genes of wheat, we developed *mlo* resistant tetraploid wheat.
To screen for *Mlo* mutations, we used a TILLING population developed in tetraploid (AABB) wheat cv. 'Kronos' for which the captured exome sequence of >1,500 lines were re-sequenced [1]. To obtain plants with *Mlo* mutations, we *in silico* screened the resequencing database (http://www.wheat-tilling.com/).
The screening of 'Kronos' lines resulted in 23 mutants for *MloA* and 29 mutants for *MloB*. Based on availability of seeds, the known barley mutations, and SIFT predictions (Sorting Intolerant From Tolerant [2]), we selected two *MloA* and four *MloB* mutants for crossings. F_2 plants were genotyped and tested for resistance by inoculation with powdery mildew in an excised leaf test. Some combinations of mutations gave resistance, others not, indicating that the SIFT analysis worked only as a guideline. A single "strong" missense mutation at pos. 163 in *MloB* had a considerable effect on resistance with clear indications of allele dosage effects.
We were able to make the highly susceptible tetraploid wheat cultivar 'Kronos' *mlo* resistant by mutating both *Mlo* homoeologs. Mlo mutations might give pleiotropic effects such as early senescence and increased susceptibility against other pathogens. Pot experiments under semi-field conditions confirmed the resistance levels of the eight different gene combinations, and we did not observe any significant signs of pleiotropic effects, such as necrotic spots. Optimal combinations of "weak" and "strong" mutations may provide *mlo* resistance without possible negative side effects.
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Functional analysis of beneficial plant microbe interaction in model plant Arabidopsis thaliana under abiotic stress condition

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Abiotic stresses such as salt stress are the major limiting factors for agricultural productivity, and cause global food insecurity. It is well known that beneficial plant-associated microorganisms can stimulate plant growth and enhance resistance to biotic and abiotic stresses. Bacterial endophytes are a group of soil bacteria that colonize the host plant and play a fundamental role in plant growth enhancement, plant resistance to different phytopathogens and abiotic stress. However, the mechanisms how beneficial and pathogenic microbes differ in their interaction with plants are poorly understood. Recently, our group reported that the beneficial Enterobacter sp. SA187 induces plant growth on Arabidopsis under salt stress conditions by manipulation of the plant ethylene signaling pathway. We therefore compared inoculation of plants by SA187 with the virulent *P.syringae* pathovar *tomato* strain DC3000 (*Pst*) and the non-virulent strain *Pst hrcC-,* that has a defect in type 3 secretion and cannot inject any effectors. While *Pst* inoculated plants were highly salt sensitive such as wild type plants, *Pst hrcC*- induced salt stress tolerance in Arabidopsis, suggesting that Pst hrcC- also contains plant growth promoting activity under stress conditions. To further elucidate the underlying mechanisms of the interaction of SA187, *Pst* and *Pst hrcC*- with Arabidopsis, RNAseq, hormone and biochemical analyses are currently carried out and will be discussed.

Can actin depolymerization actually result in increased plant resistance to pathogens?

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The integrity of the actin cytoskeleton is essential for plant immune signalling. Consequently, it is generally assumed that actin disruption reduces plant resistance to pathogen attack. However, in a previous study, it was shown that actin depolymerisation triggers the salicylic acid (SA) signalling pathway, which is interesting because increased SA is associated with enhanced plant resistance to pathogen attack. Here, we attempt to resolve this seeming inconsistency by showing that the relationship between actin depolymerization and plant resistance is more complex than currently thought. We investigate the precise nature of this relationship using two completely different plant pathosystems: i) a model plant (*Arabidopsis thaliana*) and a bacterial pathogen (*Pseudomonas syringae*), and ii) an important crop (*Brassica napus*) and a fungal pathogen (*Leptosphaeria maculans*). We demonstrate that actin depolymerization induces a dramatic increase in SA levels and that the increased SA is biosynthesized by the isochorismate synthase pathway. In both pathosystems, this phenomenon leads to increased plant resistance.

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RNA interference in a maize pathogen reduces toxin production

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The fungus *Fusarium verticillioides* can produce fumonisin mycotoxins in ears under certain environmental conditions. Because fumonisins are unhealthy for humans and livestock, control strategies with nominal risk to the environment are needed to reduce fumonisin exposure. Host-induced gene silencing is a molecular engineered technique in which double-stranded RNA expressed in the plant host is absorbed by an invading fungus; subsequently, the expression of targeted genes is reduced in the fungus. A vital preliminary step of this technique is identification of DNA segments within the gene target that can effectively silence gene expression when expressed in the fungus. Here, we used segments of the fumonisin biosynthetic gene *FUM1* to synthesize double-stranded RNA in *F. verticillioides*. Several of the resulting transformants had reduced *FUM1* gene expression and fumonisin production. Similar losses in fumonisin production resulted from double-stranded RNA constructs with segments of *FUM8*, another fumonisin biosynthetic gene. *FUM1* or *FUM8* silencing constructs were transformed into three isolates of *F. verticillioides* originating from different regions of the United States. Whole genome sequence analysis of seven transformants indicated that reductions in fumonisin production were not due to mutation of the fumonisin biosynthetic gene cluster and showed a complex pattern of plasmid integration. These results indicate the cloned *FUM1* or *FUM8* gene segments could be transgenically expressed in maize for host-induced gene silencing of fumonisin production.

Phospholipid turnover as a tool for the fine-tuning of flagellin perception and immunity

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Flagellin perception is a keystone of pathogen-triggered immunity in plant cells. It starts with flg22 peptide recognition by a plasma membrane receptor complex and is followed by a signalling cascade including protein phosphorylation and reactive oxygen species (ROS) production via NADPH-oxidase. However, the role of membrane phospholipids in these processes is currently underestimated.
We first associated the changes in membrane phospholipid turnover with classical downstream effects of flagellin (flg22) treatment like ROS production, callose deposition, gene expression changes and resistance to bacterial infection. In both plant cell suspensions and seedlings, flg22 treatment caused a rapid transient decrease in phosphatidylinositol-4,5-biphosphate levels and a corresponding parallel increase in phosphatidic acid (PA) levels. Flg22-induced PA production and ROS burst were sensitive to the effect of lipid-signalling-pathway inhibitors (U73122, R59022 and wortmannin).
Because these results strongly suggest the involvement of the lipid signalling enzymes phosphatidylinositol-dependent phospholipase C (PI-PLC) and diacylglycerol kinase (DGK) in flg22-induced responses, we performed mutant screening to investigate the connection between lipid signalling and known players in flg22-induced cascades. The receptor-kinase-deficient mutants *fls2*, *bak1-4* and *bik1* produced less PA than WT in response to flg22, but the response in NADPH oxidase-deficient *rbohD* plants did not differ from that of the control. This indicates that PA accumulation is placed downstream of receptor complex activation, but upstream of ROS generation. Among the lipid kinase-deficient lines, the *dgk5.1* mutant produced less PA after flg22 treatment, but ROS production remained at the control level. Besides, *dgk5.1* plants showed reduced callose accumulation in response to flg22 and impaired resistance to *Pseudomonas syringae* infection. The transcriptome analysis revealed a set of defence-associated genes that are under control of the DGK5 activity. Thus, we propose a new model of flagellin perception in plant cells including membrane phospholipid metabolism by PI-PLC and DGK5.

A prophage tail-like protein is deployed by Burkholderia bacteria to feed on fungi

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Bacteria are considered as one of the simplest living organisms with capacity to rapidly multiply and evolve. They are under constant evolution with other co-habiting organisms and exhibit diverse interactions ranging from mutualism, commensalism, antagonism and parasitism. Several bacteria demonstrate anti-fungal properties by producing anti-fungal metabolites, chitinolytic enzymes, siderophores, toxins. However, some of them are capable of growing and multiplying at the cost of living fungal biomass by utilizing fungal biomass as source of nutrient/energy. Such fungal eating phenomenon is known as bacterial mycophagy. Furthermore, being equipped to kill and feed upon fungi, the mycophagous bacteria could serve as an experimental tool box to facilitate discovery of novel anti-fungal molecules. We have recently isolated a bacterium (*Burkholderia gladioli* strain NGJ1) from the rice seedlings. It demonstrates broad spectrum anti-fungal activities on various phytopathogenic fungi, including *Rhizoctonia solani* (causal agent of sheath blight disease in rice). Moreover we demonstrated that the bacteria NGJ1 deploys a prophage tail like protein (Bg_9562) to feed over fungi in a T3SS dependant manner. Furthermore we observed that Bg_9562 has a broad spectrum antifungal activity on *Rhizoctonia solani* as well as various phytopathogenic fungi. This opens up a new biotechnological application of this prophage tail like protein in controlling fungal diseases in rice as well as in other plants.

Identification of novel priming agents by monitoring the expression of defence genes in rice cell suspension cultures

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Priming agents are compounds that put the plant defence system in an increase state of alertness. Upon subsequent stress exposure, primed plants activate their defence response in a faster and more robust way. Due to the current ban on a large number of traditional pesticides in the EU, discovery of novel priming agents is urgently needed.
We are constructing a platform for high-throughput screening of potential priming agents to screen libraries of natural compounds, among which potentially novel priming agents. This platform is based on a set of rice defence associated genes (DAGs), which we have identified using a 'weighted gene co-expression network analysis' (WGCNA). In this meta-analysis, more than 350 micro-array datasets of rice under a variety of biotic stresses were analysed. A list of 36 potential DAGs, activated by biotic stress, was selected for further investigation.
The upregulation of the DAGs was validated using an *in planta* experiment. Expression levels of the 36 potential DAGs were monitored by RT-qPCR, after treatment with different pathogen/damage-associated molecular patterns (PAMPs/DAMPs): lipopolysaccharides, oligogalacturonides and "NemaWater". NemaWater, a nematodal PAMP, was found to be most potent in terms of induction of the selected DAGs. A final set of 10 consistently induced DAGs was selected based on these results.
By monitoring the expression of the 10 DAGs in rice cell suspension cultures, the intensity of the plant defence response can now be investigated. As a positive control, the priming activity of β-Aminobutyric acid (BABA) is currently being validated in rice cells. To mimic pathogen attack, we add PAMPs to the cell culture, and we screen for enhanced defence gene expression upon previous exposure to BABA.

ENDOPHYTIC BACILLUS SUBTILIS IMPROVES SALT TOLERANCE IN TRITICUM AESTIVUM L. WITH INVOLVEMENT OF SALICYLATE-DEPENDENT SIGNALING PATHWAY

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Salinity is a strong damaging factor limits growth and productivity of plants. Endophytic bacterium *Bacillus subtilis* (*B. subtilis*) 10-4, producing IAA and siderophores but not active in phosphate solubilization exerts a protective action on *Triticum aestivum* L. (wheat) growth under salinity (2% NaCl) through decreasing the development of stress-induced oxidative and osmotic stresses in seedlings were found. It was suggested that anti-stress effect of the bacteria may be due to his ability to synthesize jasmonic acid (JA) or salicylic acid (SA), which are plays the role of dominant primary signals of local and systemic induced protective plant response to stresses. It was revealed that inoculation with *B. subtilis* 10-4 increased the endogenous SA level (before stress) and decreased the level of stress-induced SA accumulation in them. Proceeding from the enhancement of plants protective characteristics induced by SA and JA are associated with the accumulation of PR proteins, wherein the genes PR-1 and PR-9 act as molecular markers of systemic resistance induced by SA and JA, respectively, the effect of *B. subtilis* 10-4 on the expression of these genes were accessed. It was found *B. subtilis 10*-4 significantly induces PR-1 gene transcriptional activity in seedlings both under normal and saline conditions. At the same time, the analysis of the effect of *B. subtilis* 10-4 on the transcriptional activity of the PR-9 gene, which is a marker of jasmonate-induced plant resistance, showed no high activity both under non-saline and saline conditions. Thus, the obtained data indicated about the involvement of SA-dependent signaling pathway on the realization of endophytic *B. subtilis* 10-4-induced protective reactions on wheat plants under saline conditions.

Mycorrhizal Influence on Root Traits of Rice Seedlings

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Due to climate change, many farmers will need to begin to cultivate rice in aerobic conditions in order to maintain high yields, despite drier soils. However, our previous studies revealed that many lowland rice cultivars were not adapted to aerobic systems resulting in significant decreases in yields. Arbuscular mycorrhiza (AM) may be an important factor in improving the adaptability of lowland rice cultivars to aerobic conditions. Further experimentation showed aerobic soils more than doubled colonization rates of rice by AM compared to flooded fields. Here, we propose the use of AM to enhance rice adaptation by improving root growth, particularly, lateral root formation. Using chromosome segment substitution lines (CSSLs) it is possible to examine effects of AM on a variety of root traits. In order to determine an appropriate inoculant, rice was grown with one of four species which were isolated from farmland soils in Northern Thailand. After 3 weeks, *Glomus mosseae* (GM) had the largest impact on lateral root density at 12.3 roots/cm, compared to non-inoculated plants at 8.3 roots/cm. GM was then used alongside with 17 CSSLs contrasting lateral root traits. At 3 weeks after inoculating, the inoculated plants had no significant difference in almost all measured parameters compared to controls. However, lateral root density significantly increased with certain lines being more responsive to mycorrhizal influence. Lines 71, 53, and 78 contained 8.3, 12.8, and 9.7 lateral roots/cm, respectively under control conditions. When colonized, density increased to 18.2, 16.37, and 12. 9 lateral roots/cm respectively.Our findings suggest that colonization of seedlings increases lateral root density. Increased density may allow poorly performing varieties to acquire water and nutrients more efficiently in an aerobic setting. This knowledge has potential to help farmers in areas affected by climate change to successfully implement aerobic farming.

Functional SNPs in resistance gene with essential role in plant nematode defense response.

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Serine hydroxymethyltransferase (SHMT) is an ubiquitous enzyme present in prokaryotes and eukaryotes, including bacteria, yeasts, plants, animals and humans. SHMT is essential for cellular one-carbon and folate metabolisms, and it is involved in pathogenesis phenomena: in humans, mutations in SHMT have been associated to a wide range of disease development, whereas in plants, specific versions of SHMT can determine resistance to phytoparasitic nematodes (PPNs). PPNs cause economically important crop losses worldwide. PPNs acquire folate from their diet at developing feeding sites in the root apparatus. Modifications of the plant's folate pathway lead to a nutritional deficiency that starves the nematode and causes the degeneration the feeding cells.
In model systems such as *Glycine max L.* infected by PPNs, the role of SHMT in host-nematode interaction mediated is further supported by the characterization of two resistance loci, i.e. *Rgh1* and *Rgh4* and the identification of functional SNPs. Accessions of *Solanum Lycopersicum* L., and *Pisum sativum* L., known to be resistant to PPNs have not yet described at the SHMT loci. Therefore, we have amplified and sequenced *Rgh1* in such accessions in order to explore the presence of functional SNPs described in *gmRhg1.* The Rgh1 sequences carry amino-acidic sequence typical of the susceptible *gmRhg1*, both in resistant tomato and in pea, *i.e.* SNP130 (**R**130**P**) and SNP358 (**Y**358**N**). We have extended this analysis to tolerant accessions of *Cicer arietinum* L. to PPNs and we have confirmed what it was already known in tomato and pea. However, expression analysis in roots and shoots revealed significant up-regulation of *caRgh*1 exclusively in PPNs-infected plants. The analysis suggests that a different not-fully characterized mechanism of resistance exists in host-PPN interactions.

UNRAVELLING THE EPIGENETIC BASIS OF PLANT IMMUNITY

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In response to a pathogen attack, plants induce a variety of different defence mechanisms. In addition, after an infection, plants respond more effectively against a second pathogen encounter. This is known as acquired immunity or priming. Over recent years, evidence has accumulated that this priming of defence can be transmitted to the next generation of plants, resulting in transgenerational acquired resistance (TAR). This discovery has prompted further research to resolve the role of epigenetic mechanisms in plant immunity. Epigenetics refers to the study of inheritable changes that modify the phenotype of the organisms in the absence of changes in the DNA sequence. It has been shown that pathogen infection changes DNA methylation and that Arabidopsis mutants defective in DNA methylation express constitutive defence priming. Here, we present our latest results and future plans to decipher the epigenetic basis of plant immunity and priming. Analysis of Arabidopsis mutants has shown that TAR is regulated by RNA-directed DNA methylation (RdDM) and ROS1-dependent (de)methylation. This work also revealed that changes in DNA methylation affect nearly half of the pathogenesis-related transcriptome, illustrating the importance of DNA methylation in plant immunity. In addition, by analysing the DNA methylome of progenies from disease-exposed Arabidopsis plants, we found that the methylome responds dynamically to disease encountered in previous generations. This supports the notion that DNA methylation provides transgenerational phenotypic plasticity to biotic stress. Finally, we have studied the parental contributions and specificity of TAR in response to different (a)biotic stresses. Amongst others, this research has revealed that the durability of TAR depends on the intensity of stress exposure in the previous generations. Lastly, we will introduce some current work focused in analysing the interaction of specific defence pathways and the epigenetic machinery, in order to position them in the plant canonical defence scenario at a molecular level.

Don't let it get under your bark: exploring mechanism of defense priming in Norway spruce (Picea abies)

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As a long-lived, sessile organism, Norway spruce (*Picea abies*) is challenged by a wide range of abiotic and biotic stresses. One of the most detrimental biotic stresses is the spruce bark beetle (*Ips typographus*) along with its symbiotic necrotrophic bluestain fungi. Together this lethal team can overcome a healthy tree's defences and kill vast areas of forest. While forest management practices help to decrease the risk of such devastation, these outbreaks are predicted to escalate due to increasing temperatures. Thus, there is a need to find new ways of increasing a tree's natural defences. Spraying of adult Norway spruce bark with natural phytohormone methyl jasmonate (MeJA) has been shown to provide enhanced resistance to both the bark beetle and its bluestain fungi. This heighted resistance is observed for weeks and possible years after the treatment. Defense priming, the ability to 'remember' a previous stimulus and respond more quickly and strongly to a secondary attack, is well documented in short-lived angiosperms. However, very little is understood about the molecular mechanisms underlying priming in long-lived gymnosperms. While many of these mechanisms may be conserved across the plant kingdom, some mechanisms may differ in gynmnosperms due to their vastly different life history and genome size. To better understanding molecular changes that occur during priming, 35-year-old spruces with were treated MeJA then wounded 4 weeks later. Using multifaceted analyses including mRNA-sequencing, miRNA-sequencing, metabolite profiling, and ChIP-seq, we are trying to unveil how priming 'memory' is established in these trees. We hope that this work will contribute to a greater understanding of defence priming in Norway spruce, as well as, provide important information for the development new forest management techniques.

Can phytohormones influence the composition of the endophytic microbiome in tomato roots?

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Endophytes are microbes capable of colonizing the inner part of plants without causing disease. In some cases, they improve host plant resilience to biotic and abiotic stresses and promote plant growth. The plant-endophyte interaction involves complex mechanisms ranging from the recruitment of the microorganisms to the colonization of internal plant tissue, with the need to escape the plant immune system. These processes are regulated by different plant and endophyte signalling molecules. Phytohormones are among these signalling compounds, but little is known about the specific ways by which they influence recruitment and colonization. The current project aims to obtain a deeper knowledge of the role of signalling compounds in plant-endophyte interactions.
To understand how phytohormones influence the composition of endophytic communities, a microbiome analysis (isolation and amplicon sequencing) of endophytic fungi was conducted on roots of tomato mutants impaired in synthesis of ethylene and jasmonic acid. The amplicon sequencing analysis showed a significant effect of the phytohormones, but only the relative abundance of a few taxa was affected (*e.g. Fusarium* and *Pseudogymnoascus*). In contrast, there was a stronger effect of plant genotype (comparison of the two wild-types) where the abundance of *e.g. Thielaviopsis*, *Apiotrichum*, *Fusarium*, *Saitozyma* and *Pyrenochaeta* differed significantly. The community analysis also revealed high abundance of potential pathogens (*e.g. Thielaviopsis basicola* and *Pyrenochaeta lycopersici*) and isolated strains of these species were pathogenic when tested *in planta*. To understand why healthy plants can harbour such a high amount of pathogenic fungi, experiments with synthetic communities holding both endophytic and pathogenic isolates are currently conducted in order to elucidate possible ”natural biocontrol effects” by the endophytic fungi present in the microbiome.
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Detection of potato viruses by reverse transcription loop-mediated isothermal amplification

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Loop-mediated isothermal amplification assay (LAMP) facilitates fast and sensitive detection of DNA and RNA targets. The speed of detection of RNA can be improved by the addition of reverse transcriptase to form an RT-LAMP assay. RT-LAMP was reported as a favorable method of detection of many RNA viruses with a sensitivity similar to, or higher than, the real-time RT-PCR. The advantage of RT-LAMP is also its speed. When the assay is performed in the real-time fluorescent detection mode, the target virus can be detected in 5-30 minutes since the amplification commence. Furthermore, polymerases used in the LAMP reaction are less sensitive to amplification inhibitors than polymerases used in PCR reaction. Consequently, the RT-LAMP can be performed on crude tissue extracts or crude RNA preparations, saving the time and cost the assay. Availability of many color-changing approaches for detection of the LAMP amplicons facilitates the development of RT-LAMP variants with visual detection as a point-of-care test. Here, we present a development and optimization of RT-LAMP assays to detect the most important potato viruses including potato virus Y (PVY), potato virus M (PVM), potato leafroll virus (PLRV), potato virus S (PVS) and potato virus X (PVX). The poster will present different RT-LAMP assay procedures, dedicated to quantitative, real-time detection in the laboratory as well as for fast end-point colorimetric detection in the field.

Effects of rhizospheric bacteria on root architectural traits of maize (Zea mays L.)

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Root system architecture has been known to influence water and nutrient uptake, as a result breeding programs targeting root traits by conventional, molecular, and genetic engineering approaches have been initiated worldwide. However very few attempts are successfully implemented in agronomical settings due to laborious nature of root evaluation for plant breeding. In this study we propose the use of plant growth-promoting rhizobacteria (PGPRs) to modify root traits as an alternative method to plant breeding. Rhizospheric bacteria including *Bacillus amyloliquefaciens* R5, *B. aryabhattai* N8, *B. subtilis* N3, *B. thuringiensis* pxx13.1, *B. thuringiensis* pxx20, and *Pseudomonas reinekei* TSTRR4-2 were isolated and tested for effects on maize root architectural traits including root length, root growth angle, number of lateral root and number of crown roots. We found that all six bacterial isolates produced indole acetic acid (IAA) at different concentrations ranging from 0.9 ± 0.77 µg/ml in TSTRR4-2 to 18.0 ± 0.69 µg/ml in N8. In hydroponics, R5 decreased crown root angle by 33.4% while the pxx13.1, pxx20, R5 and TSTRR4-2 decreased lateral root density by 25.4, 40.3, 41.4 and 42.6%, respectively. The effects of R5 and TSTRR4-2 on crown root angle and lateral root density were also recognized in the soil pot system. Our results indicate that PGPRs could be used to enhance the preferable root traits which could lead to promotion of plant growth and productivity.

Endophytic fungi entering Plant Wars

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Alternative practices are urgently needed to make current agriculture more sustainable. Endophytes, referring to both bacteria and fungi that grow asymptomatically inside plant tissues, are often associated with enhanced plant growth and tolerance to abiotic and biotic stresses, making them potential tools for improving crop yields in an environmentally friendly manner. However, the actual mechanisms and effects of the complex plant-endophyte interactions remain poorly understood.
Serendipita indica (syn. *Piriformospora indica*) is an endophytic fungus with several promising agricultural and biotechnological applications. The fungus can colonise the root cortex of a wide range of plants, enhancing plant growth and modulating plant specialised metabolism. Tomato (*Solanum lycopersicum*) is an important crop, often challenged by fungal pathogens and insect pests. The wide variety of specialised metabolites produced by the plant, especially volatile organic compounds (VOCs), plays a crucial role in plant defence, helping in fighting possible enemies.
This project involves the establishment of a balanced interaction between the fungus *S. indica* and tomato plants, thus providing a model system for studying general plant-endophyte interactions. We focus on the effect of colonisation on the host metabolism, specifically the tomato VOCs. The ability of the fungus to alter production of plant VOCs during colonisation, under optimal conditions and abiotic stresses, is being tested and evaluated. In addition, *in planta* bioassays are ongoing to study the effect of endophyte-mediated VOCs on the induction of plant defence against pathogen attacks. RNAseq analyses on fungus-colonised and fungus-free plant tissues, grown under various stresses, will be the next step in order to achieve a more-in-depth knowledge on the exact mechanisms underlying the *S. indica*-tomato interaction.
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S-methylmethionine-salicylate (MMS) pretreatment contributes to the alleviation of the damages on vital crops caused by biotic stressors

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Biotic and abiotic environmental stressors can cause severe damages to plants, resulting in significant yield loss in agriculture. For this reason it is crucial to investigate the pathogen-host interactions with the aim of enhancing the defence ability of the frequently cultivated susceptible crop varieties.
Maize dwarf mosaic virus (MDMV) and powdery mildew *Blumeria graminis* f. sp. *tritici* can effectively infect the susceptible varieties of maize and wheat, respectively. Use of biologically active compounds as stress-protective agents offers a simple, environment-friend, cost-efficient and scalable solution for increasing the tolerance against different stress types, for instance biotrophic pathogens.
S-methylmethionine-salicylate (MMS) consists of two natural stress-protective component in ratio 1:1, salicylate (SA) and *S*-methylmethionine (SMM). It has been evidenced that both of these components can play major role in biotic stress response. SA, an important signal molecule in plants, induces systemic acquired resistance (SAR) against pathogens. SMM is a non-proteinogenic amino acid derivative, a key molecule in sulphur metabolism and methylation processes and affects several biosynthetic pathways that are important in stress response.
In the present work we investigated the effects of exogenous MMS and studied whether this compound can reduce the damage exerted by MDMV and powdery mildew in maize and wheat, respectively. PSII maximum quantum efficiency and chlorophyll content of wheat seedlings were monitored 3, 6 and 10 days after the fungal infection. Lengths and weights were also measured to study the effect of MMS on the development of infected wheat seedlings. In the case of the maize/MDMV interaction, virus quantity was determined three weeks after the infection, using protein-based (ELISA) and vRNA-based (qRT-PCR) methods.
Our results display that MMS-pretreatment is capable of reducing the negative effects of pathogens through increasing the innate defence capacity of the examined crop species.

A class of non-specific lipid transfer proteins of Medicago truncatula modulates the host response to rhizobia infection.

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The establishment of the symbiotic interaction between leguminous plants and nitrogen fixing bacteria is a complex process that requires a molecular dialogue between the host and the bacteria during the root infection and the colonization of the nodule, where the bacteria fix nitrogen. The formation of a functional root nodule needs a spatial and temporal coordination of all these events. Rhizobia enter root hair via a tubular plant-derived structure, named infection thread (IT), and then they are released into the cortical layers, where the bacteria enclosed in a plant-derived membrane called symbiosome, enter nodule primordia cells. The formation of the ITs as well as the release of rhizobia in the nodule cells require the synthesis of new membranes and modifications of their lipid composition. Lipid transfer proteins (LTPs) are small basic secreted proteins, characterized by lipid-binding capacity and putatively involved in lipid trafficking. In plants, the non specific LTPs (nsLTPs) are a large group of proteins implicated in various processes; most of them possess antimicrobial activity and are likely involved in pathogen defence. Recently, two LTPs, AsE246 from *Astragalus sinicus* and MtN5 from *Medicago truncatula,* have been implicated in the host regulation of N-fixing symbiosis suggesting their participation in the de-novo formation and rearrangement of infection threads and symbiosome membranes (Lei et al. 2014; Santi et al., 2017). We have identified in *M.truncatula* genome two novel nsLTPs that cluster with MtN5 in the type III nsLTP group (Wang et al., 2012), an intermediate group between the two major nsLTP1 and nsLTP2 families. We have demonstrated that these LTPs are transcriptionally induced by rhizobia infection and they can modulate the host nodulation capacity.

Effect of Bacillus subtilis and a Plant Defense Stimulator on flax protection against Fusarium oxysporum

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In Normandy, flax (*Linum usitatissimum*) is a plant of important economic interest because of its fibers. Every year, 20% of crops are lost due to *Fusarium oxysporum* infection. To date, the best-known ways to reduce *Fusarium* damages are crop rotation and the use of tolerant varieties.
In the context of the Ecophyto project, several methods have been identified to limit the use of phytochemicals on crops. One of them is the use of Plant Defense Stimulators which can be small natural molecules that act like ”vaccines”. Another way is to use Plant Growth Promoting Rhizobacteria occurring naturally in the rhizosphere.
In this work, we study the efficiency of a *Bacillus subtilis* strain to limit Fusarium wilt on two flax varieties displaying different *F. oxysporum* susceptibilities: Aramis considered more tolerant than Mélina. First, to better understand flax responses to *Fusarium oxysporum* infection, we followed the cell wall damages and reorganizations by cell wall polysaccharides immune-labelling. Interestingly, we showed differences in the cell wall organization between the two varieties in non-infected conditions. We also demonstrated, with Aramis, the possible implication of the xyloglucans and the homogalacturonans in cell wall reinforcement. Then, we investigated the potential ability of *B. subtilis* to limit *F. oxysporum* pathogenicity. We showed that *B. subtilis* was able to limit *F. oxysporum* growth *in vitro* and, in a greenhouse experiment, to significantly reduce the number of diseased plants. Finally, in a previous work, several natural compounds were screened and selected based on their abilities to activate defense-related genes and to improve resistance of *Arabidopsis thaliana* against *Pseudomonas syringae* DC3000 (Zahid *et al*., 2017) and the two flax varieties against *F. oxysporum*. Work is under way to investigate the possible synergistic effect of one of the selected compounds and *B. subtilis* on their abilities in reducing Fusarium wilt on flax.

Contribution of cell wall peroxidase-derived reactive oxygen species to Alternaria brassicicola-induced oxidative burst in Arabidopsis

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Cell wall peroxidases and plasma membrane-localized NADPH oxidases are considered as main sources of an apoplastic oxidative burst in plants attacked by microbial pathogens. In spite of this established doctrine, approaches attempting a comparative, side-by-side analysis on the functions of extracellular ROS generated by the two enzymatic sources are scarce.
We previously reported about the roles of *Arabidopsis* NADPH oxidase RBOHD in plants challenged by the necrotrophic fungus *Alternaria brassicicola* (Pogány *et al.,* 2009). Here we present results on the activity of apoplastic class III peroxidases PRX33 (*At3g49110*) and PRX34 (*At3g49120*) investigated in the same *Arabidopsis-Alternaria* pathosystem. ROS generated by *Arabidopsis* PRX33 and PRX34 peroxidases increase necrotic symptoms and colonization success of *A. brassicicola*. In addition, knocking down *PRX33* and *PRX34* transcript levels leads to a reduced number of host cells showing extracellular burst of ROS after inoculation with *A. brassicicola*. Our results also reveal an age-dependent transcript distribution of ROS-producing peroxidase and NADPH oxidase enzymes and some potential new components of the RBOHD, PRX33 and PRX34 signaling networks.

Pogány, M., von Rad, U., Grün, S., Dongó, A., Pintye, A., Simoneau, P., Bahnweg, G., Kiss, L., Barna, B. and Durner, J. (2009) Dual Roles of Reactive Oxygen Species and NADPH Oxidase RBOHD in an *Arabidopsis-Alternaria* Pathosystem. *Plant Physiol***. 151**, 1459–1475.

Arbuscular mycorrhizal symbiosis modifies cell water permeability of root cells under drought stress

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The symbiotic interaction of plant roots with arbuscular mycorrhizal (AM) fungi has been shown to be beneficial for plant tolerance to different abiotic stresses, including drought. The establishment of this symbiosis originates several modifications in root cortical cells, and previous results showed that root hydraulic conductivity and aquaporins were altered by the presence of the AM fungus within the root under drought stress conditions.
Since the regulated aquaporins can transport water, but also other solutes of physiological importance for plant performance under drought stress, we aimed to elucidate if these aquaporins were involved in the regulation of water transport in AM roots, with a role in the enhanced root hydraulic conductivity. For that, maize plants were cultivated under greenhouse conditions and inoculated with the AM fungus *Rhizophagus irregularis*. After the establishment of the AM symbiosis, a drought stress was applied to half of the plants. Cell water permeability was measured by means of cell pressure probe and protoplast swelling assay, and changes in aquaporins were analyzed by western blot analysis, ELISA and RT-qPCR.
Under water stress, the AM symbiosis enhanced significantly the cell water conductivity compared to non-inoculated plants, when measured with the cell pressure probe. Also, protoplast from AM roots showed increased osmotic water permeability, which proved the beneficial effect of this symbiosis for radial water transport under drought. Some of the aquaporin isoforms that were analyzed in this study (ZmPIP2;1/PIP2;2 and ZmPIP2;6) presented also a differential regulation due to the presence of the AM fungus, and the phosphorylation status of PIP2s increased in AM roots when subjected to drought.
Altogether, the results demonstrated that the AM symbiosis has a significant effect on cellular water permeability. Further studies should be aimed at defining the mechanisms by which the symbiosis affects membrane permeability under water deprivation.

Cellulase-induced resistance against permeabilization of the plant plasma membrane by a Trichoderma peptaibol

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Membrane-permeabilizing antimicrobial peptides are involved in pathogenic and competitive interactions between many types of organisms. However, specificity for and susceptibility of different cellular membranes are generally poorly understood. Plasma membranes of tobacco cells are permeabilized by the antimicrobial peptaibol alamethicin. It is produced by *Trichoderma viride*, a plant symbiotic fungus, and inserts as oligomers with ion channel activity into susceptible membranes. However, pretreatment of tobacco cells with cellulase from the same fungus leads to cellulase induced resistance to alamethicin (CIRA), which modifies the plasma membrane lipid composition. We have also studied roots of cultured Arabidopsis seedling. Alamethicin promoted cell membrane permeability in the epidermis of the root apical meristem and extension zone. As in tobacco cells, CIRA developed after pretreatment of the root with limited amounts of active cellulase. Mutant library screening indicates that a large number of genes are crucial for effective CIRA. The mutated genes include such that are involved in cell wall component synthesis, membrane lipid modification or transfer, and cellular signaling. The results indicate that the CIRA process involves a novel signaling pathway, which eventually modifies the plasma membrane lipid composition to resist the peptaibol.

Use of molecular dynamics in deciphering the mechanism of salicylic acid binding to soluble proteins.

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Salicylic acid (SA) has an essential role in the response of plants to the pathogens. Nevertheless, only sparse information exists on the proteins that binds SA. Yet, SA to be effective must bind proteins. Indeed, it seems that SA directly binds to soluble proteins, some of them with enzymatic activity, and thus modulate them. For instance, in plants it was shown that SA binds catalases (SA-binding protein 1 or SABP1) and glyceraldehyde phosphate dehydrogenase (GAPDH). Interestingly, SA also binds human GAPDH. Recently, by high throughput biochemical strategies, a list of hundred soluble proteins was proposed to be able to bind SA in plant cells (Manohar et al., 2015, Frontiers in Plant Science). Therefore, the understanding of the mechanism of the interaction between a protein and SA is more than ever a relevant biological question. Here we aim at answering fundamental questions: (i) What are the proteins that truly bind SA? (ii) Do they share the the same binding mode? (iii) In line with that, what are the residues responsible for the interaction between each protein and SA? (iv) Can we describe a universal pharmacophore for SA or not?
We want to answer these questions by a dialogue between molecular dynamics and biochemical studies. Tis will be illustrated on the subunit A1 of Arabidopsis GAPDH. For this protein, crystallographic structures of highly identical homologs exist. These structures will be used for homology modelling and simulation of the structure in water. *In silico* docking of SA on the protein is performed, leading to the identification of putative SA binding pockets. The force of SA binding in these pockets will be assessed *in silico*. In parallel, the heterologous production of wild-type and mutated versions of the protein will allow the biochemical validation of identification of the residues involved in SA binding.

Yr15 (WTK1) encodes a tandem kinase conferring broad-spectrum resistance to wheat stripe rust

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a devastating fungal disease that threatens global wheat production. *Yr15* of wild emmer wheat (*Triticum dicoccoides*), located on chromosome 1BS, confers resistance to a broad spectrum of *Pst* races. Comparative genomics, chromosome walking, BAC libraries (wild emmer and bread wheat), whole genome assemblies, EMS mutagenesis, and transgenic approaches enabled us to clone *Yr15* and validate its function. The Yr15 protein has a novel structure for R-genes in wheat by possessing two kinase-like domains in tandem, which we designate Wheat Tandem Kinase 1 (WTK1). We have shown that both kinase domains are essential for conferring *Pst* resistance. Macro- and microscopic observations of development and accumulation of fungal biomass suggest that the hypersensitive response plays a central role in the resistance mechanism, limiting the development of fungal feeding structures. A functional copy of *Wtk1* was found only in the B sub-genome of wild emmer wheat. Non-functional copies were identified in all three sub-genomes of modern durum (AABB) and common (AABBDD) wheats, which differ from the functional allele by the presence of indels that are predicted to create truncated proteins. These differences allowed us to design diagnostic markers that differentiate between functional and non-functional *Yr15* versions. Non-functional *WTK1* orthologs were found as well in the diploid wheat relatives *T. urartu* (AA), *Aegilops speltoides* (SS), and *Ae. tauschii* (DD), on chromosome 1R of rye (*Secale cereale*), but not on 1H of barley (*Hordeum vulgare*). Our results suggest that *Yr15* has the potential to improve stripe rust resistance in a wide range of tetraploid and hexaploid wheat germplasm. The absence of the functional *Yr15* in tested durum and common wheat varieties highlights the value of wild emmer wheat germplasm as a reservoir of resistance genes for wheat.

Guided by beneficial effectors from a plant symbiont to improve stress resilience in crops

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Beneficial plant-fungal symbioses have existed for >400 million years and enabled plants to colonise land in the harsh environment of the Mid-Palaeozoic era (480-360 mya) with its vigorous fluctuations in temperature and water availability. In co-evolution with plants, symbionts have developed abiotic stress protection mechanisms (e.g. against drought, heat) that have been and still are vital for all plants. In modern cropping systems, however, symbiont protection is often lost due to cultivation practice (e.g. tillage, pesticides). Understanding how symbionts protect plants can equip us with novel and sustainable crop protection strategies.
Using a genome-wide yeast-two-hybrid screen in combination with a plant protoplast and whole plant phenotyping approach we found that the fungus *Serendipita indica* releases effector proteins with high functional specificity in activating beneficial pathways in colonized host plants, including stress protection pathways. The broad host range of *S. indica* (e.g. all major monocot and dicot crops) suggests that underlying protection mechanisms are fundamental and possibly conserved in plants. By learning how effectors activate plant protection, our ultimate goal is to develop crops with improved stress resilience.

Dependence of wheat root mycorrhizal traits to soil tillage

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Mycorrhizas are biological tools for plants in order to expand their access especially to less mobile nutrients. The ability to adapt to a wide spectrum hosts has led to the installation of these fungi as essential symbionts in any agroecosystem. Wheat is a plant grown on large surfaces, with a large number of varieties adapted to extremely diverse conditions. The symbiotic mycorrhizal mechanisms act in plant roots starting with the early stages of growth, immediately after emergence. A multi-year experiment was conducted in order to highlight the effect of soil tillage on mycorrhizal traits in wheat roots. Based on primary results, the penetration points and the expansion of mycorrhizal mycelium into roots is largely influenced by applied soil management. Conventional soil plowing produces the fragmentation of mycorrhizal hyphal networks. This type of management leads to a discontinuous symbiosis in roots, penetration points alternating with hyphae-free areas. Plant does not benefit from a constant nutrient flow, the colonized areas of root having a much higher absorption capacity. A practice based on minimum tillage, even for a year or two leads to equilibrium in colonization and stimulates the symbiotic fungal mechanisms existing in the wheat rhizosphere. Minimum tillage management, maintained for 4-5 years, favors the formation of an extensive mycorrhizal hyphae network. Wheat plants connect to these networks, with a balanced hyphal system in root cortex providing a constant flow of plant nutrients in any part of the root. Raising the expansion of mycorrhizal hyphae in roots provides an ecosystem service with a crucial role in reducing nutrient losses and increasing crop efficiency in terms of yields and quality. Mycorrhizal patterns in roots are a valuable tool for agriculture in order to evaluate the symbiotic potential of plants and their access to soil nutrients.

How C:N ratios affect plant-microbe interactions with wheat (Triticum aestivum cv. Mulika)

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Carbon decline in agricultural soil alters C:N ratios and there is a growing concern due to associated links to degradation in soil quality. Plants supply large amounts of carbon to soil ecosystems through rhizodeposition which influences surrounding soil microbiota. Here, *Triticum aestivum cv*. Mulika was used to determine the effect of different C:N ratios (high - 32.58, medium - 17.22, and low - 9.37) on plant performance at GS31, GS39, and GS61 growth stages. Physiological, chemical and anatomical measurements were taken alongside root exudate production to measure plant response to treatment, with soil communities being characterised through qPCR and next generation amplicon sequencing. We found that low and medium C:N ratios significantly improved plant biomass (up to 200%) and leaf area (up to 50%) compared to high C:N treatment and watered controls at GS31. Overall, decreases in bacterial 16S rRNA gene abundances were found with low C:N treatment compared to watered controls, with decreases from 1.07×10^8 to 4.39×10^7 copies g^{-1} dry weight. In contrast high and medium C:N treatments showed increases in copy abundance up to 182% higher relative to control levels, with archaeal 16S rRNA showing a similar response. Increases in ammonia oxidising bacteria (*amoA*) and ITS gene copies g^{-1} DW occurred in soils treated with low and medium C:N at several time points with increases from 8.72×10^5 to 1.34×10^6 copies g^{-1} DW (amoA – 160% of control) and from 1.34×10^5 to 2.92×10^5 copies g^{-1} DW (ITS – 218% of control). Competition for bioavailable N between plants and soil microbiota may explain the differences seen in this experiment. Specifically, high C:N treatments may allow heterotrophic organisms to proliferate and utilise bioavailable N (NH_4^+/NO_3^-) before it can be utilised by plants.

Capability of electrical capacitance method to monitor the effects of symbiotic root assotiations on host plants

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Symbiotic associations play a key role in the soybean (*Glycine max*. L. Merr.) nutrition and yield production. The effectivity of bilateral and tripartite symbiotic relationships were investigated in two pot experiments on two registered Hungarian soybean cultivars (Alíz, Emese). The effect of microbial treatments on plant growth, biomass production and stress tolerance was studied. The response of plants to microbial inoculations was detected by root electrical capacitance (EC) method. The EC method is based on the correlation between the size of the root system and its electrical capacitance (C_R) measured between a ground electrode embedded in the soil and a plant electrode attached to the stem-base. The technique is suitable for monitoring root growth and activity *in situ.*
A pre-experiment was intended to find compatible symbiotic partners based on the efficiency of single and co-inoculation using two *Bradyrhizobium japonicum* strains and two commercial arbuscular mycorrhizal fungal (AMF) products. Inoculated and non-inoculated plants were grown in pumice medium. The rhizobial and AMF inoculation can improve photosynthetic efficiency and root activity, but this effect depends on the type of symbiotic assotiation. In the main experiment, treated and control plants were grown under well-watered and drought conditions in pots filled with chernozem soil. Microsymbionts significantly increased, while drought decreased the root C_R. C_R showed a highly significant linear correlation with root and shoot dry mass and leaf area. Plants inoculated with microbes reached the same weight at drought stress as non-inoculated plants at optimal water supply. Our results show the potential of EC measurement to monitor the effect of symbiotic factors influencing root growth and biomass production. This *in situ* technique provides an opportunity to follow the temporal changes in root activity and to select the efficient plant-microbe relationships.

Unravel the role of Glutathione-S-transferase as a molecular barrier of VirE2 from Agrobacterium by direct interaction during Agrobacterium mediated T-DNA transfer in Rice

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Once the *Agrobacterium-mediated* transformation has been established in the year 1994 in Rice, it's biotechnological importance overshadowed the question of recalcitrancy in monocots. Many hypervirulent strains of *Agrobacterium* have been designed for monocots which have a high copy number of virulence proteins but recalcitrancy of Rice (Monocots) is still a big question. It is well established that in *Arabidopsis* (dicot) some host proteins interact with *Agrobacterium* VirE2 in a yeast two-hybrid (Y2H) assay. All of the interactive partners interacts with VirE2 and helps in T-DNA nuclear import. In this study, we tried to identify the molecular interactors of one of the virulence protein of *Agrobacterium,* i.e., VirE2 in Rice (monocot). We identified some interactors through Y2H assay and then confirmed through BiFC assay via confocal microscopy. One of the interacting members, GST interacts with VirE2 in the cell cytoplasm. To address whether GST interaction is a molecular barrier or a molecular amplifier for T-DNA nuclear import, we studied the downstream pathway of this interaction of Vir E2 with GST. The glutathionylation substrate i.e.GSH and GSH synthesis inhibitor (BSO) is added during transformation of Rice. Upon BSO and GSH treatment to the callus of Rice, we observed a more and very low recombinant positive GUS expression, respectively. Further, we also manipulated the level of expression of this particular GST in Rice. Downregulation was done through artificial microRNA mediated silencing, and upregulation of gene was done by overexpressing under monocot-specific ubiquitin promoter for constitutive expression. miRNA mediated silencing of GST in Rice plants proves to be more prone to transformation as compared to the overexpressed GST plants. The study suggested that GST from Rice acts as a molecular barrier during *Agrobacterium-mediated* transformation and GST mediated dysfunctioning of VirE2, possibly by glutathionylation of VirE2 in rice.

Development of multiplex RT-PCR and real-time RT-PCR assays to detect three potato viruses

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Due to the vegetative way of propagation, the potato plant is a host to more than forty viruses. Among them, the most important is potato virus Y (PVY), which can reduce the tuber yield up to 80%. Similarly, a devastating impact can be also imposed by potato leafroll virus (PLRV). The dependence of this virus on its aphid vector facilitated effective control through the use of aphicides. However, it is still circulating in the environment an can propagate in seed tubers. Potato virus M (PVM) is less dangerous in terms of direct yield losses but infected plants attract fungal and bacterial pathogens contributing to the economic importance of this virus. Production of healthy seed tubers requires testing for the most important viruses, usually by DAS-ELISA. Here, we present our data on the development and optimization of sensitive and simultaneous detection of PVY, PLRV and PVM by multiplex RT-PCR and real-time RT-PCR assays.

Breeding for microbiome-mediated disease resistance

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Leaf rust (*Puccinia hordei*) is one of the most important diseases of barley (*Hordeum vulgare* L.) leading to yield losses up to 60% besides a reduction of malting quality. Resistance genes *Rph1-Rph25* are known in barley but most of these have been overcome meanwhile and the primary gene pool of barley is to some extent depleted for new resistance genes. But, priming of barley may offer an opportunity to enhance resistance to *P. hordei*. By quorum sensing of bacteria communities N-acyl-homoserine-lacton (AHL) is produced, which is known to induce systemic resistance in *Arabidopsis thaliana*. The present study therefore aims at the detection of genomic regions involved in priming capacity of barley which is one of the most important cereal crops. For this purpose a diverse set of 200 spring barley accessions is analysed in greenhouse pot experiments for priming efficiency regarding leaf rust resistance and by genome wide association studies (GWAS) to identify quantitative trait loci (QTL).
The plants are treated with bacteria, i.e. repaired *Ensifer meliloti* natural mutant *expR+ch* overexpressing AHL and transformed *E. meliloti* carrying the lactonase gene *attM* from *Agrobacterium tumefaciens* which inhibits AHL production and acts as a control. After three bacterial inoculations plants are infected with *P. hordei* strain I-80 at the three leaves stage. 12 days after infection scoring of the leaf area diseased and the infection type is conducted and biomass production determined. First results revealed significant effects ($p<0.001$) of the bacterial treatment indicating a positive effect of priming on *P. hordei* resistance. Based on the observed phenotypic differences concerning the effect of priming and 23,417 filtered SNP markers derived from the Illumina 9k iSelect-Chip and Genotyping by sequencing seven QTL involved in priming efficiency were identified. Experiments will be repeated twice to validate QTL regions and develop molecular markers for priming efficiency.

Long-lasting jasmonate induced resistance in Arabidopsis thaliana and Picea abies

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Controlling plant pests and diseases via chemical pesticides is becoming unviable due to increasingly stringent regulation, the evolution of pesticide resistance and changes in public perception. Alternative strategies have been suggested for protecting plants including induced resistance (IR), whereby exposure of plants to selected environmental stimuli increases resistance against subsequent attack. While there is a good understanding of the signalling pathways leading to IR, relatively little is known about the durability of IR over the longer timeframes which are important for real-world application. By conducting experiments in both *Arabidopsis thaliana* (Arabidopsis) and Norway spruce (*Picea abies*), we aim to enhance our knowledge of long-term within-generation IR across the plant kingdom. Our work is focused on resistance induced by exogenous application of the phytohormone jasmonic acid (JA) and its derivatives. Initial studies in Arabidopsis have demonstrated that treatment of two-week-old seedlings with JA, can provide enhanced resistance against the generalist herbivore *Spodoptera littoralis* three weeks later. However, this long-lasting resistance comes at the cost of enhanced susceptibility to both necrotrophic and hemi-biotrophic pathogens. Experiments using gene expression analysis, bisulfite sequencing and Arabidopsis mutants, have begun to decipher the molecular mechanisms underpinning the opposing resistance phenotypes. For instance, while JA seedling treatment primed defences effective against herbivores for an augmented induction upon attack, it also resulted in long-lasting repression of defences which resist necrotrophic pathogens. Furthermore, mutants impaired in DNA (de)methylation were affected in the long-term responses to JA, suggesting a role of epigenetic changes. In Norway spruce, molecular (epi)genetic analysis of three-year-old seedlings treated with methyl jasmonate one year prior to biotic stress exposure, will provide insight into whether the mechanisms underpinning jasmonate-IR are comparable to Arabidopsis. We hope that knowledge gained from this comparative study can inform the design of future pest management strategies for use in agriculture and forestry.

Sesquiterpenes produced in roots of Medicago truncatula play a role in defense mechanism against Aphanomyces euteiches

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Sesquiterpenes produced in roots of *Medicago truncatula* **play a role in defense mechanism against** *Aphanomyces euteiches***.**

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AbstractPlant roots are susceptible to a variety of microorganisms, they survive by selecting the beneficial ones and expel the pathogens by secreting root exudates. This process of selection relies on early interactions between the plants and microorganisms. The mechanism involved in this selection process is not clearly understood. In this work, we use *Medicago truncatula* as a model system as it is a natural host for the oomycetic pathogen *Aphanomyces euteiches* (*Ae*) and for the mutualistic symbiont *Rhizophagus irregularis.* After infection with the symbiotic and pathogenic spores, a differentially expressed gene caught our attention that was upregulated after infection with the pathogenic *Ae* spores. Phenotypic analysis of the plants mutated in this gene showed reduced growth after infection compared to the control plants. This indicated its putative role in plant defense. For *in vitro* product identification, coding sequence of the gene was expressed in yeast. It produced a blend of sesquiterpenes and sesquiterpene alcohols, with one major product. Products *in planta* were identified by collecting volatiles after 12 hours of Ae infection in mutant and wild type plants. Few sesquiterpenes could be identified *in planta* but major product was found missing. This major product might be modified *in planta*. In conclusion, we identified a Sesquiterpene Synthase (STS) which may have a defense function against *Aphanomyces euteiches*. Cytotoxicity effect of *STS* products is currently under study.

Towards Development of Next Generation Resistance to Soybean Mosaic Virus

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To date, 67 viruses have been identified from soybean, and 27 are considered as a threat to the soybean industry. Among them, Soybean mosaic virus (SMV) is the most prevalent virus and is recognized as the most serious, long-standing problem in almost all soybean producing areas in the world. The use of genetic resistance is the most effective means to control SMV. Extensive screening for soybean germplasm resistant to SMV resulted in the identification of three independent resistance genes, i.e., Rsv1, Rsv3 and Rsv4, each conferring resistance to SMV with strain specificities. However, recent data from our research and several other international laboratories suggest that the current resistance is very fragile and SMV mutants with a single nucleotide mutation in the viral genome may break down the resistance. Thus, incorporation of all three resistance genes in a soybean cultivar through gene pyramiding may provide durable resistance to SMV.

Bacterial endophytes improve dehydration tolerance in Vitis vinifera L. cv. Glera.

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Several endophytic bacteria are reported to induce resistance to biotic stress and abiotic stress tolerance in several plant species. In *Vitis vinifera* cv Glera different endophytic bacteria have been isolated that show multifunctional promoting traits. We demonstrated that endophytic bacteria can be able to re-colonize grapevine tissues and increase grapevine tolerance to dehydration. To better understand how bacteria induced the observed phenomena, stress-related gene expression were monitored in 8-week-old Glera grapevine plantlets after exposure to drought stress, comparing *Bacillus licheniformis* bacterized plants with non-bacterized counterparts. We observed that many specific drought stress genes were already induced by bacteria colonization, suggesting a priming effect played by endophytes. Moreover, after drought exposure, the levels of stress-related genes had declined in -bacterized plants, suggesting that the endophytes are involved in the drought acclimation process.
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Theme 7: Environmental resilience

The modulation of leaf gas exchange response and stomatal density under elevated CO2 and drought stress in monocots and dicots

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The global climate change has implications on the crop productivity due to the increased CO_2 level accompanied by soil water deficits. Elevated [CO_2] (e[CO_2]) could mitigate the drought stress by improving the water use efficiency as plants grown at e[CO_2] had high photosynthesis rate and lower stomatal conductance in spite drought stress that limits these biochemical processes. According to the leaf anatomy, C_4 plants are characterized by the Kranz anatomy that increase the [CO_2] concentration inside the cells compared to C_3 plants which reflects the different responses under e[CO_2]. The aim of this study was to investigate the modulation of the leaf gas exchange and stomatal density at e[CO_2] and drought stress in different crop species. The species selected in the experiment as dicots were quinoa (C_3) and amaranth (C_4) while as monocots were winter wheat (C_3) and maize (C_4). Plants were grown at ambient [CO_2] (400 ppm) and elevated [CO_2] (800 ppm) under two water regime, well-watered and progressive drought. The results showed that at e[CO_2] the maximal net photosynthesis rate ($A_{n\,max}$) increased significantly in quinoa, amaranth, wheat and maize by 35%, 28%, 36% and 39% respectively compared to ambient [CO_2] showing that the increment was more obvious in monocots. The maximal stomatal conductance ($g_{s\,max}$) was reduced significantly at e[CO_2] by 19% and 25% in amaranth and maize respectively, but not in C_3 plants. For most of the species, A_n and g_s become more sensitive to soil water deficits when grown at e[CO_2]. Stomatal density (SD) was increased in maize and decreased in amaranth and wheat when grown at e[CO_2], while drought increased SD in dicots and had no effect on monocot. These findings are important for underlying the mechanisms regulating leaf gas exchange of crops grown in a drier future and CO_2-enriched climate.

Effect of nursery production techniques and meshcloth shelters during plantation, on field performance of Nothofagus alessandrii, an endangered endemic Chilean specie

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*Nothofagus alessandrii*is an endangered endemic specie according to the IUCN, from the Mediterranean region of central Chile and declared a national monument. The vulnerable state of this specie urges the development of restoration programs. Although specific protocols for nursery plant production and on-field cultural treatments during plantation are largely unknown, lowering the success of restoration programs. Therefore, the effect of nitrogen fertilization and container size during nursery production and the use of meshcloth shelters during plantation were tested with the objective of improve plant quality and field survival within the specie natural distribution in central Chile, characterized by Mediterranean climate. Results showed that increase in nitrogen fertilization produced bigger plants with increased height, root collar diameter and aboveground biomass, also increasing nutrient concentration at whole plant level. While increase in container volume only increased plant biomass on each plant component. At the end of the first growing season on the field, nitrogen fertilization and the use of meshcloth shelters were the factors that increased *N. alessandrii* field survival, while container volume had no effect. Although increase in nitrogen fertilization produced plants morphologically susceptible to drought (bigger plants with higher aboveground biomass and lower belowground biomass), the application of this element increased field survival from 28% to 50%. Also, meshcloth shelters increased survival from 25% to 61% by buffering microclimatic conditions inside them, lowering air temperature, vapor pressure deficit and increasing relative humidity. These results indicate that nitrogen fertilization (at least 200 mg L^{-1} of N) during nursery production and seedling protection with meshcloth shelters at plantation are essential to improve on-field survival of *N. alessandrii* during the first growth season.

Identification of Physiological and Biochemical Markers for Salt (NaCl) Stress in the Seedlings of Mungbean [Vigna radiata (L.) Wilczek] Genotypes

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Salt stress poses a serious challenge to plant growth and development and hence influences the yield and crop productivity. This study investigates the impact of exogenous sodium chloride (NaCl) on the seedlings of six genotypes of mungbean [*Vigna radiata* (L.) Wilczek] by examining some physiological, biochemical stress indicators and some antioxidant enzymes, *viz.* superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT)**,** glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase (GST). Ten-day old seedlings were subjected to salt stress (00 – 100 – 150 – 200 −250 mM NaCl) by split application along with the half strength Hoagland's medium. The salt stress caused a decline in the fresh weight, dry weight, relative water content, photosynthetic pigments (chlorophyll and carotenoids). On the other hand, it increased the electrolyte leakage, proline, protein and total soluble sugar contents. NaCl levels were positively correlated with the production of hydrogen peroxide in leaves. The activity of SOD, CAT APX, GR, GPX and GST increased significantly upon the NaCl treatments and attained its maximum at 150mM for SOD and CAT, at 200mM for APX, GR and GST and at 250mM for GPX. The pattern of increase in the activity was similar in all the genotypes studied, though the quantitative levels were markedly different. The physiological, biochemical and oxidative stress due to H_2O_2 generation and the antioxidant enzymes activity to combat it may serve as the screening provides a basic platform for selecting the stress-tolerant genotypes in the absence of suitable salt-tolerance markers in mungbean.

The Endodermal Passage Cell Just Another Brick In The Wall?

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In old parts of the root, suberin lamellae are deposited in the cell walls of the endodermis. This occurs in a surprisingly similar manner across virtually all plant species. Intriguingly, cells adjacent to xylem poles do not always undergo suberization, and are historically characterized as ”*passage cells*” based on the assumption that they retain capacity for transport in an otherwise sealed area. The term passage cell is somewhat ill fitting, as our knowledge on them so far has remained purely descriptive. Recently, we found molecular mechanism(s) responsible for formation of passage cells in the model plant *Arabidopsis thaliana* (1), and thereby obtained tools to study their identity and whether they truly provide ”passage” across the endodermis. By performing cell-specific Translating Ribosomal Affinity Purification (TRAP), using tissue specific promoters, we found evidence that, although passage cells share developmental features with xylem, they represent an individual cell type. By further investigating the expression of enriched genes, we realized that passage cells, in combination with adjacent cortex and epidermal cells, may form trans-cellular ”funnels” that guide transport to the vasculature. Moreover, our TRAP analysis suggests that these pathways might additionally be involved biotic communication. Based on our work, we propose that the xylem pole endodermis represents a novel cell type with a divergent developmental program that leads to passage cells. The passage cells might, in turn, serve as nucleation points for bilateral communication across cell layers and with the rhizosphere. In combination, our data argue that the well-established bilateral symmetry of the root vasculature should be expanded to include outer cell layers, which provide a novel layer to our current models and understanding of nutrient use efficiency (NUE).

(1) Andersen et al. Diffusible repression of cytokinin signalling produces endodermal symmetry and passage cells, Nature, 2018.

Above- and belowground processes shaping tree resilience to drought

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Drought is a major environmental threat to plants, causing severe limitations at different scales of plant organization and function. The ability to resist the drought impact and recover after drought release determines the overall response of plants to this environmental threat, which is fundamental to the recent concept of plant and ecosystem resilience[1]. While past studies provide a large body of information on drought-induced limitations of plant growth and function, little is known about processes counteracting these limitations during drought and after drought release. Using a set of lab and model ecosystem experiments, in combination with molecular and eco-physiological approaches, we studied the above- and belowground responses of young beech, oak and poplar trees to cycles of soil water shortage and rewetting or to osmotically imposed steady-state drought[2-6]. In these experiments, we show not only the well-known limitations which trees experience under drought conditions, but also mechanisms which trees may activate in roots and leaves to minimize the adverse effect of drought on plant water relations and compensate for the loss of photosynthetic carbon gain. The obtained response patterns also suggest a close coupling of root and leaf function after drought release, in that increasing root metabolic activity triggers the subsequent recovery and stimulation of photosynthetic activity in leaf organs. From this observation, we propose that maintenance and recovery of root function play a key role in tree resilience to drought.

[1]Ingrisch & Bahn 2018, Trends in Ecology and Evolution
[2]Kuster et al. 2014, PLoS ONE
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[6]Sperisen et al., in prep.

a-Tubulin acetylation and stress-induced autophagy development in Arabidopsis thaliana: kinesin recruiting and easing microtubule interaction with autophagosomes

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The crucial role of microtubules in the realization of autophagy development is widely described. In animal cells, microtubules facilitate the process of autophagosome biogenesis and transport by changes of their dynamic state via post-translational α-tubulin acetylation and subsequent kinesin-1 recruiting. Earlier we showed that metabolic, osmotic, salt stresses and exposure to UV-B irradiation of *Arabidopsis thaliana* seedlings lead to autophagy development what is strictly accompanied by α-tubulin hyperacetylation. To explore further the interconnection of α-tubulin acetylation with the expression of genes, apparently involved in autophagy development, their profiling was conducted under the same conditions. The overexpression of *elp3*, histone acetyltransferase subunit of Elongator, was observed under all stressful stimuli. An increase of expression level of *hda14* under metabolic- and osmotic stresses, and *hda6* under osmotic-, salt stresses and UV-B were detected. Analyzing the expression profiling of plant kinesins, homologues of human kinesin-1, we observed the overexpression of certain kinesins under stress-induced autophagy. To investigate the interrelation of autophagy and programmed cell death (PCD) development the transcriptional activity of hexokinases 1, 2 and 3 was analyzed and stress-induced overexpression of certain hexokinase genes was shown. Hexokinase genes expression was down-regulated during the early stages after irradiation, and dramatically increased in 24 h after stress influence. By means of structural modelling it was shown that Atg8, the structural protein of autophagosomes, binds more tightly to hyperacetylated α-tubulin than to non-modified one. The immunohistochemical analysis of α-tubulin acetylation revealed its tissue-specific character, particularly, it is most markedly manifested in young and meristematic tissues, as well as in root tissues (root cap, epidermis and pericycle cells). Obtained results testify the critical role of α-tubulin acetylation in stress-induced autophagy by presumable recruiting of certain kinesins and easing microtubule interaction with autophagosomes; and to determine timeframes of autophagy, interconnected with PCD.

Changes in proteome profiling of potato roots upon soil drought

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Drought is one of the major abiotic stresses affecting plant growth, development and productivity. Potato (Solanum tuberosum L.) have relatively shallow root systems and is moderately drought sensitive crop (Schafleitner et al., 2007) whose yield is drastically restricted by dehydration. Recent evidence indicates that the reprogramming of gene expression results in the reorganization of plant metabolism under unfavourable environmental conditions. Since variations in drought resistance have been observed among different potato cultivars in the present experiments the up- and down-regulated proteins in drought resistant cultivar Gwiazda drought-sensitive cultivar were Oberon assayed in order to establish the molecular markers of the drought. Potato plants, three weeks after initiation of tuberisation, were subjected to soil drought by water shortage for 14 days. Proteins were extracted from potato roots according to standard protocol using TCA extraction method. Samples equal to 30 µg proteins were applied to each gel. Proteins were separated in the first dimension using the isoelectric focusing (IEF) tube gels and in the second dimension using SDS-PAGE. IEF tube gels 7 cm long with pH ranging from 3 to 7 were used. Electrophoresis was carried out at 250 V for 20 min. SDS-PAGE was performed using 12% polyacrylamide gels with 4% stacking gels at 25 mA. Finally, the gels were stained over night with Coomassie blue. Gels were scanned using ImageScanner III GE Healthcare. Protein markers were analyzed using Delta2D software 4.4. Functional distribution of identified proteins expressed in potato shoots under drought stress contained proteins involved in energy and carbohydrate metabolism, cell wall, detoxification, defense mechanism in both cultivars. In roots of resistant cultivar Gwiazda proteins were also involved in nucleic and amino acid metabolism.

Toxicity of ZnO nanomaterials for macrophytes. A physiological study

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Zinc oxide nanoparticles (NP) are increasingly released to the environment from anthropogenic sources (sunscreens, cosmetics, paints, coatings, conductors, etc.). However, little is known about the toxicity of these materials for wetland plants and the relevance of their size. Here the physiological response of *Phragmites australis* (Cav.) Trin. ex Steud to various ZnO nanomaterials was investigated. Plants were grown for 3 months in a nutritive solution containing 0.1, 1, 10, 100, or 1000 mg l^{-1} of various ZnO materials: bulk, NP <100 nm, NP <50 nm, and nanowires of 50 nm diameter. The growth, chlorophyll content, photosynthetic performance, root ultrastructure, and elemental content were analyzed. Zinc oxide treatments of 1 mg l^{-1} and above were sufficient to significantly increase [Zn] in roots and shoots, decrease the dry weight of shoots, and reduce evapotranspiration. Plants treated with 10 mg l^{-1} or higher showed reduced light-saturated net CO_2 assimilation rate (A_s), stomatal conductance (g_s), transpiration rate (E), quantum yield of photosystem II (ΦPSII), and fresh weight (FW) of roots and shoots, while the FWroot/FWshoot increased. From 100 mg l^{-1} ZnO a decrease in root length, chlorophyll content of leaves, maximum quantum yield (Fv/Fm), photochemical quenching (qP), electron transport rate (ETR), and quantum yield of CO_2 fixation (ΦCO_2) was noted, while 1000 mg l^{-1} reduced leaf water vapor deficit (VPD). Differences in the dissolution of ZnO materials were found at 100 and 1000 mg l^{-1}, with NP50 releasing more Zn into solution. Root length was lower for plants treated with NP50. Besides, NP50 and bulk ZnO induced greater reductions of Fv/Fm, ΦPSII, qP, ETR, and ΦCO_2 as compared with the other ZnO materials. In conclusion, ZnO NP are toxic to wetland plants even at the low concentrations currently found in the environment, and the size is a relevant factor for NP dissolution and phytoxicity.

Identification of novel players in rice salt-stress responses using CRISPR/Cas genome editing

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A large amount of arable land is affected by soil salinity, a major cause of crop yield reduction worldwide. Rice, an important food crop feeding more than half of the world, is highly susceptible to salinity stress. The development of salt-stress tolerant rice cultivars is essential to sustain world rice production. Therefore, unravelling key players in the complex molecular networks responsible for rice salt-tolerance is crucial to both breeding and engineering of improved salt tolerant rice. Candidate genes for salt tolerance were identified from genome wide association studies (GWAS) in our lab[1]. The GWAS was applied to a rice diversity panel[2] subjected to different periods of salinity stress. Twenty-nine genes were found to be common to all salinity treatments. Interestingly, all localized to a specific region of chromosome eight, overlapping with quantitative trait loci previously reported to be important for salinity tolerance in rice. Thus, the yet uncharacterized candidate genes in this region are highly likely to affect rice salt stress.
In order to assess the relevance of these high confidence candidate genes, we are characterizing knockout mutants for selected genes. We have used Golden Gate cloning and CRISPR/Cas genome editing to introduce deletions in the coding regions of each gene. Here we show the successful isolation of homozygous mutants for two candidate genes. The first one, *OsWAKL2* (*Wall-Associated Kinase-Like2*), belongs to the large family of plasma membrane receptor-like kinases (RLKs), regarded as potential cell wall ”sensors”. Interestingly, expression patterns in salt sensitive and tolerant rice cultivars shows that *OsWAKL2* is expressed in the sensitive lines only. The second gene, *OsUGE2*, encodes a putative Uridine-diphospho-(UDP)-Glucose/Galactose 4-Epimerase. Expression of *OsUGE2* is induced by salt treatment in different rice cultivars tested. Homozygous knockout mutants for each gene will be phenotyped under salinity stress conditions.
1.Patishtan J *et al.*doi:10.1111/pce.12975(2017).
2.McCouch SR *et al.*doi:10.1038/ncomms10532(2016).

The transcription factor, OsbHLH035, is involved in rice seed germination and recover from salt stress of seedlings through ABA-dependent and ABA-independent pathways, respectively

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The basic helix-loop-helixs (bHLHs) transcription factors (TFs) gene family are important in regulating rice growth and responses to abiotic stress. One of the TFs expression, *OsbHLH035* is induced by drought and salinity. However, its functional role in rice growth, development, and salt response is still unknown. The *in vitro* GUS-staining demonstrates that OsbHLH035 is primarily expressed in germinating seeds and seedlings. Transient or stable expression of OsbHLH035 in orchid suspension cells or rice transgenic plants revealed that this protein is located in the nucleus. The *Osbhlh035* mutants show delayed seed germination under salt stress. Also, the ABA contents are over-accumulated and the expression of ABA biosynthetic genes, *OsABA2* and *OsAAO3*, are up-regulated but the salt-induced expression of *OsABA8ox1*, an ABA catabolic gene, is down-regulated in the germinating seeds of *Osbhlh035* mutants relative to the wild type. Moreover, the *Osbhlh035* mutant seedlings are unable to recover from salt-stressed treatment. Consistently, sodium is over-accumulated in the aerial tissues but slightly reduced in the terrestrial tissues of the *Osbhlh035* seedlings after salt treatment. In addition, the expression of sodium transporters, *OsHKT1;3* and *OsHKT1;5* is reduced in the *Osbhlh035* shoot and root tissues. Furthermore, genetic complementation can restore both phenotypes of the delayed seed germination and impaired recovery from salt-treated *Osbhlh035* seedlings back to normal growth. Taken together, OsbHLH035 can mediate seed germination and seedling recovery after salt stress relief through an ABA-dependent and an ABA-independent activation of *OsHKT* pathways, respectively.

The effect of 3D-clinorotation on Brassica napus seedlings proteome and metabolome

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For plants, gravity is an important polarizing factor. Both orientation in space and development of plants are finely tuned by gravity. Thus, plant growth depends on changes in gravity field or direction of gravity vector occurred in spaceships. These conditions can be partly simulated on Earth by continuous rotation of plants. Fortunately, 3D-clinorotation proved to be an appropriate model in studies of the mechanisms of plant polar growth and development under microgravity conditions. Although plant responses to real or simulated microgravity are well-characterized, the role of microgravity in seed germination and early steps of plant development is still not well-understood. Therefore, here we address the effect of 3D-clinorotation on the germination of Brassica napus seeds by metabolomic and proteomic approaches. In the absence of clinorotation, seeds formed seedlings with normally developed hypocotyls and roots after 24 and 48 h of germination, respectively. In contrast, a 24-h clinorotation resulted in significant changes in seed biochemistry. Thus, GC-MS-based metabolomic analysis demonstrated statistically significant increase in abundances of primary metabolites – carbohydrates, organic and amino acids, in comparison to control. Additional biochemical assays revealed an increase in hydrogen peroxide tissue contents. Interestingly, observed oxidative stress did not affect the rate of seed imbibition and germination. The effects of clinorotation on seedling morphology were clearly observable after 48h. Thus, the seedlings, treated by microgravity demonstrated irregular growth and elongation patterns. But the level of most analyzed metabolites displayed no difference in comparison to control values. The LC-MS-based proteomic analysis revealed 95 up- and 38 down-regulated proteins in seedlings, subjected to clinorotation. The polypeptides, involved in protein metabolism, transport and signaling, were annotated as the functional groups most strongly affected by 3D-clinorotation.

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Hypoxia stress as activator of a primed state in cucumber (Cucumis sativus L.): transcriptome profiling

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In plants, memory is evident in altered responses to environmental stimuli after pre-exposure to the same or related stimuli, termed priming or acclimation. Abiotic stresses i.e. chilling, drought, salinity that usually occur at the beginning of a plant's life cycle, offering natural opportunity for the priming of young plants to enhance stress tolerance in mature plants. Stress memory has been defined as genetic, epigenetic and physiological changes under stress conditions. However, the molecular mechanisms underlying plant stress priming are still largely unknown. In our studies, the hypothesis was evaluated: "Plants that experience the hypoxia stress and then recover, during a second exposure to hypoxia stress, these plants 'remember' the past hypoxia experience, allowing them to get tolerance and improve survival prospects". For that, the transcriptome respond of cucumber roots to waterlogging were examined by high-throughput RNA-sequencing (RNA-Seq). The root hypoxia of two cucumber DH lines cultivated in the greenhouse condition was introduced. The duration of hypoxia was 7 days and then plants were recovered for 14 days and another 7 days hypoxia treatment were established. Genomewide transcriptomic profiling, after first hypoxia, identified 6618 and 9664 differentially expressed genes (DEGs) in the hypoxia-tolerant and the hypoxia-sensitive DH lines, respectively. In the hypoxia-tolerant DH line, there were more downregulated (4018) than upregulated DEGs. Second time stressed plants displayed increase about 23 % of DEGs in the hypoxia-sensitive DH line and decrease about 22 % of DEGs in the hypoxia-tolerant DH line. Gene ontology term enrichment highlighted DEGs exclusive to each of DH cucumber lines. Results provide information to understand molecular mechanism of hypoxia tolerance in cucumber and the effect of hypoxic pre-treatment on improving tolerance to prolong hypoxia.
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Significance of sorbitol metabolism in abiotic stress tolerance acquisition

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Changes in carbohydrate metabolism (in the contents and spectrum) are essential components of plant stress responses. In addition to ubiquitous sucrose, many plants produce, transport and utilize also other soluble carbohydrates with high potential in stress relieve. For example, sorbitol acts as a compatible solute, which participates in osmotic adjustment, osmoprotection and protein and membrane stabilization. The aim of this study is to clarify the sorbitol role in plant stress reactions. Within Plantago genus, halophytes as well as glycophytes are described. Halophytes represent a group of plants well adapted to the life in inhospitable conditions. Sorbitol or sorbitol metabolising enzymes were found also in plants, where sorbitol is not a primary photosynthetic product (e.g. *Arabidopsis thaliana*). In *A. thaliana*, the role of sorbitol haven't been clarified yet. Complete plants (*Plantago maritima, P. lanceolata* and *Arabidopsis thaliana* sorbitol dehydrogenase knock-out mutant) grew under *in vitro* conditions. Levels of soluble carbohydrates in plants exposed to selected stresses (osmotic, cold; individual and combined) were monitored with sorbitol content being the most influenced. More severe growth inhibition of the glycophyte *Plantago lanceolata* compared to the halophyte *P. maritima* was observed, but significant accumulation of sorbitol was observed under prolonged abiotic stress in both species.

Key words: abiotic stress, carbohydrate metabolism, *in vitro* cultivation, Plantago, sorbitol dehydrogenase

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Regulation of sulfur deprivation responses in Chlamydomonas and dual role of truncated hemoglobin 1

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Sulfur (S) is an essential element that plays important roles in many metabolic processes. S can be limiting in the environment and strongly influence ecosystem composition. To study fundamental problems in plant biology, the unicellular green alga *Chlamydomonas reinhardtii* has proved an excellent reference organism. During S limitation, *Chlamydomonas* cells demonstrate increased transcription of numerous genes encoding proteins associated with sulfate uptake and assimilation, internal S recycling and changes in metabolism. Moreover, we show that three truncated hemoglobins of *Chlamydomonas , THB1*, *THB2* and *THB12*, are induced under conditions of depleted S supply. Truncated hemoglobins constitute a large family, present in bacteria, in archaea and in eukaryotes. However, a majority of physiological functions of these proteins remains to be elucidated. Identification and characterization of a novel role of truncated hemoglobins in the model alga provides a framework for a more complete understanding of their biological functions. THB1 underexpression results in the decrease in cell size, as well in levels of proteins, chlorophylls and mRNA of several S-responsive genes under S starvation. We provide evidence that knock-down of THB1 enhances NO production under S deprivation. In S-deprived cells, a subset of S limitation-responsive genes is controlled by NO in THB1-dependent pathway. Moreover, we demonstrate that deficiency for S represses the nitrate reduction and that THB1 is involved in this control. Thus, our data support the idea that in S-deprived cells THB1 plays a dual role in NO detoxification and in coordinating sulfate limitation with nitrate assimilation. This study uncovers a new function for the *Chlamydomonas* THB1 in the control of proper response to S deprivation.

Endogenous ABA level modulates the effect of CO2 elevation on stomatal density and leaf gas exchange in tomato plants

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The leaf gas exchange and the stomatal morphology could be regulated by endogenous abscisic acid (ABA) level and environmental cues. The objective of this study was to examine if the endogenous ABA level was involved in the regulation of stomatal morphology and leaf gas exchange when grown under CO_2 elevation. The ABA deficit mutant of tomato (*flacca*) and its respective wild type were used in the experiment. Plants were grown in greenhouse cells with ambient atmospheric CO_2 concentration (*a*[CO_2], 400 ppm) and elevated atmospheric CO_2 concentration (*e*[CO_2], 800 ppm), respectively. The results showed that the photosynthesis rate (A_n) was significantly greater in both the tomato *flacca* and its wild type grown at *e*[CO_2] compared to at *a*[CO_2]. The stomatal conductance (g_s) and stomatal density (SD) were significantly higher while the leaf ABA concentration was significantly lower in the tomato *flacca* than the wild type at both CO_2 concentrations. Compared to plants grown at *a*[CO_2], the g_s and SD were significantly reduced when grown at *e*[CO_2] in the wild type, however this was not the case in the *flacca* mutant whose g_s and SD were not affected by the CO_2 growth environment. These results indicate that endogenous ABA level plays an important role in regulating the response of stomatal morphology and leaf gas exchange to *e*[CO_2].
Key words: Tomato *flacca*, Abscisic acid, Stomatal density, Leaf gas exchange, Climate change

Solanum and Vitis SPCH, MUTE and FAMA bHLHs as tools to modify stomatal abundance-related physiology

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Abundance, and also size and spatial distribution of stomata are anatomical traits whose influence on photosynthesis and transpiration support a growing body of data. Complex physiological and performance parameters connected to these processes –such as WUE, leaf temperature or productivity-related traits - thus relate to stomatal abundance (SA). In crops, genotypes with various SA combined with appropriate irrigation schedules may show optimized behaviors adapted to different needs.
Three Arabidopsis bHLH-type transcription factors (SPCH, MUTE and FAMA) positively regulate stomata development. Some alleles support modified SA and a few studies indicate that such changes result in modified plant physiology. Translating such alleles to crops may produce genotypes with traits of interest for future climates. But, although the partial conservation of the three Arabidopsis protein functions was determined for the *Physcomitrella patens*, *Oryza sativa* and *Brachipodium* putative orthologues, little is known for crop species, in which the genes controlling stomatal development remain largely unknown.
We identified the putative orthologues for these genes in *Solanum lycopersicum* and *Vitis vinifera*, and cloned the corresponding full length cDNAs and genomic promoter regions. For each of the genes, we fused the coding regions to their corresponding Arabidopsis promoters and mobilized them to genotypes carrying loss-of-function alleles for each of the genes, and determined their capacity for complementation of stomataless phenotypes. We also made conditional (b-estradiol-dependent) overexpression lines and examined their phenotypes. 3000-2000 kb of genomic sequences upstream from the ORFs were fused to GFP and to GUS, and introduced in Arabidopsis. Once we established the functional orthology for some of these genes, we are identifying mutant alleles in tomato and grapevine (by TILLING or eco-TILLING), searching for genotypes conferring physiological traits of interest for future climate scenarios.

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Indirect adaptation for transpiration efficiency in Australian wheat varieties

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Wheat productivity is commonly limited by a lack of water in rain-fed farming systems, especially in Australia. Although Australian breeders have historically targeted increased yield, grain quality and disease resistance, underlying drought-adaptation traits are likely to have been selected for as a by-product. To study breeding trends in Australia post-green revolution (1970's-present), a set of 15 elite wheat varieties with wide adoption and narrow phenological range has been studied in irrigated pots at normal field density. Results revealed changes in transpiration efficiency, stomatal conductance, biomass partitioning and senescence rate in varieties released over the last four decades. While plant biomass did not change significantly across cultivars, differences in partitioning were observed, with modern cultivars having greater biomass allocation to the stems and spikes, but reduced allocation to the leaves. Modern cultivars had a greater number of fertile tillers, less infertile tillers and less senesced leaves at flowering. Most importantly, a significant increase in whole-plant transpiration efficiency was observed with the year of cultivar release, giving promising results for future wheat improvement. Further study is needed to unravel the underlying physiological and genetic processes associated with increased transpiration efficiency and generate wheat lines that produce more 'crop per drop'.

Calcium and H2O2 as signal molecules in response to salt stress in rice

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Calcium and ROS are well-known signal molecules in the transduction of external stimuli, like salt stress. Specific calcium signatures have been detected in early responses to salt treatment followed by a calcium-mediated oxidative burst, likely involved in local and long-distance signalling. Reactive oxygen species generated in response to an abiotic stress, besides their well-known toxic effects, are recently been recognised to play also a role in the complex signalling network of plants' stress responses, in particularly in early signalling events.
In response to salt stress, an early and short on time H_2O_2 burst was detected in the roots of a tolerant rice variety while, in a sensitive variety, a later and long lasting H_2O_2 production was observed, and these different H_2O_2 profiles were associated with a different cell fate (survival versus cell death). The transcription factor SERF1, known for being regulated by H_2O_2, showed a different expression profile in the two varieties, so as genes involved in oxidative stress response and in sodium transport and compartmentalization.
Similarly, a difference in internal (and external) H_2O_2 profile was observed in cultured cells established from the two rice varieties in response to salt stress. Antioxidative systems were also investigated in the two cultured cell lines. The tolerant variety showed innate ROS scavenging systems. This could be the reason why ROS, in particular H_2O_2, can act as signal molecule rather than a damaging one in this variety.
In addition, plants and cell cultures of the two rice varieties, transformed with the genetically encoded Cameleon probe, were analysed upon salt stress treatment for calcium detection. Differences between the two varieties were observed in term of calcium content and dynamics.
These results clearly imply a different response to salt stress of the two rice varieties leading to different physiological fate, senescence versus adaptation.

Is phosphoenolpyruvate carboxylase processed by selective autophagy?

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Phosphoenolpyruvate carboxylase (PEPC) is a key enzyme in the metabolism of carbon and nitrogen, and shows central roles in photosynthesis, respiration, aminoacid synthesis, and the development and germination of seeds. PEPC is subjected to different post-translational modifications (PTMs); the biological function of many of them is unknown. Our group has extensively investigated some of these PTMs: phosphorylation, monoubiquitinatión, NO-related PTMs (S-nitrosylation, Tyr-nitration), and oxidative stress-associated PTMs (carbonylation). Autophagy allows the preservation of key metabolic elements under stress, meanwhile non-essential or dysfunctional elements are directed to cleavage and their components are recycled, ensuring good management of resources and preventing the effects of the dysfunction. Little information is available with respect to PEPC degradation via autophagy, or about the impact of PTMs on PEPC stability.
Two different experimental approaches were used to investigate a possible degradation of PEPC via recruitment by NBR1, which interacts with ubiquitinated proteins, towards selective autophagy. First, pull-down experiments were performed with recombinant GST-NBR1 and extracts from sorghum (leaves, root and seeds), and Arabidopsis (leaves and roots). Anti-PEPC antibodies revealed a 65 kDa peptide that interacted with GST-NBR1 in sorghum and Arabidopsis root extracts Second, the amount of protein, and the ratio monoubiquitinated to non-ubiquitinated PEPC, was measured in Arabidopsis SALK T-DNA lines specifically mutated in autophagy genes. The amount of anti-PEPC immunoreactive protein was increased in leaves of *atg18a*, *atg2*, *atg5* and *nbr1* lines. Specifically, the increase mostly concerned to the band corresponding to monoubiquitinated PEPC.
These results indicate that at least a fraction of C_3-type PEPC is degraded via selective autophagy.

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EXPRESSION OF OsMT1, A METALLOTHIONEIN FROM RICE CLASS-1 GENE CONFERS HEAVY METAL STRESS TOLERANCE IN YEAST CUP? MUTANT (DTY4) AND IN TRANSGENIC ARABIDOPSIS.

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Metallothioneins (MTs) are small (45 to 85 amino acids) , cysteine-rich (almost 30% of their amino acid content), metal-binding proteins that can bind metals via the thiol groups of their cysteine (Cys) residues and appear to be ubiquitous in diverse organisms including mammals, plants, and fungi as well as some prokaryotes. There is 3 class of MTs gene family, among them, class-1 MT play an important role in heavy metal detoxification and homeostasis of intracellular metal ions in the plant. In our study, we have done *in silico* analysis which suggests that different MTs from rice are differentially expressed during growth and development, in various tissues during biotic and abiotic stresses and may have overlapping functions. This study revealed that there is eleven Class I MT genes are present in the rice genome. We ensured the expression level of these eleven Class I MT genes during different heavy metal treatments in rice during 24 hr and 7 days. It showed that during 7-day treatment, one of the MTs, Os12g38051 ***OsMT-I-Id*** was highly up-regulated during different heavy metals (As, Cr, Cd) stresses. Further, we characterized ***OsMT-I-Id*** gene in the heterologous system [yeast cup$^{\Delta}$ mutant (MT mutant)], and *Arabidopsis thaliana*). We had done plate experiment and growth curves in yeast mutant during different heavy metals which showed that this gene gives the resistance against heavy metals. We further characterized ***OsMT-I-Id*** MT **in transgenic** *Arabidopsis thaliana* by various growth parameters and biochemicals assay between OsMT transgenic plant and wild-type plants. We concluded that this MT gene plays a very important role in detoxification of different heavy metals in rice.

Cytokinins-induced stress tolerance- a novel regulatory mechanism based on desensitization of environmental cues

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Transgenic plants were designed to express the key gene of cytokinins biosynthesis (ipt) by fusing to a senescence promoter (pSARK) and indeed displayed delay of plant senescence. Unexpectedly, these plants exhibited improved drought tolerance (Rivero et al., 2007). The autoregulatory system of cytokinins biosynthesis operating in the transgenic plants assures homeostasis of cytokinins levels and maintaining normal growth throughout plant development and aging. Since the spatial and temporal pattern of the ipt expression is dependent on promoter behavior, we have replaced the aging promoter (pSARK) with an abiotic stress-related promoter of the Arabidopsis metallothionein gene (Met). The pMet::IPT transgenic plants displayed normal growth under optimal conditions and exhibit drought and salt tolerance. To decipher the regulatory mechanism underlying the phenomenon of cytokinins-induced stress- tolerance, the following analytical approaches and tools were employed: expression of candidate stress-related genes such as antioxidants and kinases known to be involved in stress tolerance, analysis of short-term kinase activity in tobacco cell suspension, phosphoproteomics and bioinformatics analysis. The results indicate that components of stress signaling and tolerance pathways known to be activated under stress are slowing down by cytokinins either by adding exogenous cytokinins or by upregulating biosynthesis of endogenous cytokinins .Kinase activity was reduced when exogenous cytokinins (BAP) were added to tobacco cell suspension under salt stress conditions. Phosphoproteomics of tobacco cell suspension treated with BAP indicate that more than 50% of the identified phosphoproteins were downregulated under drought stress conditions. We hypothesize that upregulation of cytokinins levels under abiotic stress de-sensitize known signaling pathways that normally are being activated under stress and consequently inhibit growth. Our data support the notion that overproduction of cytokinins under stress diverts the inhibitory growth pattern into a better mode of growth and biomass production.

Nutritional and water conditioning during nursery production of Beilschmiedia berteroana plants: A specie with high ecological value and in endangered condition in Central Chile

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Beilshmiedia berteroana(Gay) Kosterm is a native evergreen chilean specie declared as endangered by the IUCN (2001). However, restoration efforts of this specie within Mediterranean environments have been unsuccessful by the lack of nursery production protocols for quality plants. Thus, our objective was to evaluate the effect of nitrogen and potassium fertilization on morpho-physiological performance of *B. berteroana* plants submitted to water restriction (WR) during hardening phase of nursery production. During rapid growth phase plants were fertilized with two nitrogen and potassium levels (100 and 300 mg L^{-1}), and during hardening stage plants were divided in non-WR treatment with plants maintained at 50% of soil water content and WR treatment that consisted in decreasing soil water content down to 25%, associated to photochemical efficiency of photosystem II (Fv/Fm) values lower than 0.55, and then rehydrate to field capacity, performing this in a cyclic fashion during a month. Results indicated that, total biomass was not affected by fertilization nor WR treatments. However, a decrease in root biomass was observed in WR plants. Nitrogen and calcium contents in whole plant increased significantly with 300 mg L^{-1} nitrogen fertilization. Also, while this fertilization treatment decreased Fv/Fm values during WR, ten days before the 100 mg L^{-1} treatment, recovery of Fv/Fm values was rapidly achieved during rehydration. Finally, we conclude that an increase in nitrogen fertilization (300 mg L^{-1}) has a positive effect for recovery of photochemical efficiency values after a stress caused by WR treatments while potassium fertilization has no effect. We strongly suggest the use of high levels of nitrogen fertilization during *B. berteroana* nursery production, specially when plant is established on Mediterranean climates, as this strategy can help plants to recover faster from drought periods.

The role of carbohydrates in drought and cold response of Fragaria sp.

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Drought and extreme temperatures are among the most common abiotic stresses that cause morphological and physiological changes limiting growth, development and productivity of plants. Different plant species and even individual cultivars often differ in the degree of tolerance to the various stresses due to a wide range of mechanisms ensuring abiotic stress tolerance or avoidance. Changes in carbohydrate metabolism undoubtedly play an important role in the stress response as carbohydrates serve not only as an energy and C reserve, but also as osmolytes, ROS scavengers or signal molecules. In this study, the link between non-structural soluble carbohydrate status and the reaction to drought, cold, and also their combination, better reflecting in vivo situations, was examined. The plants of the *Fragaria* genus were selected as model organisms, because they belong to economically important family Rosaceae, which was not, in terms of stress tolerance, intensively studied yet. Controlled conditions, targeted exposure to stress factors and changes in endogenous carbohydrate levels were obtained through the mixotrophic or photoautotrophic cultivation *in vitro.* In strawberry plants and callus cultures exposed to the experimental stress conditions, several characteristics were followed, such as relative water content, biomass accumulation, content and spectrum of soluble carbohydrates and the amount of malondialdehyde as an indicator of oxidative stress, with the aim to contribute to understanding of carbohydrate role in plant stress response.
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Flood tolerance of wheat - shoot and root traits conferring waterlogging and submergence tolerance

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Worldwide, floods have detrimental effects on yield of wheat. In course of the WheatSUB project we *i*) highlighted traits responsible for wheat waterlogging (soil flooding) tolerance, *ii*) documented contrasting submergence tolerance within wheat germplasm and *iii*) assessed the role of leaf gas films in conferring submergence tolerance of wheat.
i) Meta-analyses of literature data revealed that wheat suffers a 43% yield loss (median value, *n*=206) as sub-optimal O_2 conditions restrict root N uptake and translocation to the shoots, with N deficiency causing reduced shoot growth and grain yield. Meanwhile, some genotypes were more tolerant, resulting from higher anoxia tolerance of seminal roots, higher number of adventitious roots and from aerenchyma formation within these roots. We conclude that future breeding should focus on seminal root anoxia tolerance, root internal aeration and better N-use efficiency; exploiting the genetic diversity in wheat for these traits should enable improvement of waterlogging tolerance.
ii) In an interdisciplinary approach using physiologic, genetic and metabolomic methods we investigated if wheat cultivars hold contrasting submergence tolerance, and if submergence tolerance is linked to shoot carbohydrate consumption rates as seen in rice (*Oryza sativa*). Complete submergence of two wheat model cultivars revealed that the tolerant cultivar survived submergence 7-day longer than the intolerant. The intolerant cultivar showed accelerated leaf senescence, evident from leaf chlorophyll and metabolic fingerprinting. Surprisingly, cultivars did not contrast in carbohydrate consumption rates, suggesting that submergence tolerance in wheat is governed by other traits than in rice.
iii) Finally, assessing the role of gas layers surrounding submerged superhydrophobic leaves revealed that the presence of leaf gas films improved submergence tolerance of wheat, but that variation in leaf gas film thickness and persistence between 14 cultivars was negligible; thereby not supporting a prominent role of leaf gas films in future breeding efforts of submergence tolerant wheat.

Ascorbate enhances cadmium accumulation in a Cd hyperaccumulator Tagetes patula L.

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Role of non-enzymatic antioxidant, ascorbate, in alleviating Cd toxicity was studied in a hyperaccumulator of Cd,*Tagetes patula*. Significantly high levels of MDA, a biomarker of Cd induced oxidative stress leading to lipid peroxidation, was observed in leaves and roots of *T. patula* plants 8 days after exposure to concentration higher than 10 µM CdCl2. Similarly, significantly high levels of H2O2 was observed in leaves of 35-days-old plants exposed to 25 µM and above concentration of CdCl2 for 14 days, but no such increase in H2O2 was observed in roots of same plants. Moreover, levels of total ascorbate (AsA) significantly decreased and increased in leaves and roots, respectively. In addition, root tissues were also able to maintain high levels of reduced AsA pool as indicated by high AsA/DHA ratio. However, AsA/DHA ratios were similar across leaves of untreated and Cd treated plants. Presence of 1 mM AsA in media during Cd toxicity (25 µM CdCl2) abolished the chlorosis phenotype of plants observed under Cd stress and photosynthetic efficiency (Fv/Fm) was similar to that of control non-stressed plants. The improved oxidative stress tolerance in above ground tissue can be observed by the significant increase in total AsA level in leaves compared to Cd stressed plants. Enhanced oxidative tolerance of plants in presence of 1 mM AsA during Cd stress helped in maintaining significantly higher shoot biomass than Cd stressed plants though significantly lower than that of control untreated non-stressed plants. The increase in shoot biomass translated in higher accumulation of Cd in above ground tissue under Cd stress. The results indicate that the growth of *T. patula* plants as well as Cd accumulation can be improved under Cd stress by enhancing the ascorbate pool in above ground tissue.

Water use and water use efficiency of spring wheat lines near-isogenic for the reduced-tillering tin trait across a wide range of water supply conditions

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Water use and water use efficiency of spring wheat (*Triticum aestivum* L.) near-isogenic lines for the reduced-tillering tin (tiller inhibition gene) trait were examined using boundary line analysis with published data from across the Australian wheatbelt. The minimum water required to obtain a measurable yield was less in reduced-tillering than free-tillering lines (70 vs. 95 mm). After this, for every mm increase in water supply, grain yield in free-tillering lines increased more rapidly than reduced-tillering lines (15.4 vs. 12.6 kg ha^{-1} mm^{-1}). The maximum yields were 5.7 and 6.4 t ha^{-1} for reduced-tillering and free-tillering lines, respectively. This result suggests that reduced-tillering wheat is likely to produce more yield in the cropping areas with water supply of less than 200 mm. This needs to be taken into account when planning for reduced-tillering wheat in dryland cropping systems.

Increased xylem sap pH, rather than ABA content and regulation, reduces stomata opening in elevated CO2

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Elevated CO_2 is an important signal regulating stomatal movement and plant transpiration. Abscisic acid (ABA) is a plant hormone best known for its involvement in stress response and stomatal closure, which, along with intact ABA signalling, has been found to be a requirement in stomatal responses to elevated CO_2. We show here that in tomato (*Lycopersicum esculentum*) plants grown under elevated CO_2 (1000 ppm), stomatal apertures are closed to a greater degree than control plants grown under ambient CO_2 (400 ppm), despite having a lower total ABA concentration in the leaves. Plants grown under elevated CO_2 had higher xylem sap pH than control plants, which may be involved in the mechanism inducing stomatal closure. Increased xylem sap pH has previously been shown to induce stomatal closure, independent of ABA concentration. Growth in elevated CO_2 furthermore caused some changes in ABA metabolite regulation; plants grown in elevated CO_2 showed a trend towards lower ABA:ABA-glucose ester (ABA-GE) ratios during both the day and the night, and higher night-time deactivation of ABA to phaseic acid (PA) and dihydrophaseic acid (DPA) than control plants.

Rice classIII peroxidase (OsPRX38) mediated defence response in Arabidopsis thaliana during Arsenic stress

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Class III peroxidase are higher plant-specific peroxidase enzyme, which exists as large multigenic families. They are involved in various physiological and developmental processes like various biotic, abiotic stress and cell elongation by governing several biological progressions for instant lignification and seed germination. In the present study, we identified a secretory class III peroxidase (*OsPRX38*) from rice which excessively increases their expression during arsenic stress condition. Presence of N-terminal signal peptide targets it to the secretory pathway. The 3-D structure model of *OsPRX38* detected the presence of Ca^{+2} ion binding site and a heme prosthetic group at the central position which facilitate their function. Arsenic responsive function of *OsPRX38* is characterized by overexpressing *OsPRX38 in Arabidopsis thaliana.* We observed the increased arsenic stress tolerance in heterologous overexpressing transgenic *A. thaliana* lines compared to wild-type (WT). Further, we investigated the biochemical machinery which bestows the arsenic resistance property of the transgenic plants by analysing the antioxidant network. Transgenic plants showed high SOD, POD activity, low H_2O_2 and maliondealdehyde content, less electrolyte leakage, increased chlorophyll and proline compares to wild type plant. *OsPRX38* gene overexpression also affect the plant production by increasing in total biomass and seeds production in transgenics than WT in arsenic stress condition. In order to investigate the increased plant biomass, we performed some biochemical and microscopic analysis and observed that *OsPRX38* gene also plays role in lignification directly or indirectly. Overall study indicates that overexpression of class III *OsPRX38* in *Arabidopsis,* activates the signaling network of different antioxidant system during the stress condition and enhances plant tolerance.

Active multi-component antioxidative system including robust carbohydrate status is the essence of tobacco tolerance to arsenic stress

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Contamination of soils with arsenic (As) leads to its accumulation in food chain and decreases agricultural production. The significant component of As toxicity is an oxidative stress. Plants have developed a highly organized system to quench free radicals, based on both antioxidant enzymes and antioxidant molecules, including saccharides belonging among the substances with high antioxidant activity. Using two contrasting tobacco genotypes, the project focused on the arsenic-stress responses and followed arsenic forms distribution within plants, growth characteristics and oxidative damage along with the levels of antioxidant system components. The As-tolerant genotype exhibited lower contents of ROS and malondialdehyde (indicator of membrane damage) together with higher contents of glutathione and carbohydrates compared to sensitive genotype. Further, there were significant differences in antioxidant enzymes distribution patterns: in tolerant tobacco, the activities were higher in roots, while activities prevailed in the leaves of the sensitive one. The data support the conception of the ability to cope with oxidative stress being basic part of tolerance to arsenic stress. The stable carbohydrate metabolism was among others viewed as an important protection mechanism against oxidative stress that accompanies arsenic exposure. We assume the obtained results to be valuable for phytoremediation conception, which subsequently can serve to limit contamination of the food chain.

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The effect of GR24 on expression of strigolactone and antioxidant system related genes in dependence on phosphate nutrition

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Phosphorus in a form of phosphate (Pi) represents an essential macronutrient for plants. Its lack or inaccessibility restricts plant development and is considered as a nutrient stressor. Stress conditions in general may lead to an oxidative damage. To prevent this plants have evolved a complex antioxidant system which serves as a protection against unfavorable conditions. Strigolactones (SLs) represent a novel group of phytohormones which is associated with plant responses to Pi deficiency.
The effect of synthetic SL GR24 on *Arabidopsis thaliana* gene expression was investigated during Pi deficiency and in full Pi nutrition (100 µM). Concentration range 0 – 5 µM GR24 was used in 7-day experiment. Genes of interest were divided into 3 groups: SL-related; Pi transporters and the antioxidant system-related.
In leaves, Pi deficiency itself led to up-regulation of *PHT1;4.* This Pi transporter was most likely acting in remobilization of Pi. Simultaneously, thylakoidal ascorbate peroxidase (tAPX) was down-regulated probably as a result of lower rate of photosynthesis. Application of 1 and 2.5 µM GR24 caused similar positive changes in gene expression of SL biosynthetic gene *MAX3* and Pi transporters *PHT1;4* and *PHO1* in the lack of Pi as in full Pi nutrition. In contrast, Pi deficiency had significant impact on expression of SL repressors *SMXL6* and *SMXL8*, as well as on karrikin repressors *SMAX1* and *SMXL2*. The antioxidant system related genes *CAT1*, *CAT3*, *SOD1* and *RBOHD* were up-regulated by 1 µM GR24 only in the lack of Pi. 1 µM GR24 proved to be the most effective in generating changes in gene expression under low Pi conditions in leaves. Application of 5 µM GR24 led to down-regulation of *MAX3*. No negative/toxic effect of the high 5 µM GR24 concentration was observed.
The study was supported by the Charles University, project GA UK No 1086217.

Vegetative and reproductive performance along a climatic gradient does not explain GP in Ranunculus kuepferi.

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Apomictic taxa are mainly polyploid and produce seeds asexually. They often show geographical parthenogenesis (GP) which means that they have latitudinally and elevationally larger distribution ranges than their sexual relatives, and tend to colonize previously glaciated areas more frequently. Various models try to explain this phenomenon, among those polyploidy which might give apomicts a fitness advantage over the diploid parents in stressful habitats. However direct evidence is missing. We therefore pursued an experimental ecological approach by testing the response of the sexual/apomictic model plant *Ranunculus kuepferi* Greut. et Burd. to different climatic conditions. The self-compatible tetraploid apomicts are widespread in the Alps whereas the self-incompatible sexual relatives are restricted to the Western Alps. Experimental plots with apomictic and sexual individuals were established along an elevational gradient of 1000m in the Austrian Alps. Growth performance and reproductive success were investigated in 3 consecutive years.
Growth parameters did not indicate a fitness advantage of polyploidy over diploidy in *R. kuepferi*. Diploids tended to develop even more ramets and thus more leaves per individual and showed a higher specific leaf area than tetraploids. Only the rhizome biomass and the number of roots significantly increased with elevation in tetraploids but not in diploids.
Apomictic tetraploids developed slightly more flowers per ramet and about twice as much carpels per flower than sexual diploids. Nevertheless, seed output was higher in diploids than in tetraploids – mainly due to the high number of malformed ovules.
Overall, for *R. kuepferi*, the study did not confirm the hypothesis that polyploid apomicts show a higher developmental plasticity and therefore cope better with harsh environmental conditions than diploid sexuals. It has to be assessed whether other intrinsic factors such as the capacity for uniparental reproduction can explain the successful spread of the apomicts in the Alps.

How crops regulate sugar export from leaves in response to environmental cues

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In most crop plants, the product of photosynthesis is sucrose, which is transported to other parts of the plant, including fruit and grain, via the highly specialized cells of the phloem. Sucrose transporters (SUTs) are responsible for loading the sucrose into the phloem and are, therefore, the key proteins for determining the rate of carbon export from leaves. So far, little is known about the regulation of sucrose transporters. Specifically, the question of how environmental cues control SUT activity remained unanswered.
Here, tomato, wheat, maize and Arabidopsis plants were exposed to different light conditions and various types of abiotic stress, including drought, heat and salt stress. The amount of exported sugars was quantified with two independent techniques. This data was complemented with measurements of photosynthetic rate, soluble sugar levels in the leaf lamina, *SUT* gene expression and SUT protein abundance.
The data revealed that SUT regulation happens mostly on the transcriptional level. However, environmental cues that increase photosynthesis and sugar export, such as transfer to high light conditions, acted post-transcriptionally. The increased amount of active SUT was shown to be due to reduced protein break-down. Furthermore, the results indicated that environmental cues usually affect SUT activity via the change in leaf sugar status. However, additional experiments also indicated that certain environmental cues affect SUT activity via hormone action.
The present results on the molecular mechanism of the regulation of carbon export from leaves can facilitate the biotechnological improvement of carbon utilization in crop plants growing under challenging environmental conditions.

CO2 elevation retards the response of leaf gas exchange to progressive soil drying in tomato plants

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The objective of this study was to investigate the response of tomato leaf gas exchange to progressive drought stress under ambient (*a*[CO_2], 400 ppm) and elevated (*e*[CO_2], 800 ppm) atmospheric CO_2 concentration. The fraction of transpirable soil water (FTSW) was used to evaluate soil water status in the pots. The stomatal conductance (g_s) and transpiration rate (T_r) were significantly lower while the net photosynthetic rate (A_n) was significantly higher in plants grown under *e*[CO_2] than those under a[CO_2] at onset of drought stress. Along with soil drying, these gas exchange parameters become less sensitive to soil water deficits in plants grown under e[CO_2] as compared to those grown under a[CO_2]. The intrinsic water use efficiency (*WUEi*, A_n/g_s) and leaf water use efficiency (*WUEleaf*, *An/Tr*) of plants grown under *e*[CO_2] was significantly higher than those under *a*[CO_2]. Under *e*[CO_2], the drought-stressed plants had greater leaf area (LA), dry matter (DM) and water use efficiency (WUE) than those grown under *a*[CO_2]. With the decrease of FTSW, concentration of abscisic acid in leaf ([ABA]*leaf*) and xylem sap ([ABA]*xylem*) increased exponentially. At moderate water deficit, i.e., 0.3 < FTSW < 0.5, g_s decreased linearly with increasing [ABA]*xylem*, and g_s in plants grown under *a*[CO_2] declined faster than those grown under *e*[CO_2], indicating that at moderate drought stress the g_s of tomato leaves was mainly regulated by the root-to-shoot chemical signaling under both CO_2 environment, and g_s was more sensitive to [ABA]*xylem* for plants grown under *a*[CO_2]. The results give some predictions of plant retarded response to drought stress in future CO_2-rich climates.

ANTIOXIDANT DEFENSE SYSTEMS AND BIOCHEMICAL MARKERS IN SWEET CORN PLANTS (Zea mays L. saccharata) UNDER SALINITY STRESS

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Sweet corn (*Zea mays* L. *saccharata*) is a variety with high aggregate value and production potential, proving to be an alternative for small and medium-sized farmers. The objective of this work was to investigate the effect of salinity on the growth of two sweet corn genotypes (Tropical Plus and BR 427III). In addition, we sought to compare antioxidant responses, malonic aldehyde content (MDA), electrolyte extravasation and proline content in different plant tissues (root and leaf) submitted to high levels of salinity. The experiment was developed in a completely randomized design, in a 2x6 factorial arrangement (2 sweet corn genotypes with 6 NaCl concentrations - 0, 25, 50, 100, 150 and 300 mM). The results showed a decrease in shoot and root length, fresh and dry shoot mass, stem diameter and chlorophyll index in both genotypes, as the salinity increased. The sodium (Na) and potassium (K) content analysis revealed an increase of (Na) in the roots and leaves of both sweet corn genotypes, and a decrease of (K) in root and leaf, except for the Tropical Plus genotype, in which the analysis of regression was not significant for leaf. An increase in the proline accumulation was noticed when the treatments ”control” and ”300 mM” were compared. The total antioxidant capacity (Ferric Reducing Antioxidant Power - FRAP) and the phenol content increased in response to the stress severity, for root and leaf, in both sweet corn genotypes evaluated. The Tropical Plus genotype showed reduction of MDA in root and leaf, as the salinity increased; and increased electrolyte extravasation. In this work, the antioxidant mechanisms tested did not differ in the response between the different evaluated organs (root and leaf). However, the investigation of these defense mechanisms may contribute with the indication of genotypes that are more tolerant to abiotic stresses.

The Solanum chilense guanine nucleotide exchange factors SchMON1 functions in endocityc trafficking and protection against salt stress.

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During saline stress, plants generally activate different tolerance mechanisms associated with the control and reduction of excess sodium in the cytosol, for example, preventing the influx, increasing the efflux, or maximizing the compartmentalization of sodium in vacuoles. Each strategy requires a constant movement and replacement of membranes and proteins as cation/proton antiporters, proton pumps, and water channels, among others. This is possible thank to an active traffic of vesicles, directed and regulated by master proteins denominated Rab GTPases. To work, the Rab proteins switch between two conformations, an inactive form bound to GDP, and an active form bound to GTP, being this form directed by specific guanine nucleotide exchange factors. In this context, we have identified a gene from tomato wild relative species *Solanum chilense,* MON1 homolog, denominated *SchMON1*. MON1 is a guanine nucleotide exchange factor capable of activating proteins of the RabG sub-family, which participates in the regulation of vesicular traffic between the multivesicular body and the vacuole. Here, we show that the pattern of expression presented an early up-regulate in leaves and roots during saline stress in plant of *S. chilense*, and ectopic overexpression of *SchMON1*, under the control of a specific root promoter, enhanced tolerance to salt stress in transgenic *Arabidopsis thaliana*. This tolerance is consequence of a higher rate of endocytosis, of acidification levels and of accumulation of sodium in the vacuoles of the root cells. Our results suggest that *S. chilense* would use endocytosis and vesicular trafficking as a mechanism of protection against salinity stress, and that *SchMON1* could be used in programs of genetic improvement for the development of tolerance to salinity in crop plants.

A role of phospholipases in plant autophagy

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Autophagy is involved in plant responses to abiotic and biotic stresses and in nutrient remobilisation during both natural and stress-induced senescence. Autophagy is a proteolytic process that is involved in the bulk of degradation of organelles and long-lived proteins and involves formation of phagophores and autophagosomes, double membrane compartments able to enclose and internalize areas of cytoplasm (including other compartments and organelles).
Phospholipases are grouped into three main families designated as phospholipase A (PLA), phospholipase C (PLC) and phospholipase D (PLD) according to their sites of phospholipid hydrolysis. Phospholipase C can be further distinguished according to their substrate. Non-specific phospholipase C (NPC), use structural phosphoglycerolipids such as phosphatidylcholine (PC) as a substrate. By contrast, phosphatidylinositol-specific phospholipase C (PI-PLC) use minor membrane phospholipid phosphatidylinositol-bisphosphate as a substrate. Phosphatidic acid (PA) is the common product of PI-PLC, NPC/diacylglycerol kinase and PLD pathways. In mammals, PA is considered as regulatory molecule participating in formation of autophagosomes. Whether PA is also involved in plant autophagy machinery remains unclear.
Collection of loss-of-function mutants of NPCs, PI-PLCs and PLDs was screened for phenotypic changes related to autophagy. Carbon starvation and etiolated hypocotyl growth assays were used. Two members of phospholipase D family were identified as hot candidates to be involved in plant autophagy machinery.

Effects of potato spindle tuber viroid infection on phytohormone and antioxidant responses in potato (Solanum tuberosum L.)

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Viroids are the smallest known plant pathogens that despite of the relative simplicity of their genomes (246 to 401 nt-long circular, single-stranded RNA molecules) and lack of protein-coding capacity, have the ability to cause diseases in several plant species. The subviral pathogen *Potato spindle tuber viroid* (PSTVd) causes serious disease worldwide in potato, tomato and some other economically important herbaceous and woody plants. When infected by PSTVd, potato develops symptoms such as leaf reduction and distortion, stunting of the whole plant, and abnormal development of tubers. Although transcriptome studies in tomato and potato infected by PSTVd have recently been reported, the physiological responses of plants to viroid infections have not yet been fully explored. In this study, changes in endogenous phytohormone content and antioxidant enzyme activity in different plant parts of Solanum tuberosum cv. Désirée in response to PSTVd infection were examined. The results showed that the endogenous jasmonic acid significantly increased in leaves while castasterone significantly increased in tubers of systemically infected plants compared to that of mock-inoculated control plants 7 to 8 weeks after inoculation. Endogenous indole-3-acetic acid moderately increased only in tubers of infected plants. No difference in endogenous salicylic acid and abscisic acid content was observed between infected and control plants. The trends observed for endogenous phytohormone content were compared with the expression level of selected phytohormone biosynthesis genes using real-time PCR. PSTVd infection also enhanced the activity of guaiacol peroxidase in leaves and ascorbate peroxidase in tubers of infected plants. The activity of catalase decreased in leaves of infected plants, while superoxide dismutase activity remained steady regardless of the treatment and organ type. In summary, the results suggest that PSTVd infection induces changes in endogenous JA, CS and IAA content and peroxidase activity, which might be associated with defense response and symptom development in potato.

The beneficial effects of Si on iron deficiency stress alleviation in barley: modulation of Strategy II genes expression and metal redistribution

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The beneficial effects of silicon (Si) on various abiotic and biotic stresses in plants are well established; however, molecular mechanisms are not completely understood. An ameliorative effect of Si on iron (Fe) deficiency stress has only been shown on plants which use the reduction-based strategy (Strategy I) for Fe acquisition. The aim of our study was to investigate influence of Si on Fe deficiency stress alleviation in a cereal plant which uses the chelation-based strategy (Strategy II) for Fe acquisition, and barley was chosen as a representative.
Si successfully ameliorated Fe deficiency in barley, attenuating chlorosis and biomass loss of the youngest leaves, as well as ROS accumulation, accompanied with the recovered activities of antioxidative enzymes, ascorbate peroxidase and catalase. Si increased Fe content in the youngest leaves of Fe deprived plants, as well as Fe concentration in the water-soluble (w-s) fraction. On the other hand, w-s concentration and total content of optimally supplied microelements, Mn and Zn, were decreased in Si supplied plants. The expression of Strategy II genes was modulated under the influence of Si. An expeditious increase in the gene expression was detected in Fe deficient roots. Moreover, a dramatic Si-promoted upregulation of some of the investigated genes was detected in leaves.
Fe deficiency in plants due to low Fe availability in soils has a considerable impact on both yield and nutritional value of crops. New findings presented in our study may support development of strategies to overcome this substantial agricultural problem.

Effect of abscisic acid or osmotic stress on polyamine metabolism in wheat plants

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Investigations on compounds capable of reducing the stress sensitivity of plants are of great importance in the ever changing environment. Polyamines play a critical role in plant stress adaptation, and it has been increasingly shown that abiotic stress tolerance is chiefly influenced by the role of polyamines in signalling processes rather than by their accumulation. However, recent studies also indicated that polyamine signalling is involved in direct interactions with other metabolic routes and hormonal cross-talks, but the precise mechanism by which polyamines play role in plant responses are largely unknown. According to these, understanding the regulation of polyamine metabolism is of major interest. In the present study, the effects of abscisic acid treatments either alone or in combination with polyethylene glycol-induced stress were investigated in young wheat plants. It was observed that abscisic acid plays a role in the coordinated regulation of the proline and polyamine biosynthetic pathways, which compounds are related to each other through a common precursor. Abscisic acid pre-treatment induced similar alteration in the pattern of polyamine contents as it was found after osmotic stress, namely increased the putrescine, but decreased the spermidine contents in the leaves. These changes were mainly related to the polyamine cycle, as both the synthesis and peroxisomal oxidation of polyamines have been induced at gene expression level. Although abscisic acid and osmotic stress influenced the proline metabolism differently, the highest proline accumulation was observed in the case of abscisic acid treatments. It was also revealed that proline metabolism partly regulated independently and not in an antagonistic manner from polyamine synthesis. This work was supported by GINOP-2.3.2-15-2016-00029.

Damages of Zinc limitation and excess can be relieved by Silicon nutrition in barley

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Silicon is usually not considered as an essential nutrient for higher plants; nevertheless, it is regularly used as fertilizer. Its beneficial role in stimulating the growth and development of several plant species has been well documented, resulting in a mitigation of various biotic and abiotic stresses.
Zinc is an essential micronutrient, and its deficiency causes several problems to plant growth and development; however, excessive Zn levels in soils can result highly toxic to plant cells interfering with metabolism.
The aim of this work was to investigate the effects of Si nutrition in barley plants exposed to Zn deficiency and toxicity. Plants were hydroponically grown in a nutrient medium at variable Zn concentrations, from 0.001 mM (deficiency) to 100 mM Zn (toxicity), with and without 1 mM Silicon.
Zn deficiency resulted in severe decrease of photosynthetic rate; as consequence, ROS scavenging systems were particularly activated, as observed for APX and GR expression and activities. Zn toxicity affects photosynthesis; furthermore, activities, occurrence and expression of antioxidant enzymes were increased.
Silicon supply severely changed these behaviours both on Zn deficiency and toxicity: the oxidative stress was extremely reduced, and photosynthetic efficiency ameliorated.
Remarkably, nitrogen metabolism was affected by the different Zn concentrations: in both stress conditions, free NO_3^- and NH_4^+ contents increased, and nitrate reductase and glutamine synthetase enhanced in their expression and activity.
Intriguingly, Silicon supply mitigated the effects of Zn stress on nitrogen metabolism, specifically increasing NR activity and resuming GS activity at control rates. Silicon increased the levels of free nitrate, possibly improving nitrate uptake and storing in the leaf vacuoles.
In conclusion, silicon supply in the growth medium resulted in a better adaptation of barley plants to Zn limitation and toxicity reducing the effects of stress and improving nitrogen assimilation which is severely affected by Zn availability.

A comprehensive analysis of the Korean fir (Abies koreana) genes expressed under heat stress using transcriptome analysis

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A comprehensive analysis of the Korean fir (*Abies koreana*) genes expressed under heat stress using transcriptome analysis

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Korean fir (*Abies koreana*), a rare species endemic to South Korea, is sensitive to climate change. Here, we used next-generation massively parallel sequencing technology and *de novo* transcriptome assembly to gain a comprehensive overview of the Korean fir transcriptome under heat stress. Sequencing control and heat-treated samples of Korean fir, we obtained 183,094,162 and 161,685,060 clean reads, respectively. After *de novo* assembly and quantitative assessment, 324,825 unigenes were generated with an average length of 441.07 bp. In total, 6,401 differentially expressed genes were detected, of which 2,958 were up-regulated and 3,443 down-regulated, between the heat-treated and control samples. A gene ontology analysis of these unigenes revealed heat-stress-related terms, such as ”response to stimulus”. Further, in depth analysis revealed 204 transcription factors and 189 Hsps as differentially expressed. Finally, 12 regulated candidate genes associated with heat stress were examined using quantitative real-time PCR (qRT–PCR). In this study, we present the first comprehensive characterisation of Korean fir subjected to heat stress using transcriptome analysis. It provides an important resource for future studies of Korean fir with the objective of identifying heat stress tolerant lines.

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Physiological mechanisms of adaptation of conifer seedlings to water deficiency of different intensities

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Global climate warming is accompanied by the aridization of the upper soil. This makes it important to study the mechanisms of adaptation of the main forest-forming coniferous species of Eurasia to the water deficit. We used the water culture with polyethylene glycol for a comparative analysis of the resistance of Scots pine and European spruce seedlings to water deficit of varying intensity (-0.15, -0.5, -1.0 and -1.5 MPa). In both species, an active dose-dependent increase in the concentration of osmolytes in both the roots and the needles was observed. Water stress exerted a strong damaging effect on the root systems of both species, which manifested itself in the fall of the esterase activity of the root cells, the loss of potassium and a sharp increase in calcium content in root cells under severe water stress. A characteristic feature of spruce under conditions of weak (-0.15MPa) water stress was a more than 2-fold increase in the average length of the root hairs. The main root elongation in pine was suppressed already at -0.15MPa, being the most sensitive process to the water deficit among all those studied. Stability of the growth processes in shoots of both species was closely related to the ability for cell wall adjustment. Seedlings of pine, in which this ability was better developed, were able to maintain the growth of the aerial part even with a significant decrease in its water content under strong water stress (down to -1.0 MPa), and the growth of the spruce seedlings was completely inhibited by the decrease of the water content of the aerial part. These differences can be associated with significant differences in habitats for these species, because pine pine in nature are strongly competed by herbaceous plants and therefore must maintain high growth rates in the early stages of development.

Energy metabolism is crucial for heat stress tolerance of Arabidopsis seedlings

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Temperature is one of the most important abiotic factors affecting plant growth and development, and in the context of global warming, the frequency and severity of heat waves are likely to intensify. To counteract the impact of heat stress on membrane and protein stability, plants implement acclimation mechanisms known as the ”heat stress response”, which includes the induction of heat shock proteins and antioxidant systems. We aim to decipher the contribution of energy metabolism as a component of heat acclimation mechanisms that allow plants to survive otherwise lethal temperatures. Using a robust system, whereby *Arabidopsis* seedlings are grown in liquid medium under conditions that cause developmental arrest at the cotyledon stage (Benamar *et al.*, 2013), we have shown that a priming treatment (2h at 38°C) enables seedling survival when, 24h later, they are given a 2h heat shock at 43°C. Respiratory and photosynthetic activities measured right after heat shock were partly protected by the priming treatment. Full recovery was observed one day later in primed seedlings, while damage was irreversible in non-primed seedlings. The preservation of energy transduction thus appears to be essential for heat acclimation. Indeed, based on oxygraphic measurements 2h after heat stress, it is possible to predict long-term survival. By integrating multi-level omics data (transcriptome, proteome and metabolome), we will also discuss the signalling and metabolic pathways which seem to be determinant in deciphering seedlings fate (death or survival).

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Effect of 1-aminocyclopropane-1-carboxylic acid (ACC) on polyamine homeostasis of the Arabidopsis glutathione peroxidase-like5 (Atgpxl5) mutants

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Glutathione peroxidases (GPXs) are important antioxidant enzymes found in animals and fungi. The plant GPXs are mostly like animal phospholipid hydroperoxide glutathione peroxidases (PHGPX). These PHGPXs play a very important role in protecting against oxidative damage of membranes. Plant GPXs use GSH (glutathione) as the reducing substrate but most of them prefer thioredoxin as electron donor rather than glutathione. Moreover, in contrast to animal GPXs, plant glutathione peroxidases contain cysteine in their active site instead of selenocysteine, thus plants contain GPX-like (GPXLs) enzymes. The polyamines (PAs) putrescine (Put), spermidine (Spd) and spermine (Spm) are low-molecular-weight organic cations found most frequently in plants and other in a wide variety of organisms. In plants, PAs are involved in different physiological processes such as growth, tuber formation, cell division, root initiations, fruit ripening, embryogenesis, flower development and response to abiotic and biotic stresses. Polyamines and ethylene share a common precursor in biosynthetic pathways, suggesting that alteration in ethylene production can affect polyamine homeostasis.
Both PAs and *GPXLs* are regulators of ROS homeostasis, they also may participate in redox signalling and important components of cellular responses to stresses. Based on the literature, it has been come to know that the role of polyamines in signalling processes rather than their accumulation chiefly influences abiotic stress tolerance. Our aim was to investigate the role of *AtGPXL5* in response of ACC treatments using *Atgpxl5* insertional mutant plants.
Arabidopsis thaliana wild type and mutant plants grown for 6-week in hydroponic system were treated with 1µM ACC for 24 hours. Beside measurements the changes in free polyamine levels, the activities of diamine oxidase (DAO), polyamine oxidase (PAO) enzymes were determined. The highest differences were found between the wild type and mutant plants in the amount of Spm indicating the relationship between the GPXL5 and ACC and/or ethylene synthesis.

Modelling yield and water use of isohydric and anisohydric barley cultivars for a Nordic and a Mediterranean site in Europe

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Barley (*Hordeum vulgare* L.) is a major grain crop worldwide and the greatest share (ca. 60 MT/yr) is produced in Europe. However, growth rate in yields has flattened and future harvests are threatened by climate change associated with more frequent agroclimatic extremes, especially drought. Plants have developed different water management strategies to deal with reduced water availability: (i) an isohydric water-conserving strategy and an anisohydric risk-taking strategy. Since utilizing genotypic diversity is considered an important strategy to adapt crops to climate change, the the EU-wide ClimBar project set out evaluated > 200 barley lines on different traits, including drought tolerance. Aim of this study was to apply ecophysiological crop modelling to barley genotypes representing typical isohydric (IH) and anisohydric (AH) water response types and analyse their water use and yield performance using historical weather.
Typical barley water response ideotypes were defined and run for two contrasting soils (clay loam; sandy soil) for Jokioinen in SW Finland and Lleida in NE Spain. First, the sensitivity of barley yield and water use to systematic changes in the soil water depletion fraction (hydricity) of barley was performed using crop parameter values from cultivar Scarlett. After testing, simulations for the two water response ideotypes and systematic variations were performed using baseline weather data 1980-2010. Preliminary results indicate that different degrees of hydricity hardly affect yield and water use at the humid Jokioinen site contrary to the semi-humid Lleida. Differences in soil types and effective precipitation had little effect at Jokioinen but considerably influenced yield and water use at Lleida – in particular in dry years. On average, at both sites, the anisohydric ideotype had higher yields and water use than the isohydric one.
Our study suggests meaningful linking of modelling and experimentation can support breeding of drought tolerant barley cultivars under contrasting future conditions.

Isolation and preliminary characterization of the phytochelatin synthase from the liverwort Marchantia polymorpha

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The enzyme phytochelatin synthase (PCS) is a ubiquitous enzyme in land plants, and it is responsible for the synthesis of phytochelatins (PC). Previous studies suggest that PCS likely plays a pivotal role in the response to metal homeostatic needs and toxicity in all tracheopytes, but until now only a few handful investigations have been addressed to functional characterization of PCS from bryophytes. In the study performed here, we cloned the only *PCS* gene present in the genome of the model liverwort *Marchantia polymorpha*. The gene (*MpPCS*) codes for a predicted protein of 530 aminoacids with an overall identity of 49% to *Arabidopsis thaliana* PCS1 protein. In line with all other known PCS, also MpPCS has a highly conserved N-terminal domain and a poorly conserved C-terminus encompassing approximately half of the protein. Overexpression of *MpPCS* in a mutant yeast strain susceptible to heavy metal toxicity increases its tolerance to both cadmium and excess zinc. We are now going to verify, by mass-spectrophotometry, if significantly higher amounts of PCs are synthesized both in cadmium-exposed *M. polymorpha* gametophytes and in the overexpressor yeast strain, compared respectively with metal-untreated gametophytes and yeast transformed with an empty vector, thus demonstrating that the liverwort possesses the ability of producing PCs and that the encoded enzyme is indeed a functional PCS. Even more so, we want to demonstrate that, when overexpressed in *Arabidopsis thaliana cad1-3* mutant (which is lacking PCS1), *MpPCS* can rescue the mutant phenotype of increased susceptibility to cadmium, indicating a remarkable evolutionary conservation of PCS function between *A. thaliana* and *M. polymorpha*.

Global analysis of small RNA level changes and identification of RNA targets in barley upon Pi starvation

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Phosphate (Pi) homeostasis maintenance is controlled by transcription factors like PHR (Phosphate Starvation Response), phosphate transporters, non-coding RNAs, microRNAs (MIR399 and MIR827 genes), protein modifiers and PHO2 (PHOSPHATE2) enzyme involved in Pi -related proteins degradation. We are interested in the role of small RNAs in Pi starvation response in barley. As already known microRNA399 and microRNA827 play an important role in Pi level regulation. These microRNAs target barley mRNAs encoding PHO2 (ubiquitin-conjugating E2 enzyme) in the case of miR399 and SPX-MFS (implicated in Pi sensing or transport) in the case of miR827. We analyzed changes in small RNAs expression profiles in barley roots and shoots during Pi starvation. We performed PARE (Parallel Analysis of RNA Ends) analysis to find a correlation between small RNA level changes and sequences that could be recognized by small RNAs and further cleaved by AGO proteins. Firstly, we identified barley microRNAs which expression was changed significantly during Pi starvation (CLC Genomics Workbench, EDGE test: Bonferroni and FDR P-value correction). The microRNAs were only just over a dozen in number. Most of them were microRNA399 and microRNA827 isoforms. The other barley small RNA sequences with significant changes in expression level were mapped to the defined sequences deposited in Ensembl Plants database (release 38). The mapped small RNAs matched mostly to protein-coding sequences in barley roots and shoots. We identified also novel barley targets for microRNA399 and microRNA827. Our main task was to identify if these small RNAs were involved in mRNA degradation, translation inhibition or they were just the products of RNA degradation. We present barley Pi-starvation response network composed of small RNAs, their RNA targets, and protein products.
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Effect of CO2 elevation on the responses of wheat genotypes to progressive soil drying at anthesis

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Drought is one of the major constraints to wheat yield globally. Elevated CO_2 concentration may enhance the yield, water use efficiency and alleviate the impact of drought on wheat crops. Yet, the combined effects of drought and CO_2 elevation on contrasting drought resistant wheat genotypes have not been investigated. The current experiment was designed to study the response of six spring wheat lines originated from diverse crosses with varied drought resistance. The plants were grown in 4 L pots in greenhouse cells with either ambient (400 ppm) or elevated (800 ppm) CO_2. At anthesis, in each of the cell half of the plants were given drought stress by withholding irrigation and the other half were well-watered at 95% of pot holding capacity. Daily evapotranspiration (ET) of each pot was recorded by weighing. The soil water status in the pot was expressed as the fraction of transpirable soil water (FTSW). A linear-plateau model was used to estimate the response of daily ET to progressive soil drying. The FTSW thresholds at which the relative ET started to diverge from 1 were ranged from 0.4 to 0.64 at ambient CO2 and from 0.56 to 0.74 at elevated CO2 (eCO_2), indicating the plants grown under eCO2 were more sensitive to soil water deficits. Although higher photosynthesis was observed for plants grown at eCO2, the grain yield and shoot biomass were not positively affected in relation to plants grown under ambient CO2. In contrast, relative water contents, grain yield and biomass showed positive correlation with the threshold value of FTSW while stomatal conductance showed negative association.

Auxin homeostasis as a mechanism of abiotic stress adaptation

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Drought and increased salinity are considered as main abiotic stresses causing the most serious problem for global agriculture effecting plant growth, development and crop yield. Plant responses to osmotic and salinity stress are represented by a number of cellular and molecular processes which are regulated by a complex network of phytohormones. Among them, phytohormone auxin (indole-3-acetic acid, IAA) is the key one mediating plant responses to stress conditions. In order to clarify mechanisms regulating auxin homeostasis in response to osmotic and salinity stress, we have focused on the reversible auxin conjugation as a mechanism of stress adaptation. The role of specific genes of auxin homeostasis in abiotic stress response was addressed, namely functional research was performed on the *Arabidopsis thaliana* lines that carry mutations in the genes for auxin amidohydrolases (ILR, IAR3 and ILL). Furthermore, the potential to invoke salt and osmotic stress tolerance by over-expression of selected auxin amidohydrolases was evaluated. To screen auxin activity and distribution under stress conditions auxin reporter line (*DR5::GFP*) was used. Local changes in the auxin levels in the root are connected to the re-adjustment of the auxin homeostasis on the whole plant level in response to salt and osmotic stress.

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Impacts of elevated temperature and CO2 concentration on growth and phenolics in the sexually dimorphic Populus tremula (L.)

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Future climatic changes may alter the balance between the sexes of dioecious species due to differential effects on resource allocation to growth and defense. Our purpose was to study the impacts of elevated temperature and CO_2 concentration on the relative changes in growth and phenolics accumulation in stem bark of the dioecious *Populus tremula*, a keystone species for boreal forest biodiversity and one that is browsed by many mammalian herbivores. In a greenhouse experiment, four female and four male genotypes of *P. tremula* were grown under single and combined treatments of elevated temperature (1.5 °C on average) and CO_2 concentration (720 ppm) for one growing season. Elevated temperature increased the height, diameter, leaf and stem biomass, and in addition decreased the concentration of phenolics including salicylates, flavonoids, phenolic acids, salireposide and lignan. Elevated CO_2 concentration, on the other hand, reduced the height growth (other growth parameters were unchanged) and increased the concentration of phenolics, especially salicylates and phenolic acids. In the combined treatment, *P. tremula* tended to grow more, but elevated temperature counteracted the effect of elevated CO_2 concentration on phenolics accumulation. Although statistically not significant, males tended to have greater growth and a lower level of phenolics than females. The smaller sexual differences were also not strongly affected by climatic factors. Under future elevated temperature and CO_2 concentration, both sexes of *P. tremula* will probably grow more and possibly accumulate lower levels of phenolics, but intersexual differences in growth and phenolics accumulation may be more pronounced after sexual maturation.

Insights into epigenetic environmental stress response and adaptation of barley (Hordeum vulgare)

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Plant epigenetics has gained vast interest, not only as basic research but also as a possible new source of beneficial traits. The mechanisms of epigenetic regulation could be exploited to broaden plant phenotypic and genetic variation, which could improve long-term plant adaptation to environmental challenges. Adverse environmental conditions, e.g. drought, causes plants to prematurely senescence with precarious recycling and major losses in yield. In the last years, it became obvious that in plants, major developmental programs like flowering, seed development, germination and adaptive responses to the environment are under control of epigenetic mechanisms. Our work conducted on barley (Hordeum vulgare) aimed to reveal the epigenetic indexing at senescence and stress responsive gene HvS40 in wildtype and transgenic barley plants with a knockdown of WHY1 (Hvwhy1kd) lines exhibiting a delayed senescence. Drought is causing in wildtype a significant increase in the levels of euchromatic mark H3K9ac all over the analyzed gene regions which correlates with a massive induction of HvS40, while no substantial increase of H3K9ac in why1kd plants was observed. The results suggest that drought induced expression of HvS40 is under epigenetic control but the heritability of these histone modifications loading has to be studied especially because it is known that heritability of epigenetic modifications offers an attractive possible mechanism of adaptive processes.

Different application of salicilc acid can induce different protection during heat stress in Brachypodium plants

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Different application of salicilc acid can induce different proteczion during heat stress in Brachypodium plants
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Salicylic acid (SA) has been known as a signal molecule in the induction of defence mechanisms in plants for a long time. SA is a promising compound for the reduction of abiotic stress sensitivity in plants, since several methods of application (soaking seeds in SA prior to sowing, adding SA to the hydroponic solution, irrigating or spraying with SA solution) have been shown to protect various plant species against abiotic stress factors. Previous results showed that SA and its sodium salt (NaSA) induced various defence mechanisms during Cd stress and the best protection was provided by NaSA. In the present work the effects of various NaSA treatments (soaking seeds in NaSA or spraying with NaSA solution) were investigated during mild heat stress in Brachypodium plants. The maximum efficiency of PSII was not affected by the heat but the Quantum Yield of PSII increased in the control and seed soaking plants at elevated temperature but did not changed in the sprayed plants. Seed soaking and spraying with NaSA affected the antioxidant systems in different ways. Changes in the endogenous SA level and flavonol content were also investigated. A decrease in the abscisic acid level was also observed during heat stress and it was more pronounced in NaSA treated plants especially after spraying.
This work was supported by Grant No. GINOP-2.3.2-15-2016-00029.

EFFECT OF DIFFERENT PHOSPHORUS AND POTASSIUM FERTILIZATION RATES ON SUGARCANE (VAR- CI 4730) TISSUE NUTRIENT CONTENT FOR DIFFERENT MINERAL SOILS IN THE EVERGLADES AGRICULTURAL AREA

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Sugarcane is the main crop in the Everglades Agricultural Area, where it is grown in high organic matter soils known as muck soils. Different problems with muck soils have led to sugarcane being grown in soils with different characteristics. These soils, normally sandy soils, lack updated fertilizer recommendations. The objective of the project was to determine the best extraction method that relates with leaf phosphorus and potassium content. Four different soil series were used with four different fertilizer phosphorus or potassium levels in two separate experiments with samples collections every four months through 12 months. The experiment was an open field pot trial in which each pot contained a combination of the soil series and the fertilizer rate, and the pots were arranged in to four replications or blocks. The experiment was divided in to two different trials with the same setup, one trial for phosphorus and one for potassium. Leaf tissue and soil samples were collected and analyzed for nutrient content. The soil extractable nutrients where determined using four different extractants: acetic acid, ammonium acetate, Mehlich 3 and water. Leaf tissue nutrient concentrations were determined through dry ashing digestion. The sampling date was significant, so the study was analyzed individually for each sampling date to determine factors that were significant for each sampling date. Fertilizer rate was significant for leaf phosphorus concentration in the first sampling date, however, soil series became more significant in the last sampling date. Fertilizer rate was significant for leaf potassium concentration on the two first sampling dates but not in the last where the soil series was significant. The extraction method that had the best correlation for soil extractable phosphorus and leaf phosphorus concentration was Mehlich 3 extractant. However, more research will be required to determine the best extractant for soil test calibrations.

Cadmium(Cd)-induced changes in salicylic acid content and its relation with the expression of putative salicylic acid-induced protein kinase after polyamine treatments in wheat

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Cadmium is a toxic heavy metal, which induces several physiological changes in plant. Salicylic acid (SA) plays a role in the signal transduction during the plant response to heavy metal stress. SA treatment has been found to provide protection against Cd stress; furthermore, concentration dependent increase in the SA content has been reported.
Polyamines (PAs) are small, aliphatic amines. Putrescine (PUT) belongs to the most abundant compounds. Cd-induced accumulation of PAs and their protective effect during heavy metal stress have been already demonstrated (Tajti *et al*. 2018, EES).
SIPK (salicylic acid-induced protein kinase) is known as a ”central master switch” for stress responses. It can be induced inter alia by salicylic acid, jasmonic acid or ozone. The coding gene was characterized among monocots so far only in the rice (OsSIPK). **The expression of a new, putative SIPK gene (TaSIPK) was investigated in wheat identified by sequence homology.**
The main aim of the present experiment was to reveal relationship between SA accumulation and the gene expression level of SIPK during Cd stress in wheat plants. We also would like to investigate the influence of PA pre-treatments on Cd-induced changes in the SA content and the gene expression pattern of SIPK.
Cd-treatment increased the endogenous SA level**. If the putative sequence indeed is the ortholog of OsSIPK, we can be the first one to demonstrate that the expression of SIPK can be induced not only by exogenous SA but also by the Cd-induced accumulation of endogenous SA in wheat**. Interestingly, this tendency was observable only in the root, but not in the shoot. For PUT+Cd treatment the expression was lower compared to Cd treatment, which may be associated with the protective effect of PUT during Cd treatment, which was also manifested in photosynthetic and biomass parameters.

Inducibility, tissue-specificity and product variation of three phytochelatin synthase homeologs from the cadmium-tolerant reed A. donax L.

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Phytochelatins (PC) are a family of Cys-rich oligopeptides constituting the main defence of plants against toxicity of heavy metals and metalloids like cadmium and arsenic. PCs are non-ribosomally synthesized from glutathione by the enzyme phytochelatin synthase (PCS). Dicotyledonous PCS have been characterized in detail, while much less information is available on monocotyledonous ones. In this study, we characterized three different *PCS* genes from giant reed (*Arundo donax* L.), a biomass/bionergy crop with remarkable tolerance to cadmium, to study the evolution of this trait in monocots. Phylogenetic reconstruction with PCS genes from fully sequenced monocotyledonous genomes indicated that the three *A. donax* PCS, *AdPCS1-3*, are most likely homeologs - resulting from lineage-specific whole-genome polyploidization. *AdPCS1-3* genes are tissue-specifically expressed, and *AdPCS1* is expressed about 5 times more than *AdPCS2* and *AdPCS3*. All three genes displayed cadmium-responsive expression in roots, and coded for functional PCSs, as once overexpressed in yeast they confer enhanced tolerance to cadmium stress. Overexpression of *AdPCS1-3* in *Arabidopsis thaliana* further confirmed the typical phenoytype associated to overexpression of functional PCS genes. Mass-spectral analyses detected statistically significant differences in the amount and spectral feature of the PCs synthesized, with AdPCS2 and AdPCS1 producing, respectively, the highest and lowest amount of total PCs in yeast cells. AdPCS1 synthesized the same amount of PC2, PC3 and PC4, while both AdPCS2 and AdPCS3 enzymes produced significantly higher amounts of PC2 and PC3 compared to PC4.
Taken together, these results indicate that the genetic bases of *A. donax* high capability to tolerate the presence of heavy metals is, at least in part, related to the high functional specialization of its *PCS* genes from a transcriptional as well as enzymatic point of view. Thus, transcriptional neofunctionalization and specialization seems to have played a major role in the evolution of Cd tolerance in *A. donax*.

Expression of the yeast vacuolar Na+/H+ antiporter VNX1 in plants improves salt tolerance.

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Accumulation of Sodium inside vacuoles is an important mechanism to overcome salt stress in plants. *Arabidopsis* vacuolar (Na^+,K^+/H^+) antiporters NHX1 and NHX2 were plausible candidates to fulfill these roles but plants in which both NHX1 and NHX2 are disrupted accumulate more Sodium in response to salinity while the growth defects in *nhx1nhx2* double mutant could be partially rescued by supplementing the plants with Na^+ ions (1, 2). This indicates that some other unknown transport system is responsible for vacuolar Na^+ accumulation.
We recently identified the yeast protein Vnx1p, and determined that disruption of the gene resulted in an almost complete abolishment of vacuolar Na^+/H^+ exchange (3). Yeast Vnx1 is member of the type II CAX family, found in fungi, Dictyostelium and lower vertebrates, but we could not demonstrate Ca^{2+}/H^+ exchange activity of Vnx1p (4). While the protein with a C-terminal GFP tag is mostly retained in the ER, use of an internal or N-terminal tag shows that the protein has a vacuolar localization (3, 5)
Overexpression of the gene responsible for vacuolar sodium accumulation in plants could provide a way to improve salinity tolerance. However, as it is still not known how plants exactly accumulate sodium inside the vacuole, overexpression of the yeast vacuolar (Na^+,K^+)/H^+ antiporter VNX1 could be our next best option.
Therefore we engineered a constitutively activated version of Vnx1p by deleting an autoinhibitory N-terminal domain, and used it to transform *Arabidopsis* plants. Transgenic plants showed enhanced Na^+ accumulation, and performed better when exposed to high salt concentrations.
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Biosensor-based imaging technologies reveal in vivo the impact of impaired Fe nutritional status on subcellular processes

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Mitochondria play a central role in the modulation of Fe deficiency-induced responses in plants. The information available on the link between Fe deficiency and mitochondria has been obtained for the most by using traditional *in vitro* approaches such as biochemical and molecular characterization as well as omics analyses. However, mitochondria are highly dynamic organelles in both their function and their morphology. Therefore, the opportunity to observe *in vivo* and with less invasive methods the physiological responses of mitochondria to stress is of paramount value.
In this work the effect of Fe stress (deficiency or excess) on mitochondrial functions was investigated *in vivo* by using Arabidopsis lines expressing Cameleon, roGFP2 and cpYFP biosensors localized in the mitochondrial matrix for the monitoring of calcium ion content, redox status of the glutathione (GSH) pool and matrix pH, respectively. Plants were grown under Fe deficiency, control and Fe excess conditions. Wide-field fluorescence microscopy analysis revealed that Fe stress significantly affected the calcium ion content, GSH redox status and matrix pH of root mitochondria.
Furthermore, we investigated the calcium ion dynamics under Fe deficiency by using the Light Sheet Fluorescent Microscopy (LSFM) technique, which allows i) a fast acquisition at high resolution with high depht optical sectioning of the specimen even respect to confocal laser scanning microscopy and ii) the analysis of *in vivo* single cells and even sub-cellular compartments offering limited manipulation of the sample under examination. LSFM results revealed that mitochondrial calcium ion content increased under Fe deficiency mainly in the external layers of root tips.
Even though these results are preliminary, biosensor-based bioimaging technologies represent an effective approach to monitor Fe-induced metabolic responses *in vivo* with a high subcellular resolution.

Phytoremediation of milk serum by Chlorella minutissima: nitrogen reduction and lipid accumulation in a batch system

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Chlorella minutissima is one of the major microalgae used for intensive production of algalbiomass, for its high growth rate and the ability to accumulate critical amount of mono and polyunsaturated fatty acid under mixotrophic growth conditions. Annually the Italy dairy chainproduces over 6 M tons of whey, a special classified waste by-product due to its high amount of organic compounds (lipids and carbohydrates 4-6% of d.w.) and nitrogen compounds (proteins 0,6- 1% of d.w.). In this study the growth of *C. minutissima*, between different concentrations of whey, in *batch* culture condition, was evaluated. As well as the consumption of lipids, carbohydrates and protein as sources of carbon and nitrogen. In addition, qualitative and quantitative analysis was conducted on microalgae fatty acids accumulation, through gas chromatography techniques with GC coupled with MS and FID detector. Results show an important reduction of the serum protein content (80%) after 13 days at both concentrations of serum tested. However, there was no significant reduction of the carbohydrates content (lactose) in the growth medium. Finally, it was observed a 4-fold increase in concentration of the main fatty acids (C16:0, C18:0 and C18:1ω-9) than the concentration of the identical fatty acids in microalgae control cultures. The data collected allowed to assume the hypothetical use of *C. minutissima* in a phytoremediation process of dairy wastewater, using whey as low-cost and low pollutant valid alternative for mixotrophic growth.

Identification of transcription factors involved in the regulation of deacclimation in Arabidopsis thaliana

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Due to the sessile nature of plants, adaptation to temperature changes is essential for their survival. It has been established that plants can adapt to cold, non-freezing temperatures, by increasing their freezing tolerance in a process known as cold acclimation. However, a decrease in freezing tolerance after a cold stress is essential to maximise the fitness of the plant. The loss of freezing tolerance during warmer temperatures has been defined as deacclimation. While changes at the metabolic and molecular levels have been studied in detail for cold acclimation, the regulation of deacclimation remains largely unknown. Through previous qRT-PCR- profiling of 1880 Arabidopsis transcription factors (Pagter et al. 2017, BMC Genomics 18, 731) 32 genes have been selected as candidates for the regulation of deacclimation. Corresponding knock-out mutants were phenotyped for changes in the rate of deacclimation. These experiments allowed the identification of several potential regulators of the deacclimation process. Of these regulators transcriptions factors *JUB1* and *MBF1c* have been studied in more detail. This work marks the beginning of a comprehensive investigation of the regulation of deacclimation in *Arabidopsis thaliana.*

Winter snow cover is essential for flower bud survival in high mountain plants

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The majority of perennial high mountain plants initiates flower buds in the year before anthesis which, depending on the species, pass winter in different developmental states. High mountain plants usually pass winter below the snow at temperatures between 0 and -5°C. Growing on rocks and windblown ridges, however, they experience free air temperatures which can reach -25 to -30°C. Vegetative organs are usually sufficiently hardy to survive such temperatures, but almost nothing is known about the winter frost tolerance of reproductive structures.
We investigated winter frost tolerance of dormant flower buds in three common plant species in the European Alps with different site preferences. Plants were exposed to short-term (ST, one night) and long-term (LT, one week) frost between -10 and -30°C in temperature-controlled freezers. Reproductive buds were isolated and examined for frost damage using vital staining. In addition, ice nucleation temperatures were recorded by IDTA (Infrared Differential Thermal Analysis). A part of the individuals were returned to the mountain site and, at the time of anthesis, flowering frequency was determined.
The IDTA-analysis showed that short-stem shoots of both saxifrages did not supercool and froze out between -5 to -7°C. Flower bud vitality decreased significantly with temperature and exposure time. Already -10°C LT-frost caused initial frost damage in floral apices. Below -20°C the proportion of injured apices ranged between 30 and 70% (ST-frost), and 50 and 97% (LT-frost). Flowering frequency essentially reflected the results from the vitality test.
It can be assumed that in mountain plants without sufficient snow cover a part of the flower buds do not survive winter which reduces flower abundance and thus the reproductive output in summer. This problem gains particular relevance in the context of winter periods with low precipitation and winter warming events leading to the melting of the protective blanket of snow.

Transcriptomic and metabolic profiling in the proliferative shoot basal region of Nona Bokra, a high salt-tolerant Indica rice

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Salinity tolerance is a complex agronomic trait and diverse mechanisms are associated with different aspects of salt stress. Although SKC1 allele to maintain high K + /Na + ratio is a key determinant of salt tolerance of Nona Bokra, other factors which contribute to seedling survival under saline conditions remains to be elucidated. Salt stress inhibits proliferative activity and stop growth and differentiation of organs. It is observed that shoot explants of Nona Bokra has a distinguished ability to develop roots and shoots under high NaCl condition. To understand a molecular mechanism, we compared time-dependent changes in the transcriptomic and metabolic profiles in the basal proliferative region of the young shoots of rice subjected to salt stress. Compared to Nipponbarre, the salt stress-induced transcriptional repression of cell cycle genes were significantly attenuated in NonaBokra. JA is known to mediate the inhibition of cell elongation and cell division by salt stress. We found that expression of JAZ subfamily of TIFY genes were differentially regulated by salt stress in NonaBokra. Metabolite profiling in the shoot basal region was conducted by LC-Q/TOF MS spectrometer and mass data acquisition was performed in both ESI(+) and ESI(–) modes. Multivariate statistical analysis showed separation of metabolites between Nipponbarre and NonaBokra during salt stress. Potential markers were selected by comparing quantitative differences of mass ions and differential metabolites were identified. Supported by a SSAC grant (PJ013182032018) from RDA.

Transcript, lipid and metabolite changes contribute to low temperature memory in Arabidopsis

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Cold priming, also called cold acclimation, the adaptation of plants to low, non-freezing temperatures, is an important aspect of winter survival of plants. In addition, a tightly regulated deacclimation process, i.e. the subsequent loss of freezing tolerance in spring, is necessary to combine the transition to reproductive growth with the need for maintained freezing tolerance to be prepared for recurring cold periods. The molecular and metabolic basis of cold priming has been investigated in detail, but hardly anything is known about memory of a cold event during a subsequent warm spell. We show that cold priming at 4°C followed by an intervening lag phase at 20°C improves the freezing tolerance of the Arabidopsis accessions Col-0 and N14 after the occurrence of a second cold trigger compared to the primed plants. For the identification of possible molecular determinants of this improved freezing tolerance transcripts, metabolites and lipids were investigated after priming, memory phase and triggering by Illumina-based RNA-Seq, GC-MS metabolite profiling and UPLC FT-MS-based lipidomics. Both accessions showed differences in transcript, lipid and metabolite content when comparing triggered with primed plants. Unique changes after triggering included 93 and 128 differentially expressed genes in Col-0 and N14, respectively, with an overrepresentation in functional categories such as lipid and secondary metabolism, stress, redox and cell wall related reactions in Col-0. Furthermore, in Col-0 and N14 three and six lipids showed significant differences in content. They included three arabidopsides as unique triggering responses in N14. In addition, one metabolite in N14 was identified as a unique triggering response. Possible functions of these candidates will be discussed. This is to our knowledge the first report on molecular and metabolic changes accompanying cold stress memory and triggering by a second cold stress.

Theme 8: Genome Evolution

Polyploidization - way to increase genetic diversity of the crops.

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Nowadays, widening of the assortment of the crops is very important question in horticulture. Many new breeding programs are appeared recently, but when genetics works with new perspective crops they have many problems. One of this problem is a different number of chromosomes even in nearest species inside genes. So, polyploidization is a probably good solution for this problem. Using chemical substance, such as colchicine, for example, we can change the number of chromosomes in target species with the aim to get plants with equal number of them. In some families of useful horticulture plants we observe species with different genomes, but if we will doubled the genome we can have interspecific hybrids. For example, in Brassicaceae family we have a big amount of presence and perspective species that have not equal number of chromosomes. One of the perspective genus is Lunaria. This plant have a very valuable nervonic acid in it oil. Amount of this acid is the biggest among the family. At the same time the number of chromosomes is the highest too (2n=30). But, according to literature sources, in some natural populations we can find plants with a 28 or with 32 chromosomes. At the Zaporizhzhia National University (Zaporizhzhia, Ukraine) several years ago we start works with this new crop. We obtained the interspecific hybrids inside this genus Lunaria. And starts to work with another Brassicaceae species with the goal to obtain polyploidy forms of this species using colchicine and after that create an interspecific hybrids trying increase an amount of nervonic acid in their oils. Now, we have plants of three mustard species that was growing after colchicine treatment of the seeds and we are starting studies of these plants.

Physiological and epigenetic responses to drought in winter wheat

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Drought stress severely affects growth, development and production of crop plants, making tolerance to dehydration an important selection trait in breeding programmes. Identifying morphological, physiological, biochemical and molecular changes in crop varieties that differ in drought tolerance is essential to understanding the mechanisms of plant survival during drought periods.
We evaluated fifteen Bulgarian winter wheat varieties subjected to severe but recoverable drought stress at the seedling stage. The assessment of relative soil water content, leaf water deficit and electrolyte leakage displayed that three of the varieties (Guinness, Katya and Yoana), behaved as drought tolerant, while the other three (Farmer, Bojana, Dobrudjanka), behaved as drought sensitive. We found drastic differences between drought-tolerant and drought-sensitive varieties regarding the photosystem II (PSII) photochemistry, the effective dissipation of untrapped excitation energy from the active PSII reaction centers, and the efficiency of Q_A^- reoxidation. Analysis of leaf epidermal features showed changes in stomatal density and guard cell length, which were consistent with the contrasting drought tolerance. We also examined expression levels of the wheat DNA methyltransferases *TaMET1*, *TaMET2a*, *TaMET2b*, *TaCMT*, *TaMET3* and ATP-dependent DNA helicase *DDM1.* The epigenetic marks, such as DNA methylation, have been recognised as a critical component of plant drought tolerance. Our results showed genotype-specific changes in the expression pattern of the studied DNA methyltransferases and *DDM1*, which suggest the potential role of genotype-specific DNA methylation patterns as another key regulatory mechanism of crop responses to drought.

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Evolution of plant photoperiod transcriptome

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Measuring photoperiod (day length) confers a strong fitness improvement to plants allowing them to anticipate diurnal cycles and to take decisions in a daily manner (1). Closely linked to signals coming from the circadian clock and photoreceptors, day length sensing is also a precise way to detect the changing seasons and take crucial developmental decisions such as the floral transition or the dormancy period (2). Light sensing can be traced back to cyanobacteria, but in eukaryotic algae, an evolutionary tool kit arose that has developed into the complex photoperiodic system of higher plants (3). Using gene coexpression networks (GCN) from algae and plants a conserved machinery to respond to external light clues has been unveiled (4). Here we will discuss the most recent discoveries about the evolution of light perception, measuring photoperiod and the close link to the circadian clock along the evolution of the eukaryotic green lineage (5).
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INFLUENCE OF SALT STRESS AND ALGAL DERIVATIVES ON RETROTRANSPOSON MOBILIZATION IN MICRO-TOM PLANTS

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Solanum lycopersicon L. specie is widespread and it has a high economic importance (i.e. fresh consumption and idustrial transformate. So furter investigations on its genome organization are uspicable. It's well known that tomato genome is predominantly constituted by retrotransposon elements and recent evidence, suggests the possible generation of genetic plasticity in response to anbiotic stresses by retrotransposon mobilization.
The aim of our study was the investigation via Sequence- Specific Amplification Polymorphisms profiling (SSAP) of *Tvv1* retroelement mobility and conseguently insertional polymorphism in *Solanum lycopersicum* cv. Micro-Tom.
The seedlings were grown in presence of two different algal extracts as organic fertilizer (Bioatlantis and Agriges) and different salt concentrations (0-5-10 gL^{-1}).
Results showed that the use of bio-fertilizer with or without salt stress produce new polymorphic bands indicative of different *Tvv1* subfamilies. Using sequence similarity research in MiBase database, we noticed that new transposition events appear to be preferentially inserted in the retroelements belonging to LINEs and Ty1-*copia* family (70%).
Interestingly, three new insertions were found upstream to the start codon of lipase gene (2000 bp) and of *Rcd1* gene (9oo bp) and within the transit-polypeptide belonging to chloroplastic protein Ycf15.
In- *silico* analysis of U3 *Tvv1* region promoter showed the presence of three putative *cis*-acting elements including CAAT, HSE, TC rich- repeat motifs. These putative transcription factor binding sites displayed highly similar sequence to motifs involved in the transcriptional activation of defense genes in plants.
Our results represent the first direct demonstration that bio-fertilizer are able to generate new polymorphisms through retrotransposon mobilization. Our study, therefore, improves the hypothesis that stress modulation of retrotransposons could play a role in generating host genetic plasticity in response to environmental stress.

Analysis of the diploid Thinopyrum elongatum using molecular markers

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Perennial wheatgrasses represent a rich potential source of many agriculturally significant characteristics. These species are important gene pools for wheat improvement. *Thinopyrum* genus are known to possess genes conferring resistance to various diseases, such as leaf and stem rusts, wheat streak mosaic virus, barley yellow dwarf virus, Fusarium head blight, etc.
Thinopyrum elongatum can be easily crossed with wheat, making it suitable for wheat improvement. In pre-breeding programs it is essential to identify the alien introgressions in the wheat background. Based on the recently reported FISH based karyotype of the *Th. elongatum* it is possible to identify the chromosomes individually. These commonly used *in situ* hybridization methods are powerful techniques; but they are less efficient to screening large populations. To increase the frequency of the plant breeding process, marker-assisted selection is an adequate method.
The aim of this study was to identify chromosome arm-specific COS markers for the E genome and to study evolutionary rearrangements between the genomes of *Th. elongatum* and bread wheat. There is only a small number of cost-efficient and chromosome specific marker available for the E genome. We were analysed *Th. elongatum* disomic and ditelosomic chromosome addition lines in Chinese Spring wheat background to identify chromosome specific COS markers for the E genome.
In total we tested 114 COS markers and could assigned 50 markers to various E genome chromosomes. Besides we investigated the macrosyntenic relationship between wheat and *Th. elongatum*. We found several rearrangements in chromosomes 2, 5, 6 and 7 but not in the case the chromosome 1 and 4. The selected COS markers will make it possible to identify gene introgressions in breeding programs and will be useful in the development of new chromosome-specific markers, evolutionary analysis and gene mapping.

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Respiratory type II NAD(P)H dehydrogenases in plants - NDB type

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Plants have several alternative enzymes involved in the respiratory processes, as compared to animals. These alternative respiratory enzymes provide multiple routes for electrons to be transferred in the mitochondrial inner membrane and bypass the oxidative phosphorylation. The alternative enzymes that oxidize NAD(P)H are named type II NAD(P)H dehydrogenases (DHs). Type II NAD(P)H DHs affect cellular NAD(P)H redox status that has vital biological roles in energy metabolism, ROS production, anti-oxidation and reductive biosynthesis.
The regulatory mechanisms of external type II NAD(P)H dehydrogenases (NDB1 and NDB2) by cytosolic Ca^{2+} and pH are studied, with isolated potato mitochondria and *E. coli* membrane contain expressed AtNDB1 as material. Additionally, we recently investigated purified NDB1 and NDB2 to compare.
Evolutionary analysis of the eukaryotic NDB-type proteins revealed ancient and recent reversions between the motif observed in proteins specific for NADH (acidic type) and NADPH (non-acidic type), and that the clade of enzymes with acidic motifs in angiosperms derives from non-acidic-motif NDB-type proteins present in basal plants, fungi and protists. The results suggest that Ca^{2+}-dependent external NADPH oxidation is an ancient process, indicating that it has a fundamental importance for eukaryotic cellular redox metabolism. In contrast, the external NADH DHs in plants are products of a recent expansion, mirroring the expansion of the alternative oxidase family.

Ca2+-dependent external NADPH oxidation is an ancient process compare to external NADH oxidation in plants

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Most eukaryotic organisms, except some animal clades, have mitochondrial alternative electron transport enzymes that allow respiration to bypass the energy coupling in oxidative phosphorylation. The energy bypass enzymes in plants include the external type II NAD(P)H dehydrogenases (DHs) of the NDB family, which are characterized by an EF-hand domain for Ca^{2+} binding. Here we investigate these plant enzymes by combining molecular modeling with evolutionary analysis. Molecular modeling of the Arabidopsis thaliana AtNDB1 with the yeast ScNDI1 as template revealed distinct similarities in the core catalytic parts, and highlighted the interaction between the pyridine nucleotide and residues correlating with NAD(P)H substrate specificity. The EF-hand domain of AtNDB1 has no counterpart in ScNDI1, and was instead modeled with Ca^{2+}-binding signal transducer proteins. Combined models displayed a proximity of the AtNDB1 EF-hand domain to the substrate entrance side of the catalytic part. Evolutionary analysis of the eukaryotic NDB-type proteins revealed ancient and recent reversions between the motif observed in proteins specific for NADH (acidic type) and NADPH (non-acidic type), and that the clade of enzymes with acidic motifs in angiosperms derives from non-acidic-motif NDB-type proteins present in basal plants, fungi and protists. The results suggest that Ca^{2+}-dependent external NADPH oxidation is an ancient process, indicating that it has a fundamental importance for eukaryotic cellular redox metabolism. In contrast, the external NADH DHs in plants are products of a recent expansion, mirroring the expansion of the alternative oxidase family.

A draft genome analysis reveals gene copy number variations as signatures of specialized flavonol metabolism in tartary buckwheat

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We report a draft genome assembly of a high-rutin tartary buckwheat (*Fagopyrum tataricum*), which included 43,771 protein-coding gene models captured in 526 million base-pairs (Mbps). The diploid genome showed a signature of a single whole genome duplication dated before its divergence from common buckwheat (*F. esculentum*) and after the divergence from Amaranthaceae family crop species. Comparative analyses identified an enrichment of transcription factors among Fagopyrum28 specific gene families. The genome of tartary buckwheat included higher copy numbers of genes encoding enzymes synthesizing precursors of rutin than those of common buckwheat and Amaranthaceae family crops. Notably, we identified an inverted tandem duplication of gene loci encoding paralogs of flavonol synthase 1 (FtFLS1) that showed flower-specific expression patterns in the tartary buckwheat. The inverted tandem duplication was also present in the co-linear region in the genome of grape, another species known for high flavonol content, but absent in genomes of common buckwheat and Amaranthaceae crops. The tartary and common buckwheat genomes included uniquely expanded additional copies of enzymes representing specialized metabolic profiles specific to each species. The tartary buckwheat genome has expanded gene families encoding nitrate and phosphate transporters as well as enzymes synthesizing phenylpropanoid and terpenoids, exemplifying an adaptive strategy that optimize growth with a buildup of defense molecules low in nitrogen and phosphorous in a nutrient-poor habitat. Our draft genome, the pan-genomes of buckwheat and the comparative analyses with other plant genomes provide insight and resources for studying the genomic basis of adaptive evolution specially related to flavonoids.

Identification of transcription factor genes and genomic regions involved in anthocyanin biosynthesis in carrot (Daucus carota L.) using whole-genome sequencing and RNA-Seq data

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Anthocyanins are commercially important pigments commonly used for food coloring, and they account for a wide range of colors such as red, purple and black. DNA-binding R2R3-MYB transcription factors, basic helix–loop–helix (bHLH) transcription factors, and WD40 repeat proteins are known to form a MBW complex, which activates the transcription of structural genes in the anthocyanin pathway. Although black cultivars of carrots (*Daucus carota L.)* can accumulate large quantities of anthocyanin in their storage roots, the regulatory genes responsible for their biosynthesis are not well characterized. The current study aimed to analyze whole-genome sequencing (WGS) and RNA-Seq data, and mined MYB, bHLH and WD40 genes that may function as positive or negative regulators in the carrot anthocyanin biosynthesis pathways. WGS data was obtained from carrot samples of various colors, and RNA-Seq data was obtained from differently colored calli, as well as tissue samples from taproots of various cultivars across the course of development. Genome-wide association (GWA) and differential gene expression analyses were performed using WGS and RNA-Seq data, respectively. Differential expression analyses revealed a total of 11 transcription factor genes that were consistently down- or upregulated in the purple color-specific manner, and the expression of these genes was significantly correlated with anthocyanin content. GWA analyses revealed multiple SNPs linked with the purple color located within one of these transcription factor genes, RAX2. The results of this study provide insights into regulatory genes that may be responsible for carrot anthocyanin biosynthesis, and suggest that future focus on them may help improve our overall understanding of the anthocyanin synthesis pathway.

Characterisation of the pan-genome of Vitis vinifera using Next Generation Sequencing

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Analyses of structural variations have shown that a single genome alone does not reflect the complete genomic complement of a plant species, leading to the concept of pan-genome. The latter is composed of a Core Genome (CG), common to all individuals of a species, and a Dispensable Genome (DG), absent from at least one individual. DG appears to be largely the youngest and most dynamic component of the pan-genome. Smaller deletions and insertions, due to recent movement of transposable elements and larger variants referred to as Copy Number Variants (CNVs) contribute to high levels of structural variation. In plants, the dispensable fraction of the genome may be widely influenced by the very active transposable elements. We re-sequenced more than 50 *Vitis vinifera* varieties and two related species and, based on a variety of approaches, we produced a catalogue of Single Nucleotide Polymorphisms (SNPs) and Structural Variants (SVs). SNP markers were used to explore the grapevine population structure, the geographical patterns of diversity, and to assess the genetic relationships between varieties. In order to gain knowledge about DG composition, structural variants of different sizes were detected using paired-end mapping information. We will describe the dispensable fraction of the grapevine pan-genome, its composition and extent, and its phenotypic and epigenetic effects. Using transcriptomic data, we also analysed the effects of gene copy number variation and invasion of transposable elements into the gene space on measures of gene expression. Furthermore, we will explore the mechanisms that generate the dispensable portion. Gaining insights into the composition and function of the DG will contribute to understand the mechanisms that create genetic diversity and phenotypic variation.

DNA repair in plant mitochondria - A complete base excision repair pathway in potato tuber mitochondria

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Mitochondria are one of the major sites of reactive oxygen species (ROS) production in the plant cell. ROS can damage DNA and this damage can lead to mutations. In many organisms such damage is mainly repaired by the base excision repair (BER) pathway. We know very little about DNA repair in plants especially in the mitochondria. Combining proteomics, bioinformatics, Western blot and enzyme assays we here demonstrate that the complete BER pathway is found in mitochondria isolated from potato (Solanum tubersosum) tubers. The enzyme activities of three DNA glycosylases and an apurinic/apyrimidinic (AP) endonuclease were characterized with respect to Mg^{2+} dependence and, in the case of the AP endonuclease, temperature sensitivity. Evidence for the presence of the DNA polymerase and the DNA ligase, which complete the repair pathway by replacing the excised base and closing the gap, was also obtained. The activity of the BER pathway enzymes was higher per mg protein in mouse liver mitochondria than in potato tuber mitochondria suggesting that low mtDNA repair activity is not the explanation for the rapid accumulation of mtDNA mutations with the age of the individual and with evolutionary age in mammals.
We tested the effect of oxidative stress on the mitochondrial BER pathway by incubating potato tubers under hypoxia. Protein carbonylation increased significantly in hypoxic tuber mitochondria indicative of increased oxidative stress. The activity of two BER enzymes increased significantly in response to this oxidative stress consistent with the role of the BER pathway in the repair of oxidative damage to mtDNA.

Genome-wide identification and characterization of the SNARE gene family in tomato (Solanum lycopersicum) revealed induction of genes under salt stress.

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Intracellular vesicular trafficking ensures the exchange of lipids and proteins between the membranous compartments. Under salt stress this is important, since both the removal of transporters and ion channels from the plasma membrane and the compartmentalization of toxic ions requires the formation of vesicles. Soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are the central components of the machinery mediating membrane fusion and key factors for vesicular trafficking in all eukaryotic cells. Taking advantage of the available whole genome sequence of the plant *Solanum lycopersicum*, 63 genes encoding putative SNARE proteins were identified in its genome. The phylogenetic analysis allowed the classification of SNAREs in five groups in tomato as observed in Arabidopsis. Then, the analysis of the structure of the genes, the syntenic relationships and the location in the chromosomes were made for its characterization. In addition, the expression profiles of the SNAREs in different tissues and under saline stress conditions were investigated using microarray-based analyzes. The results indicated that several SNAREs genes had a higher induction in leaf, root, flower and mature green fruit. Along with this, the analysis of comparative expression between a sensitive plant and a tolerant high salinity revealed a differential expression profile under salt stress. Finally, to contrast this expression patterns were analyzed the transcript levels by qPCR of a salt-tolerant wild tomato *Solanum chilense* under salt stress that showed that some of these SNARE genes are induced in early-times. Taken together, these results summarize about the genome-wide identification of SNAREs as well as their differential response to salt stress. Even more, these data can also be utilized to identify potential molecular targets for conferring tolerance to various stresses in tomato.

Identification and characterization of regulatory elements in grapevine

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The identification and characterization of regulatory elements in plants remains a largely unexplored area, complicated by both plant species diversity and also by the differences in genome structure as compared to well-studied animal models. Grapevine (*Vitis vinifera*) presents a useful plant system in which to study such regulatory dynamics, being both a culturally and economically important crop and one that has a suitably complex genome consisting of both euchromatin and heterochromatin. Here, we use multiple approaches to identify and characterize potential regulatory elements in the 487 Mb grapevine genome. A chromatin accessibility assay (ATAC-seq) revealed the presence of more than 19,000 open chromatin regions, with more than 6,000 of these regions in intergenic regions, making them potential enhancer candidates. A motif search revealed the presence of several transcription factor binding sites in these regions, with those for TCP family proteins in greatest abundance. Bisulfite sequencing showed that these regions are hypomethylated for all three cytosine contexts, and ChIP-seq indicated that they are enriched slightly for H3K27ac compared to surrounding regions, but far less than levels of H3K27ac found at the TSS. These possible enhancer candidates are typically within 10 kb from their nearest promoter, and a gene ontology analysis indicated that nearest promoters are significantly enriched for transcription factor genes. Furthermore, a nucleotide diversity analysis indicated that these intergenic accessible regions can be classified into four groups, with varying levels of sequence diversity in the flanking nucleotides as compared to the accessible region. Together, our data provide evidence for the presence of intergenic regulatory regions in grapevine and contribute new information to the growing knowledge of plant enhancers.

Molecular regulator mechanisms associated with bud dormancy in Quercus suber

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To survive the winter, perennial plants enter a dormant state in order to acclimatise to cooler temperatures. The release of bud dormancy happens after exposure to a certain number of chilling hours during the winter ensuring the transition from vegetative to reproductive growth in spring. *Quercus suber*, one of the most economically important forest species in Portugal, also displays seasonal cycles of bud dormancy in winter and bud burst with new vegetative growth and emergence of the flowers in spring. The knowledge of the molecular machinery controlling the shift between bud set and bud burst is crucial to fully understand the reproductive success of this species. Evidence from other plant species suggest that MADS-box transcription factors encoded by *DORMANCY ASSOCIATED MADS-box (DAM)* genes, as well as epigenetic modifications such as DNA and histone methylation, may play a role in this process. In this work, we used the genomic resource generated by the Portuguese Cork Oak ESTs Consortium to identify *Q. suber* homologues of dormancy associated genes and to characterise the phylogenetic relationship of epigenetic modifying enzymes such as DNA Methyltransferases. Using functional and expression analysis approaches we suggest that in *Q. sube*r, *QsDAM* genes may be implicated in the establishment of bud dormancy. Moreover, changes in the expression pattern of epigenetic modifying enzymes and the detection of epigenetic marks during bud dormancy in *Q. suber* studied either by qRT-PCR and immunolocalization techniques suggest that epigenetic regulation may be implicated in bud dormancy and swelling.
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Molecular Phylogeny of Endemic Genus Litsea (Lauraceae) in Western Ghats of India inferred from the analysis of nrDNA ITS and ETS sequences and its morphology

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A species complex group *Litsea* is a dioecious tree species characterized by having aromatic leaves, coriaceous inflorescence, introse anthers with hairy stamens. In the Western Ghats areas of Karnataka state, India; 12 species of *Litsea* are distributed. These species are almost identical in nature and are difficult to identify using only the morphological characters that to when they are not bloomed. Many researchers are trying to resolve the systematic complexity of the genus by different approaches. In the present study, morphological and molecular data were used to explain the systematic complexity and phylogeny of the group. In this study we have used both morphological characters as well as molecular phylogeny characterization by using certain primers. For morphological data set we have used 16 phenotypic characters among 12 *Litsea* species along with two outgroup species collected from Central Western Ghats. For molecular studies total genomic DNA was isolated by freshly collected leaf samples, amplified and sequenced by using different primer combination for comparing the phylogeny results. The sequence fragments of each taxon were first edited individually then analysed and aligned in the software Sequencher 5.2. The combination of ITS1, ETS1 and 18S-IGS primers data was found to be very useful to get phylogenetic tree. Addition of more species from different geographical regions may yield a clear phylogeny of the genus.
KEY WORDS: DNA, ETS, *Litsea*, Phylogeny, Western Ghats

Conservation and diversification of the CONSTANS gene family in photoperiod-mediated flowering

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In temperate regions with strong seasonality, plants need to precisely coordinate their flowering with favorable conditions. Temperate grasses such as barley and wheat flower in response to long photoperiod in the spring. Barley and wheat belong to the grass subfamily Pooideae, which dominate temperate regions. How Pooideae as a subfamily, whose common ancestor is likely a tropical short day plant, evolved into temperate plants flowering in long days is unknown. Studies in *Arabidopsis thaliana,* which flower in response to long days, show that at the center of flowering is the gene CONSTANS (CO). Under long days, expression of CO is regulated by circadian clock genes and the coinciding expression of CO and the FLOWERING LOCUS T (FT), the main floral integrator gene in angiosperms, induces flowering. This mechanism provide a molecular explanation for the external coincidence model. In this study we selected three early diverging Pooideae species to test if the CO external coincidence model holds true for non-model Pooideae grasses and thus contributes to the success of Pooidea species in temperate regions. Two were long-day species (*Piptatherum miliaceum* and *Melica nutans*) and one was a short-day species (*Nassella pubiflora).* In detail, we investigate the expression profiles of the CO homolog in plants growing in long and short days. We predict that in long-day plants expression of CO will similar to *A. thaliana*, whereas it will be altered in the short day responsive species. Possible transcriptional activators for CO genes, the circadian clock gene pseudoresponse regulator protein 37 and Phytochrome C are otherwise central genes involved in day length dependent flowering in model Pooideae species. We included those genes in our expression analyses to test if their role is conserved throughout the Pooideae. This study will contribute to the understanding of evolution of seasonal flowering.

Theme 9: Natural Products

Light quality affects biomass and biochemical characteristics in in vitro-cultured *Cnidium officinale* callus

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Callus cultures of *Cnidium officinale* Makino were maintained on the Murashige and Skoog medium with 0.5 mg·L^{-1} each of 2,4-D and BA under monochromatic (red or blue), mix (red + blue), or white (control) light or dark provided by LEDs. Callus thereby treated showed different responses, with respect to biomass, contents of total phenol and flavonoids, antioxidant activity, and importantly expression of antioxidant enzymes. Dark-grown callus after 4 weeks displayed maximum 2 g of callus fresh weight that was comparable to 1.4 g in the mix light. Total phenol and flavonoid contents increased in the mix light than white light or dark condition. However, DPPH free radical scavenging activity remained the same among different light conditions. The total protein content significantly increased (4.3 mg·g^{-1} FW) in the red than white light. It was noted that ascorbic acid peroxidase activity was significantly higher in the white light and was comparable to that of the mix light, while activities of catalase (CAT) and guaiacol peroxidase (GPX) were higher in the dark than the other treatments. The results are potentially useful for practical applications. This is the first report documented the importance of light quality on the production of phenolic acids and flavonoids in *C. officinale* Makino in in vitro cultures. Further studies are recommended to make these findings amenable at an industrial scale for production of secondary metabolites.

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Nepetalactones profiling and putative iridoid synthase expression analysis indicate trichome specific localization and developmental regulation of iridoids biosynthesis in leaves of Nepeta rtanjensis

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The majority of species belonging to genus *Nepeta* (fam. Lamiaceae) produce and accumulate nepetalactones as the main secondary metabolites. Nepetalactones are iridoid monoterpenoids recognized for their wide spectrum of high-value bioactive properties, namely antimicrobial, cytostatic, anti-inflammatory, phytotoxic, etc. However, biosynthetic pathway of these compounds in plants remains to a great extent unexplored. Most probably they derive through general iridoid biosynthetic pathway leading to nepetalactol, a common intermediate of all plant-produced iridoids, that is formed in a reaction catalyzed by iridoid synthase (*IS*, EC 1.3.1.99). Present investigation entails homology based degenerative primer approach which led to the identification of two promising iridoid synthase candidates (*NrIS1* and *NrIS2*) in leaves of *Nepeta rtanjensis* Diklić & Milojević, rich in *trans,cis*-nepetalactone (*trans,cis*-NL) and dehydronepetalactone (DNL). Comparative UHPLC/qqqMS profiling of nepetalactones in detached trichomes and abraded leaves, complemented with Raman spectroscopy and dichloromethane dipping experiment highlighted trichomes as the main site of their accumulation. This was further supported by the significantly high trichome/abraded leaves expression ratio of *NrIS1* (~31) and *NrIS2* (~25), which strongly indicated the involvement of these genes in nepetalactone biosynthesis. High correlation between *NrIS1* expression profile and *trans,cis*-NL and DNL contents in five developmental stages of *N. rtanjensis* leaves confirmed developmental regulation of nepetalactone biosynthesis. Concentrations of *trans,cis*-NL and DNL were 22-fold and 80-fold lower in the oldest leaves investigated than in the youngest leaves, respectively. In addition, the expression pattern of the *NrIS1* followed the same trend exhibiting 7.4-fold higher expression in the oldest leaves compared to the youngest ones. The present study on spatial and developmental regulation of the nepetalactones production and accumulation in *N. rtanjensis* leaves, as well as the identification of two *IS* candidate genes is a proof-of-the-concept research that serves as a cornerstone for future studies on elucidation of the nepetalactones biosynthesis.

Triterpenoid saponins in a wild crucifer - biosynthesis, evolution and structure activity relationships in plant-insect interactions

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It is well known that plants produce specialized metabolites for defense, yet we know little of how they actually evolved. Certain classes of chemical defense compounds have evolved repeatedly in plants, facilitated by recruitment of genes from a few old gene families. This was probably mediated by gene duplications, either locally or by whole-genome duplications, followed by selection on their enzyme substrate and product specificity. The initial assumption that divergent evolution is the major player is being challenged, and it appears that convergent evolution is surprisingly common in specialised metabolite pathways (Pichersky and Lewinsohn 2011).
Triterpenoid saponins comprise a highly diverse group of specialised metabolites, derived from 2,3-oxidosqualene, the precursor for sterols in most eukaryotes. They are widespread in different plants families suggesting that their biosynthesis has evolved convergently.
We have developed the wild crucifer *Barbarea vulgaris* as a model system to study the biosynthesis of triterpenoid saponins and cloned genes encoding key steps in the biosynthesis using a combination of QTL mapping and transcriptomics/genomics. A draft genome sequencing revealed that the genes in the pathway are highly duplicated but unlinked in the genome which is in contrast to other known triterpenoid pathways. Reconstitution of the encoded enzymes in yeast and/or tobacco leaves showed that the enzymes in the *B. vulgaris* saponin pathway produce multiple products explaining how a few genes can produce the observed structural diversity. LC-MS-NMR identified more than 49 saponin structures, of which only some correlated with insect resistance. Structure-activity relationships studies using *in vitro* produced saponins or saponin reconstituted in tobacco leaves presented to insects, such as the crucifer pest Diamond back moth, has facilitated to unravel which chemical features of saponins influence toxicity to specific insects.

Expression analysis of flavonoid metabolism in response to abiotic stress in two tomato species

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Plant secondary metabolites (SM), such as the phenolic flavonoids that can be induced by stress, have been shown to possess beneficial properties for mankind and are therefore valuable resources for pharmaceutical applications. One beneficial property of plants secondary metabolites is antioxidant activity, associated also with the group of flavonoids. The aim of this study was to investigate the effect of several abiotic stress treatments and treatment combinations on leaves of young tomato plants with a special focus on expression analysis of structural genes related to flavonoid biosynthesis and antioxidant activity.
A commercially relevant, cultivated tomato (*Solanum lycopersicum* var. Lyterno) and the wild relative *Solanum pennellii* LA0716 were subjected to several abiotic stresses, such as nitrogen starvation, decreased temperatures, elevated light radiation and combinations thereof. Growth data and the total antioxidant capacity (TAC) were investigated to estimate the plant's stress level. Candidate genes for expression analysis were selected on the basis of transcriptomics data. The relative expression of structural flavonoid metabolism genes was analysed with quantitative real-time PCR and validated mutually with transcriptome data. Decreased temperature and nitrogen deficiency, and combinations, were identified as abiotic stress treatments that contribute to decreased growth and elevated TAC. Nitrogen starvation (also in combination treatments) caused the most effective upregulation of flavonoid biosynthesis genes. Observed differences in the regulation of single structural genes between the studied species indicate that abiotic stress treatments can be used to induce specific, valuable SM in tomato plant residues. The results suggest that abiotic stresses have each a characteristic impact on SM synthesis in the studied species, that thus can be used to increase SM in tomato plant residues for possible extraction in industrial applications.

In vitro and in vivo transformation of Centaurium erythraea Rafn extracts and resulting bioactivities

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The main secondary metabolites of *Centaurium erythraea* Rafn are biologically active secoiridoid glycosides (SGs) swertiamarin, gentiopicrin and sweroside. Removing the non-reducing terminal glucosyl residues from glycosylated compounds (deglycosylation) leads to the formation of highly active, but unstable aglycones.
The present study was designed to evaluate the differences in biological activities between non-hydrolyzed and hydrolyzed methanol extracts (ME and HME, respectively) and major SGs of *C. erythraea*. Metabolite profiling of extracts following their β-glucosidase mediated enzymatic hydrolysis revealed significant changes in β-d-glucoside/aglycone ratio of secoiridoid compounds and phenolics. Developed and validated UHPLC/DAD/ + HESI−qqqMS method for the identification and quantification of SGs and their aglycones in both ME and HME revealed gentiopicral and erythrocentaurin as the major aglycones. The same metabolites also appeared after the hydrolysis of swertiamarin, the dominant SG of *C. erythraea*.
The present study was further aimed at ascertaining the differences in biological activities between ME and HME. High antimicrobial activities of both ME and HME, which surpassed the effects of the reference antibiotics and antimycotics, could be attributed to SGs in either of the glycosylation forms. Plant extracts and pure SGs were especially effective against most of the tested Penicillium species. Nonetheless, *Penicillium funiculosum* developed a mechanism to exceed sublethal concentrations of SGs and to overcome the fungitoxic effects of SGs, which involved their biotransformation and complete degradation.
Interestingly, antioxidant activities of swertiamarin and sweroside recorded in ABTS assay increased after the compounds have been hydrolyzed, which highlighted their possible antioxidant role during ingestion. Thus, present work contributes to the clarification of the role and fate of *C. erythraea* SGs after digestion within *in vivo* systems, and further promotes the applications of this remarkable plant in pharmaceutical and food industry.

Cytokinin arabinosides and their biological properties in various bioassays

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Plant hormones, such as cytokinins, regulate many aspects of plant growth and development. Cytokinins have been known for a long time and they play an important role during the cell cycle as well as they influence numerous developmental processes.
As we are aware, 6-Benzylaminopurine (BAP), which is routinely used in plant biotechnology, and its endogenous metabolites can negatively influence rooting and acclimatization of some plant species[1]. Based on our previous research of naturally occurring aromatic cytokinins in plants, we synthesized a new group of cytokinin derivatives with high activity in different cytokinin bioassays to solve this problem.
These compounds were prepared by the reaction of hypoxanthine arabinoside with corresponding benzylamines and they were characterized by various instrumental methods (NMR,HPLC-MS, EA, etc.). Biological activity of prepared derivatives was tested on *A. thaliana* AHK3 and AHK4 receptors and in three cytokinin bioassays such as leaf senescence assay, *Amaranthus* betacyanin assay and tobacco callus assay, to confirm their ability to improve selected plant micropropagation and acclimatization processes. Moreover, measurement of chlorophyll fluorescence parameters was performed in detached wheat leaves kept in darkness for 6 days to establish senescence-related changes in photosynthetic activity, namely in activity of Photosystem II (PSII). Next, we adopted similar approach for evaluation of photosynthetic activity in the detached leaves of Arabidopsis. These results showed that our compounds had significant senescence-delaying effects that were specific for tested plant species. While one compound showed high activity preferentially in wheat, another compound showed better protective function in Arabidopsis leaves. Therefore, second compound was selected for future whole transcriptome differential expression study using Arabidopsis as a model system.

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Regulatory links between defense chemistry and development

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Plants utilize numerous internal and external signals allowing them to plastically control metabolism and development. As energy availability is an essential cue controlling growth, photo-assimilates, such as glucose and sucrose, are monitored on a nearly continuous basis and their internal levels are used to determine the growth potential by partitioning just the right amount of sugars between immediate use and storage. Yet, plant plasticity is not limited to responding to the internal energy status, but also to a vast array of external environmental inputs. For example, plant defense against biotic organisms requires coordination with plant development and with other organisms. This is vital for the plant as a metabolic defense response to one organism can entrain an ecological cost making it more sensitive to a different organism. The current model is that developmental decisions hierarchically regulate defense metabolism with little to no feed-back from defense metabolism to development. However, work on the glucosinolate defense compounds is beginning to suggest that defense metabolites can equally modulate development.
The evolution of the core of glucosinolate biosynthesis is relatively young, and specifically modified glucosinolate structures are even more recent. Using purified compounds, we screened for endogenous signaling properties among short-chain methionine-derived aliphatic glucosinolates by testing their ability to induce visual phenotypic responses in *Arabidopsis thaliana* seedlings. We identified novel signaling properties specific to individual glucosinolate structures. One distinct modified glucosinolate, 3-hydroxypropyl (3OHP) glucosinolate, or derived compounds, reversibly inhibits root growth and development across the plant kingdom. Mutants in the Target of Rapamycin (TOR) pathway display altered sensitivity to 3OHP glucosinolate supporting the hypothesis that 3OHP glucosinolate-associated signaling proceeds through the TOR complex. Thus, plants might link evolutionarily new defense metabolites to ancient signaling pathways to optimize energy allocation.

Novel Plantago mucilages for health and industrial uses

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Plantago species are myxospermous meaning that their seeds extrude a mucilage when they encounter an aqueous environment, probably aiding seed dispersal and germination. The mucilage of some Plantago species have been used for hundreds of years to provide herbal remedies and in modern times we are probably most familiar with psyllium which comes from Plantago ovata. Psyllium is the papery husk removed from the dry mature seed. This husk predominantly contains heteroxylan, a branched polysaccharide that has significant water-holding capacity and which is valuable as a dietary fibre supplement providing bulk and aiding laxation, but is also a versatile hydrocolloid added to processed food. As well as its use in ice cream and as a key ingredient in gluten-free foods, psyllium is also finding environmental applications in green concrete and as a corrosion inhibitor. We have built a unique set of Plantago resources which are allowing us to successfully use the mucilage accumulating cells (MACSs) in the seed as a proxy for the plant cell wall, and also to produce novel mucilage types for future applications in human and animal health and food manufacturing. We have recently discovered that the way the mucilage is made, deposited and released in Plantago is completely different to Arabidopsis which has dominated studies of any myxospermous species. We have made a mutant Plantago ovata population using gamma irradiation and have screened the mucilage of many lines for visible and chemical changes using stains, antibodies and monosaccharide profiles and NIR techniques respectively. We have found exciting mutant phenotypes and using our PacBio genomic scaffold are now uncovering the identity of causative lesions. We are also now able to tackle the agronomic improvement of Plantago ovata based on successful field trials in Australia, including mutant lines possessing superior cultivation phenotypes.

Carvacrol as a potential phytotoxin: study of its effects on Arabidopsis thaliana seedlings

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The study of secondary metabolites with bioherbicidal potential has exploded due to the great need of finding more sustainable alternatives to synthetic herbicides, as controlling weeds to increase crop productivity and quality should not in turn lead to problems linked to the emergence of weed resistance, soil contamination and the presence of undesired chemicals in the food chain.
The essential oils components have aroused great interest as phytotoxins due to their structural diversity and action mechanisms, and their a priori less toxicity. Carvacrol is an oxygenated monoterpene present in essential oils of some plants of the Lamiaceae family whose phytotoxic mode of action is still unknown. Therefore, this work has been developed to initially approach the mode of action of carvacrol on *Arabidopsis thaliana* seedlings at a structural and physiological level. Growth and germination bioassays were performed with *A. thaliana* seedlings treated with concentrations of carvacrol between 0 and 1200 µM for 14 days to elaborate the dose-response curves, calculate the IC_{50} (202 µM) and IC_{80} (315 µM) values (concentrations responsible for the 50% and 80% inhibition, respectively) and morphologically analyze the seedlings with a magnifier. To complete this study, *Arabidopsis* seedlings were treated with the IC_{50} for 7 and 14 days to calculate the D_W/F_W ratio, and structurally and ultrastructurally study the roots by light and electron microscopy. Chlorophyll a fluorescence and H_2O_2 (DAB staining) and cell death (trypan blue staining) were also analyzed to detect structural and physiological alterations. The results revealed dehydration effects, photosynthetic alterations, oxidative damage and increase of death cell in roots after treatment with carvacrol. In conclusion, carvacrol showed a strong phytotoxic potential on seedlings of *Arabidopsis thaliana*.

Transcriptional Regulation of Saponin Biosynthesis in Barbarea vulgaris

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Insect pests and pathogens cause major losses in agriculture, therefore, there is a critical need to develop novel and sustainable methods to reduce these losses. Plants produce a vast diversity of specialized chemicals that deter antagonists, whose potential for pest management is still largely unexplored. We need to understand better how such defense compounds are synthesized by plants and what biotic and abiotic conditions trigger and regulate their production, in order to develop and use them sustainably. Here, we explore the genetic and molecular regulation of triterpenoid saponin biosynthesis, using the insect-resistant wild crucifer *Barbarea vulgaris* as a model system. We show that a previously reported QTL associated with resistance to herbivory, but not with higher content of deterrent saponins, harbors two transcriptional factors. Both of these regulators are co-expressed with saponin biosynthetic genes and they are related to proteins regulated by jasmonate signaling in plant defense. Currently, we are generating plant lines with altered expression of these regulators in order to explore their role in saponin biosynthesis in *Barbarea vulgaris*. Additionally, we are investigating how saponin biosynthesis and its regulation is affected by herbivore attacks using the larvae of the diamondback moth, *Plutella xylostella*. Our findings will provide insight into the regulation of saponin biosynthesis and means for manipulating these metabolites to develop crops with modified content of saponins and ecologically appropriate defense reactions.

A comprehensive approach to the production of cucurbitacins

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Cucurbitacins are a diverse group of highly oxygenated tetracyclic triterpenoids. While, they are widely distributed in the plant kingdom, they are mostly found in the plant family Cucurbitaceae. These compounds act as a potent feeding deterrent, helping plant defense against insects. Furthermore, cucurbitacins possess strong pharmacological properties such as antitumor, anti-inflammatory and hepatoprotective effects. However, this potential is hampered by their low availability and high price. Previous studies have identified genes involved in the oxygenation, reduction and acetylation steps that succeed the cyclization of the triterpenoid skeleton, cucurbitadienol, in species of the family Cucurbitaceae. Nevertheless, due to the complexity of their chemical structure, their complete biosynthetic pathway has not yet been described. We have characterized additional P450s and an acetyl transferase from a plant species of the plant family Brassicaceae that produce alternative cucurbitacin intermediates. Building on these foundations, in this study we will further investigate cucurbitacin diversity and elucidate additional steps in their biosynthesis pathway. Additionally, ”hairy root” cultures will be used as a platform to enhance the production of cucurbitacins in controlled *in vitro* conditions. Preliminary results show that hairy root cultures engineered for the overexpression of genes involved in the early steps of cucurbitacin biosynthesis lead to a significant accumulation of cucurbitacins.

Carrots for production of natural food colors

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Food coloring is widely used to influence the perceived flavor of food and make them more attractive to consumers. However, due to concerns over food safety over recent years, there has been an increasing interest among both consumers and manufacturers to replace the synthetic dyes with natural colors. Development of crop varieties with high concentration of pigments, therefore, has a huge economic potential as raw materials in the production of natural food colorants.
This project aims to develop new varieties of carrot with a high concentration of pigments (beta-carotene and anthocyanins). The carrots are developed through selection within existing varieties over several generations. Selected roots with a high content of pigment have been polycrossed by open pollination, and the progeny grown and compared in order to identify new cultivars with improved color concentration.
The polycross approach has been used to maximize the number of cross combinations that can be represented among the progeny. The polycross, however, lacks genetic control with complete loss of paternity information among the progeny. Simple sequence repeat (SSR) marker-based paternity analysis is proposed as an effective molecular tool for identifying paternity. Progeny from each polycross family has been genotyped along with the parents, using 14 previously described SSR markers.
The objective of this study is to demonstrate that the paternity of polycross progeny from carrot can be determined by using polymorphic SSR markers. The ability to identify paternity information allow for a rapid assessment of diversity at the genome level and for a targeted selection of parental plants in carrot breeding programs.

Post-harvest UV-B treatment of White Sultanina grapevine berries

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The grapevine berries are an important source of phenolic compounds. In this study, White Sultanina berries were treated with UV radiation and followed quantitative change of these secondary metabolites. In the skin of cv. White Sultanina table grapes, a phenolic acid and several mono-glycosilated flavonols were identified by HPLC-DAD. The aim of this work was to alter this profile, to improve antioxidant properties. Resveratrol contents of berry skins have already been successfully increased using UV-C or UV-B radiation (Cantos et al. 2000) in another variety (cv. Napoleon). In this study, we report a UV-B-inducible increase in flavonoid-glycosides observed 2 h after 30 min exposure to 11.5 W/m^2 radiation flux (physical dose) from a narrow band source (VL-215M centred at 312 nm, Vilbert Lourmat, France). This was accompanied by higher antioxidant capacities of berry skin extracts, in accordance with the observed strong antioxidant capacities of quercetin-glycosides in vitro (Csepregi et al. 2016, Csepregi and Hideg 2018). Differences between UV-B-treated berries and untreated controls were less pronounced when assayed after a longer storage period following irradiation at 20 ℃ under low fluxes (60 µmol $m^{-2}s^{-1}$) of photosynthetically active radiation. Berry skin photosynthesis, measured as photochemical yield using imaging PAM (Heinz Walz GmbH, Effeltrich, Germany) temporary increased after the UV-B treatment, then declined, suggesting a possible metabolic source of increased flavonol biosynthesis. These experiments suggest that UV-B irradiation of table grapes can be beneficial in terms of increasing the content of potentially health-promoting flavonol derivatives.

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B6 vitamers facilitate leaf defence against UV-B

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Solar UV-B is an important regulator of plant growth and development, but in combination with other abiotic factors, a synergistic effect may lead to oxidative stress via ROS (1). Our previous results suggest that effective scavenging of H_2O_2 and $^{\bullet}OH$ is needed for a successful UV-acclimation, since solar wavelengths of UV-B are able to increase metabolic H_2O_2 concentrations and can also photo-convert H_2O_2 to $^{\bullet}OH$ (2). Vitamin B_6 is an important factor of plant development and stress tolerance (3). Besides its crucial role in several biosynthetic pathways, B_6 derivatives are efficient ROS scavengers (4).
Arabidopsis thaliana mutants (*rsr4-1*) reduced in B_6 biosynthesis were used to investigate how B_6 contributes to acclimation to supplemental UV-B. In response to UV-B, both mutant and wild type leaves altered their antioxidant profiles – including increases in B_6 derivatives, but only the mutants suffered oxidative damage and showed decreased leaf photochemistry. Unlike the wild type, *rsr4-1* plants showed elevated catalase and markedly decreased SOD activities, but these UV-responses were insuffcient to neutralize H_2O_2 effectively in vacuoles or chloroplasts. Responses are also discussed in terms of changes in leaf B_6 profiles and ROS reactivities of B_6 vitamers.

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Chemical composition of Brunfelsia uniflora leaves extracts cultivated in vitro

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Brunfelsia uniflora (Pohl) D.Don is a perennial shrub, woody, 2-3 m high, native to Brazil South/Southeast. It is important to indigenous communities who use it for medicinal purposes (Schultes, 1979). The objective of this study was to characterize chemical composition along developmental stages *in vitro*. Meristems removed from lateral branches were disinfested and transferred to Murashige and Skoog medium with growth regulators according to developmental stage. Explants were placed in chamber for 240 days with artificial light and a crude hexane extract was prepared with 2 cm leaves long for the first stage. The explants were in vitro cultivated until rooting. Leaves with 8 cm long were used for the second hexane crude extract. The extracts were obtained by the dynamic maceration method with solvent depletion followed by rotary evaporation (Martins et al., 2009). The chemical constituents obtained were identified by GC-MS. The chemical composition was determined by the analysis and comparison with mass spectra with the Wiley spectrophotometer 275 and by comparing the retention indices with the literature (Adams, 2012). Chromatographic analysis obtained in the first development stage indicated the presence of 3-nonanol (15.37%), 1-undecene (12.89%), decane (8.41%), 3-hexanol, 5-methyl- (7.41%) and 6-tridecene, (Z)- (6.01%), related to primary plant metabolites (Jorge, 2009). In the second stage, the presence of esters of fatty acids (7.91%), phytosterols (3.73%); compounds carveol (0.36%), iso-pinocarveol (0.49%) which are part of the monoterpenoids biosynthesis (Kegg pathway); and phytol (0.11%), a chlorophyll diterpenoid compound (Jorge et al., 2017). The results indicated that it was possible to promote the plant in vitro development, and to verify the chemical compounds produced by the plant in the initial phase of cultivation, with production of primary metabolites, and in the secondary phase, with secondary metabolites production.

Paclobutrazol and methyl jasmonate enhanced anthocyanins content in black corn (Zea mays L.)

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Anthocyanins are plant's natural product found in vegetables and fruits that play roles as pigments and UV-B protectant. Anthocyanins are considered therapeutic agents that associated with the scavenging of free radicals and protective effects against cardiovascular diseases. Anthocyanins biosynthesis can be influenced by a variety of environmental factors such as plant hormones, mineral nutrition, light and temperature. Paclobutrazol is a growth retardant that increased coloration in several fruits, while methyl jasmonate also enhanced anthocyanin biosynthesis in several species. This research evaluated the effect of paclobutrazol and methyl jasmonate on growth, yield and anthocyanin content of black corn (*Zea mays* L.). The experiment was conducted by factorial 4 x 4 designs. Black corn seeds were germinated in a polybag, one seed per polybag, and at 2 and 4 weeks after planting, paclobutrazol of 0 ppm, 5 ppm, 10 ppm or 20 ppm were applied as a soil drench. Methyl jasmonate of 0 ppm, 2.5 ppm, 5 ppm or 7.5 ppm were applied at cob emergence. The results showed that paclobutrazol reduced plant height but increased chlorophyll content. Two major anthocyanins found in black corm were cyanidin 3 O-β-D glucoside and peonidin 3 O-β-D glucoside. The anthocyanins content in black corn were increased by both paclobu trazol and methyl jasmonate treatments.
Key words : black corn (*Zea mays* L.), paclobutrazol, methyl jasmonate, antosianin

Co-expression of squalene epoxidases with triterpene cyclases boosts production of triterpenoids in plants and yeast

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Triterpene cyclases catalyze the first committed step in triterpene biosynthesis, by forming mono- to pentacyclic backbone structures. Squalene epoxidase precedes this cyclization by providing the oxygenated and activated substrate for triterpene biosynthesis. Three squalene epoxidases from *Cucurbita pepo* (*CpSEs*) were isolated and shown to have evolved under purifying selection with signs of sites under positive selection in their N- and C-terminal. They all localize to the ER and produce both (3*R*)- and (3*S*)-2,3-oxidosqualene as shown when expressed in yeast. Of the three SEs, expression of *CpSE2* in the yeast *erg1erg7* knockout mutant resulted in the highest amount of 2,3-oxidosqualene. Co-expression of the *CpSEs* with four different triterpene cyclases, either transiently in *N. benthamiana* or in yeast, showed that *CpSEs* boost triterpene production. *CpSE2* was the best performing in this regard, which could reflect either increased substrate production or superior channeling of the substrate to the triterpene cyclases. Fluorescence Lifetime Imaging Microscopy (FLIM) analysis with C. pepo cucurbitadienol synthase (CpCPQ) revealed that there was a specific interaction only with CpSE2 but not the other CpSEs. When *CpSE2* was stably transformed into *C. pepo* hairy root lines cucurbitacin E production was increased 2-fold compared with control lines only transformed with the corresponding empty vector. This study provides new insight into the importance of SEs in triterpene biosynthesis, suggesting that they may facilitate substrate channeling, and demonstrates that SE is a new tool for increasing triterpene production in plants and yeast.

The role of plant homologue of USP protein family as a novel member of phytohormone-mediated growth responses

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Plant growth and development, as well as stress tolerance, is controlled by phytohormones and protein regulators under diverse environmental conditions. Universal stress proteins (USPs) were first discovered in bacteria to play an important role in survival under various stresses. In plants, proteins containing USP domain with the specific structure of nucleotide-binding Rossmann-like fold were found, but not all of USPs able to bind nucleotides. Several USP plant homologues are expressed in response to phytohormones and abiotic stresses. However, biological function of plant USPs still remains unknown.

We characterized the transgenic plants with low expression of *At3g58450* gene encoding a homologue of the USP family. This protein shared identity to *Arabidopsis thaliana* USP-proteins however does not extend through all the known secondary structure elements of USP domain. The transgenic plants demonstrated prolonged vegetative phase and delayed flowering partially reversed by gibberellins (GA) treatment. Consistent with this, mutants showed reduced content of bioactive GAs (GA_1 and GA_3) and altered expression of GA metabolic pathway genes (GA-dioxygenases). These results show the involvement of AT3G58450 protein in regulation of gibberellins-mediated growth responses. On the other hand, gene expression analysis demonstrated *At3g58450* gene to be induced in response to abscisic acid (ABA) treatment and to be suppressed indirectly by GA. These results may indicate the involvement of AT3G58450 protein not only in abiotic stress responses as universal stress protein, but in regulation of GA-dependent processes of growth and development in normal conditions. We suggest this protein to play possible role in regulation of growth and development by involvement in crosstalk between GA and other plant hormones.

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Tapetal Biosynthesis and Transport of Flavonol-3-O-sophorosides

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In Arabidopsis, two pollen specific flavonol-3-*O*-(*1,2*)-diglucosides: kaempferol- and quercetin-3-*O*-sophoroside are produced by two uridine diphosphate dependent glycosyltransferases (UGTs) in the tapetum. In addition to phenolamides these glycosides accumulate on the surface of pollen grains. Accumulation of both types of compounds is highly conserved in Angiosperm pollen. UGT78D2 and UGT79B6 perform the stepwise condensation of two glucose units to the aglycone forming a characteristic *1→2* inter-glycosidic linkage. HPTLC and LC-MS-based analysis showed that a recently UGT knockout and UGT/SHT (spermidine hydroxycinnamoyl transferase) double knockout lines have been generated and display a strongly impaired phenylpropanoid accumulation pattern on the pollen surface. Promotor-Localization studies based on stable GFP-reporter lines using a putative 1000bp UGT79B6 promotor proved the tapetal specific expression of the UGT79B6 as the terminal enzyme in flavonol-3-*O*-sophoroside biosynthesis. Both UGTs expressing genes were functionally expressed in *E. coli* and the recombinant proteins were used to produce the corresponding flavonol-3-*O*-sophorosides as substrates in search for putative flavonolglycoside transporters in the anthers. Candidate transporter genes screened by database mining, co-expression data, RT-PCR and sequence analysis were identified as members of the multidrug and toxic compound extrusion protein family (MATE) or as ATP-binding cassette proteins (ABC). Individual T-DNA insertion lines of all putative transporter candidates were analyzed particularly for changes in the anther phenylpropanoid profile by LC-MS. The functional *in vitro* characterization of these putative flavonoid specific candidates was achieved by a microbal uptake assay based on ^{14}C-labeled flavonol-3-*O*-sophorosides. Efforts are undertaken, to replace the detection of the radioactive substrates by a fluorescence based technology using fluorogenic flavonoids.

A day in the vineyard - Short term responses of leaf phenolic compounds to environmental factors

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Sun acclimated leaves of grapevines are facing the risk of oxidative stress by a combination of high PAR and solar UV on each clear day. Long-term stress avoidance is supported by, among other metabolites, phenolic compounds. These metabolites act as UV screening compounds in the epidermis [1] and as antioxidants possibly in all tissues [2,3]. In a recent study we have shown that the main environmental regulator of phenolic contents in Pinot noir leaves is cumulative UV radiation during leaf development from bud-break to veraison [4]. The present work explored short term, hourly changes in photosynthesis, phenolic profiles and antioxidant capacities of South-facing Pinot noir leaves between 7 am and 7 pm during a clear summer day at Pécs (N46.071°, E18.156°). Adaxial flavonoid content (measured as Dualex flavonoid index) showed strong positive correlations with PAR, UV, leaf temperature and net CO_2 assimilation; but was not significantly connected to leaf antioxidant capacities. Total extractable phenolic contents increased during the day, due to an increase in the main flavonol component (quercetin-glucuronide, measured with HPLC-DAD), resulting in increased leaf antioxidant capacities. Positive correlators of these changes were air and leaf temperature but not light conditions, indicating that long term environmental plasticity and short term responses are driven by different factors.

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DIFFERENT BREEDING MODES ALTER THE ANTIOXIDANT CAPACITY OF HYSSOPUS OFFICINALIS

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Hyssop (*Hyssopus officinalis* L.) is a well-known medicinal, aromatic and culinary herb of the *Lamiaceae* family. Presently is applied in pharmaceutical and food industry as a source of important biologically active substances with antioxidant and antimicrobial properties. The antioxidant capacity of *H. officinalis* plants conventionally cultivated from seeds, *in vitro* propagated and adapted under field conditions plants and those from natural habitats was compared. In order to provide a better understanding of the impact of different cultivation method on Hyssop performance, changes in the pattern and activity of the main antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; guaiacol peroxidase, GPO), were evaluated. The activities of the investigated antioxidant enzymes were significantly higher in the leaves of plants than in the flowers. The activities of SOD and APH in the plants *in vitro* propagated were statistically equal to those of the natural habitat. CAT activity reached the highest values in the leaves of naturally grown plants, while in GPO the opposite tendency was expressed. Leaf and flower extracts of *H. officinalis* in all three plant breeding modes showed different antioxidant potential. The highest values of ferric reducing antioxidant power (FRAP) assay was observed in plants *in vitro* propagated. 2, 2-diphenyl-1-pycril-hydrazyl (DPPH) free radical scavenging method indicated the same tendency of antioxidant capacity in the leaves and flowers of *in vitro* propagated and naturally grown plants.

Alkaloid biosynthesis as an additional fitness advantage of certain nodulated Fabaceae?

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Plants within the family Fabaceae are well known for their biotic interactions with nitrogen fixing rhizobia that result in the formation of specific organs, the nodules, to host these symbionts. Species of the genistoid lineage produce natural products like quinolizidine alkaloids (QAs) or pyrrolizidine alkaloids (PAs) as part of their chemical defense against herbivores.[1,2] The knowledge about enzymes involved in these two biosynthetic pathways is limited. However, for the first specific step in PA biosynthesis, homospermidine synthase (HSS) was characterized. Recent studies on PA-producing species of *Crotalaria* showed that PA biosynthesis is induced by nodulation[3]. Therefore, we analyze future species of the Fabaceae to understand the link between alkaloid-production and nodulation. These analyses include the physiology of PA production and accumulation in nodules (e.g. by immunolabeling) and of QA production in dependency of various environmental factors.

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Role of red light in carotenoid and anthocyanin biosynthesis during development and ripening of bilberry

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Development and ripening of fruits include metabolic and structural changes such as accumulation of pigments and flavor compounds. Fruit ripening processes are controlled by plant hormones, most importantly ethylene and abscisic acid (ABA), but also affected by environmental factors, such as light. Bilberry (*Vaccinium myrtillus* L.) is an economically important wild berry species in boreal forests of the Northern Europe. Bilberry fruits are rich with health-beneficial flavonoids, especially anthocyanins that accumulate berries during ripening.
Our recent investigations have focused on developmental and environmental regulation of ripening and anthocyanin biosynthesis in non-climacteric bilberry fruit. Our studies have shown that during bilberry ripening both carotenoid and anthocyanin biosynthesis is up-regulated but only anthocyanin content increases. This inconsistency is most likely due to apocarotenoid formation from carotenoids during the berry ripening, such as formation of ABA and berry flavor compounds. The ABA concentration increases highly at the onset of bilberry fruit ripening preceding anthocyanin accumulation indicating that ABA plays an important role in the regulation of ripening and anthocyanin biosynthesis in bilberry fruit. Light is known to induce anthocyanin biosynthesis and in our studies intense white light conditions also increased the expression of the carotenoid biosynthetic genes and also the expression of the carotenoid cleavage genes, especially in unripe fruits. Red light wavelengths were efficient in inducing anthocyanin accumulation in ripening bilberries. The mature bilberry fruits responded specifically to red/far-red light wavelengths by inducing the expression of both the carotenoid biosynthetic and the cleavage genes indicating tissue and developmental stage specific regulation of apocarotenoid and anthocyanin formation by light quality.

Sterol 24-isomerase, a DWARF1 paralog, catalyzes the first committed step in the biosynthesis of withanolides branching off the general phytosterol pathway

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Withanolides are C_{28} isoprenoids produced by the Indian medicinal plant *Withania somnifera* and several other solanaceous plants. Withanolides share an upstream biosynthetic pathway with general phytosterols; 24-methyldesmosterol [1] and 24-methyldesmosterol [2] are known precursors.
Based on genome and transcriptome data we have identified a key enzyme in the biosynthesis of withanolides; a DWARF1 paralog that catalyzes the initial step in withanolide biosynthesis branching off the general phytosterol pathway. DWARF1 catalyzes a two-step isomerization-reduction reaction from 24-methylenecholesterol to campesterol in brassinosteroid biosynthesis [3]–[5]. *Solanum* plants have two DWARF1 homologs, known as sterol side chain reductase (SSR) 1 and SSR2 [6]. SSR1 is identical to DWARF1, while SSR2 has $\Delta^{(24\text{-}25)}$-reductase activity involved in cholesterol biosynthesis. We have identified a third DWARF1 homolog in solanaceous plants that produce withanolides. Phylogenetic analysis suggests that this gene arose before the speciation of the *Solanoideae*, and that *Solanum* plants later lost this gene again. Using heterologous expression in yeast, we showed that the enzyme is a 24-isomerase (24ISO), catalyzing conversion of 24-methylenecholesterol to 24-methyldesmosterol. Even though this is an isomerization reaction with no net consumption of NADPH, this cofactor is required for activity and could not be substituted with $NADP^+$ in *in vitro* assays. 24ISO localizes to the ER in tobacco cells, corresponding to the site of phytosterol biosynthesis. Silencing of 24ISO in *W. somnifera* lead to reduction in 24-methyldesmosterol and withanolide accumulation.

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Transcription Factor Families Regulate the Anthocyanin Biosynthetic Pathway in Raphanus sativus

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Transcription of anthocyanin biosynthetic pathway genes is required the expression of at least one member of each of three transcription factor families: MYB, bHLH and WD40. These transcription factors form a complex that binds to structural gene promoters, thereby modulating gene expression. *Raphanus sativus* L. (radish) displays a wide spectrum of tissue-specific anthocyanin pigmentation, making it a useful model for the study of anthocyanin accumulation. To investigate the genetic basis for tissue-specific pigmentation, we used quantitative real-time polymerase chain reaction to evaluate the expression of eight anthocyanin biosynthetic and three regulatory genes in leaves, root skins and root fleshes from three radish varieties. Transcript level of biosynthetic genes including *RsCHS*, *RsF3H*, *RsDFR* and *RsANS* was significantly high in anthocyanin-accumulating tissues. Moreover, it was significantly associated with the transcript level of *RsMYB1* and *RsTT8*. These results showed that differential expression of *RsMYB1* as well as *RsTT8* occurs coincident with anthocyanin accumulation in leaves, root skins and root fleshes from radish. Heterologous co-expression of both *RsMYB1* and *RsTT8* in tobacco leaves dramatically increased the expression of endogenous anthocyanin biosynthesis genes and anthocyanin accumulation. Furthermore, a yeast two-hybrid assay showed that RsMYB1 interacts with RsTT8 at the MYB-interacting region (MIR), and a transient transactivation assay indicated that co-expression of RsMYB1 and RsTT8 activate the *RsCHS* and *RsDFR* promoters. RsMYB1-overexpressing Arabidopsis plants exhibited the red pigmentation throughout the plant and had a higher antioxidant capacity than did non-transgenic control plants. In addition, complementation of the Arabidopsis *tt8-1* mutant, which lacks red pigmentation in the leaves and seeds, with RsTT8 restored red pigmentation, and resulted in high anthocyanin and proanthocyanidin contents in the leaves and seeds, respectively. Together, these results demonstrate that *RsMYB1* and *RsTT8* perform a function as active anthocyanin regulators.

Manipulation of Sex genes increases starch phosphate in potato tubers

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Starch is an important plant product used by industry. The amount of covalently bound phosphate helps to determine its physicochemical properties and its usability in industrial processes. It has been known for many years that phosphate is incorporated by glucan and phosphoglucan, water dikinases. More recently two enzymes (SEX4 and LSF2) that remove phosphate from both starch and its degradation products have been identified through the analysis of starch excess Arabidopsis mutants. We identified orthologs of these genes from potato and removed their activities in stably transformed plants using an RNAi approach. As in Arabidopsis the potato plants were repressed in their capacity to degrade leaf starch. The amounts of starch in tubers from the plants were unaltered as was the proportion of amylose within the starch. Starch phosphate content was greatly increased in all lines; however, the position of the phosphate was altered depending on the manipulation. Plants lacking SEX4 contained more phosphate at the 6-position, while those repressed in LSF2 contained more ta the 3-position. Interestingly there were also alterations in starch structure and average granule size. RVA analysis indicated that this leads to altered physical properties in the starch and improved functionality. Despite the inhibition of starch degradation in leaves of the plants, tubers stored at 4°C were unaltered in cold induced sweetening.

Auxin-like behavior of norharmane on Arabidopsis metabolism

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Norharmane is an indole alkaloid that can be found in terrestrial plants, in some marine organisms or cyanobacteria. Although algicide, antibacterial and pharmacological activities of this secondary metabolite have been broadly demonstrated, its herbicidal activity has been little investigated. Therefore, the phytotoxic activity of different concentrations of norharmane (12.5, 25, 50, 75, 100, 200 and 400 µM) on *Arabidopsis thaliana* (L.) Col-0 seedlings was analyzed in this work.
Treated *Arabidopsis* seedlings showed a strong growth decrease with an IC_{50} value (concentration responsible for 50% inhibition) of 62 µM, and presented an altered morphology of the roots with left torsion and symptoms of necrosis. The ultra-structure analyzed under transmission electron microscopy (TEM) showed differences between treated and control seedlings. Roots treated with the IC_{50} norharmane showed tissue disorganization, altered division planes with incomplete cell walls and multinucleated cells. The alteration of the microtubules was confirmed by immunolabeling with Alexa 488, while cell death suggested by the observation of necrotic areas was confirmed by Trypan Blue staining.
Treated seedlings showed an altered morphology characterized by a decrease in the length of the main root and an increase in the formation of lateral and adventitious roots, and root hairs. These alterations suggested the possibility that norharmane alters production or transport of auxins. A bioassay with the anti-auxin PCIB confirmed this possible alteration of auxins by reversing the effect of norharmane. However, the indole acetic acid quantified by GC-MS, decreased in the seedlings treated with the IC_{50}, which may indicate that the compound is mimicking the effect of auxins. GFP mutants for the different PIN transporters and for the display of auxins were subjected to the IC_{50}, observing an alteration for the PIN3 and PIN7 transporters.
Based on these results, norharmane appears as an interesting candidate to be an auxinic bioherbicide for weed control.

Phytochemical analysis, antioxidant properties and DNA fingerprinting of pistachio skin from six Pistacia vera L. kinds growing in the subtropical line

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Among Anacardiaceae family the genus *Pistacia* contains at least 12 tree and shrub species, of which only *Pistacia vera* produces edible nuts. Results from *in vivo* studies showed a positive correlation between pistachio intake and reduced risk of cardiovascular disease. This protection has been ascribed to fatty acid profile that can have a favorable influence on serum concentration and profile of triglycerides, and total and LDL cholesterol. Otherwise, experimental data showed that pistachio consumption significantly improves oxidative status of healthy individuals and lowers the levels of circulating inflammatory biomarkers, suggesting a positive correlation with the content of hydrophilic phytochemicals having antioxidant and anti-inflammatory activities. Due to the kernel contains over 50% of lipids, polyphenol compounds in pistachio are mostly found in the skin, generally removed when pistachio is used as a confectionery ingredient. Instead, this study investigated the phytochemical profile and the antioxidant properties of pistachio nut skin of six kinds of *Pistacia vera* (Bronte, Kerman, Kern, Larnaka and Mawardi). TPC varied from 66.02 to 262.81mg GAE per g of skin, with the highest values being observed in Italian cultivar Bronte (262.81±11.93mg/g). The AOA of SHEs were measured by DPPH, ABTS, FRAP and CAA methods, showing significant differences among the cultivars. Moreover, qualitative and quantitative chemical analysis by HPLC-DAD-ESI-MS/MS showed differences in the amount of phenols, but not in the quality. Moreover, the quantification of anthocyanins and proanthocyanidins by pH-jump and DMAC suggested that these classes of compound contributed almost exclusively to TPC. Finally, in order to investigate the possible action mechanism of CAA, qPCR analyses were carried out on the expression of genes involved in AOA (CuSOD, MnSOD, GPx and CAT). At least, in order to discriminate closely-related kinds of *Pistacia vera*, the chemical data were compared with PCR-RFLP analysis of the ITS and 5SP sequences of nrDNA.

Veronica austriaca L. ssp. jacquinii (Baumg.) Eb. Fisch. - GC-MS Peaks of Volatile Compounds

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Genus Veronica L. is divided into 13 subgenuses according to morphological traits and it belongs to the Plantaginaceae family. There are 40 species of Veronica in Croatia. They are cosmopolitan and ecologically diverse species spread on a variety of habitats from aquatic, marshy and forest to rock, rock cracks, fields and ruderal habitats. The investigated Veronica jacquinii belongs to Southeast-European-Mediterranean hemicryptophytes. Plant material was collected from Ličko polje (Croatia) and was air dried and water distilled. Composition of volatile compounds was analyzed by GC and GC/MS (Adams, 2007) using VF-5ms capillary column. The peaks appeared from 30 – 59 minutes. Thirteen compounds were determined representing 89% of the total oil. Composition of this essential oil is characterized by a high concentration of hydrocarbon derivatives: hexadecanoic acid (63.5%), followed by octadecanoic acetate (8.9%), oleic acid (6.2%) and heptatriacotanol (3.5%). In our previous research in the oil of V. spicata one of the most abundant hydrocarbon compound was heptacosane (Dunkić et al., 2015). According to a literature review GC-MS studies have been performed only on Veronica thymoides subsp. pseudocinerea and the most abundant constituent was hexatriacontene (21%) (Ertas et al., 2015). The present study gives additional knowledge about volatile compounds of the genus Veronica.

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NPF - A LONG-SOUGHT FAMILY OF SPECIALIZED METABOLITE TRANSPORTERS

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Synthesis and accumulation of plant natural compounds often take place in separate organs, cells and subcellular compartments. Given the vast diversity in plant specialized metabolism, this suggests the existence of a plethora of dedicated transport proteins with key roles in plant specialized metabolism. Yet, the transporters involved have remained largely unknown. Recently, we made several discoveries that suggest subclade 2.x of the NPF (nitrate and peptide transporter family) is a veritable ”treasure-chest” of transporters of natural compounds. The glucosinolate transporters AtNPF2.9-2.11 and strictosidine transporter CrNPF2.9 exemplifies that this subclade encodes i) plasma membrane-localized proton-driven active transporters controlling the distribution patterns of final products across tissues and ii) tonoplast localized passive transporters controlling biosynthesis by facilitating movement of intermediates across the tonoplast. Identification of the NPF2.x subclade provides a foundation for homology-based identification of transporters of natural compounds. This has been exploited to identify transporters of cyanogenic- and iridoid glucosides in cassava and *Catharanthus roseus*, respectively. Interestingly, we have also identified NPF2.x transporters from Arabidopsis thaliana capable of moving exogenous natural compounds across membranes. Thus, from a structure-function perspective, the large diversity in chemical structures transported suggests an enigmatically high degree of plasticity in the substrate-binding site of the NPF2.x subclade. We believe that the triplet of glucosinolate transporters provides a super-model system for studying the structure-function relationship of the NPF2.x subclade and eventually of the entire NPF family. I will outline the questions we seek to answer; some of the experimental approaches we are pursuing and how we envision our work will lead to identifying molecular determinants of e.g. substrate specificity and ion coupling. Knowledge of the NPF2.x subclade can be used to control distribution of natural compounds *in planta*; provide a repository of transporters for synthetic biology approaches and eventually enable us to design transporters according to our needs.

Understanding the diversity and the key drivers of cyclotide production in butterfly pea (Clitoria ternatea)

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Cyclotides are knotted, head-to-tail cyclized peptides composed of ~30 amino acids. They are currently being exploited as ultrastable scaffolds for producing peptide-based pharmaceuticals due to their exceptional stability. Cyclotides also merit utility in agriculture as they exhibit insecticidal activity, a role in which they are hypothesized to have evolved for. To date, cyclotides are reported in five angiosperm families. Of interest in this study, are the cyclotides found in butterfly pea (*Clitoria ternatea*), which is currently the only species in the legume family that is known to produce cyclotides. Why the butterfly pea cyclotides are produced in such great abundance, and how these are produced and metabolized, are not well understood. To shed light on these questions, the cyclotide profiles of 23 butterfly pea accessions obtained from 11 countries were compared. Results showed that the different accessions have variable cyclotide expression profiles—some of which do not produce CterM, typically the most highly-expressed cyclotide in the vegetative tissues. Sequencing revealed that CterM of these accessions contained numerous missense mutations and variability in the regulatory elements, likely contributing to the lack of CterM expression. Furthermore, we examined the regions upstream of several cyclotide gene precursors to determine the key factors that drive cyclotide production. Interestingly, we discovered that there are two unique sequences upstream of CterM, suggesting that there are two gene precursors encoding CterM, which may contribute to its high expression. The upstream sequences of CterM and the other butterfly pea cyclotide precursor genes contain cis-regulatory elements for transcription factors that are associated with abiotic and biotic stress conditions. Induction experiments on wild type butterfly pea seedlings showed significant increase in cyclotide production when exposed to stress factors such as salicylic acid and ethylene biosynthetic precursor, among others. Overall, this study recapitulates the hypothesis that cyclotides indeed function in plant defense.

Effects of a two-component, biologically active compound, S-methylmethionine-salicylate against cold stress in maize

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Plants of tropical origin, including maize, could be exclusively sensitive to low temperature and thus cold stress may result in significant yield loss. Use of biologically active compounds as stress-protective agents offers a simple, environment-friendly, cost-efficient and scalable solution for increasing the tolerance against different stresses, for example chilling. We aimed to examine the effects of the two-component *S*-methylmethionine-salicylate (MMS) against short-term cold stress in young maize plants. MMS contains two stress-protective compounds: *S*-methylmethionine (SMM), which is a non-proteinogenic amino acid, and the phenolic derivative salicylate (SA), which plays role in the signalization during biotic and abiotic stress. Stress-protective effects of SMM have been evidenced by our research group, while support in signalization and further stress-related characteristics of SA have been proved by many authors. The two compounds were combined in the new material MMS, for testing the combined effect and for stabilization of SMM. In this recent work physiological parameters such as photosynthetic activity were studied in cold-stressed maize plants, with or without MMS-pretreatment. A microarray analysis was also carried out for gathering deep information about the changes in gene expression patterns. More than 4000 genes were found, that showed at least two-fold expression change in any experimental relation (low temperature treatment with or without MMS-pretreatment). Since oxidative stress is a common secondary effect in chilling, changes in the antioxidant system were also studied, carried out in targeted, qRT-PCR-based measurements of gene expression and photometric monitoring of enzyme activities of ascorbate peroxidase, glutathione reductase and superoxide dismutase. Our results have shown that MMS is capable of increasing the defense potential of cold-stressed maize plants.

Species identification of plant components in teas, spices and herbal remedies using DNA metabarcoding

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Plants are the key components of many food products, in particular, teas, spices and herbal remedies. The inconsistency between declared and real composition, which often occurs in such products, can lead to serious health problems. A usual approach to quality control of food products that include plant components is the botanical analysis. It has however several limitations (not suited for highly processed products, lacks resolution for many plant taxa). Analysis based on the sequencing of genome regions that are variable at species level (DNA barcoding) seems to be the most promising approach successfully tested on honey and herbal supplements. To expand it on larger set of plant products we analyzed 6 teas, 6 spices and 6 herbal remedies available on Russian market. We tested three methods of DNA extraction to choose one that minimizes its degradation and the presence of PCR inhibitors. The composition of samples was determined by sequencing of nrITS1 sequences and their alignment on local nrITS reference database. For DNA library preparation optimized primers for nrITS1 were used that selectively amplify plants only and not fungi. For PCR we used two polymerases, one of which is high-fidelity and optimized for the amplification of GC-rich regions and other is less accurate but highly processive and resistant to PCR inhibitors. Sequencing was performed on two platforms - Illumina (MiSeq) and semiconductor sequencing (Ion S5). We found that most of the labeled plants were found in analyzed products, but three products had significantly different contents than declared by the manufacturer. Certain discrepancies in the results between the two HTS platforms were also found. In addition, we found that one of the tea blends contains inhibitors that can completely inhibit PCR with high-fidelity polymerase, even after additional DNA purification, but could be amplified with the robust one.

Contamination of Cadmium and Lead in Medicinal Herbs Commercialized in Turkey

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Medicinal herbs play an important role in various traditional medicine and recently they are increasingly used in the primary health care intervention. However, herbs may be subjected to natural and chemical contaminations during the growing process which is increasing concern on the safety and toxicity of natural medicinal herbs. Therefore, it is has been a growing interest of monitoring heavy metal contamination in herbs. The aim of this study is to determined the **l**evels of lead and cadmium in frequently used medicinal herbs. Totally thirty-nine samples of ginger, turmeric, liquorice and nutmeg were purchased during the spring and summer period of the year 2016 from traditional bazaars in Istanbul, Turkey. The levels of cadmium (cd) and lead (Pb) were analyzed by inductively coupled plasma technique with optical emission spectrometry (ICP-OES) after microwave-assisted digestion with Berghof method. According to the results lead in the concentration range of 4.218-4.964 mg/kg, 4.229-4.658 mg/kg; 4.226-4.487 mg/kg and 4.233-4.336 mg/kg and cadmium in the concentration range of 0.302-0.481 mg/kg, 0.312-0.784 mg/kg, 0.295-0.462 mg/kg and 0.425-0.541 mg/kg was found in ginger, turmeric, liquorice and nutmeg samples, respectively. It may be suggested that there is a potential global danger to the health, therefore, heavy metal content of medicinal herbs should be carefully considered.

The enzymes OSC1, LUP and CYP716A263 produce a high variety of triterpenoids in the latex of the dandelion species Taraxacum koksaghyz

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The dandelion species *Taraxacum koksaghyz* produces a plethora of secondary metabolites in its latex, the milky cytoplasm of laticifers. Among them are natural rubber, phytosterols and pentacyclic triterpenes. Pentacyclic triterpenes exhibit extraordinary biological activities against fungi and bacteria, making them highly attractive also for agricultural and pharmaceutical applications. In order to gain deeper insight into their biosynthesis, genes encoding for pentacyclic triterpene-synthesizing and triterpene-modifying enzymes were investigated via spatial expression analysis in different tissues of *T. koksaghyz*. Moreover, functional characterization was carried out in *Nicotiana benthamiana* and in an engineered *Saccharomyces cerevisiae* strain to determine product specificity of the corresponding enzymes. Here, we present the analysis of the multi-functional oxidosqualene cyclases TkOSC1 and TkLUP which are highly active in *T. koksaghyz* latex and capable of synthesizing at least eight of the latex-predominant pentacyclic triterpenes. Additionally, a cytochrome P450 oxidase (CYP) was identified which is also highly active in *T. koksaghyz* latex and capable of oxidizing a variety of pentacyclic triterpenes. The resulting compounds, including a newly identified pentacyclic triterpene and its derivative, were isolated and will be tested for anti-inflammatory and anti-microbial activities in future approaches.

Bioherbicide potential of harmaline on Arabidopsis metabolism

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The role of phytochemicals in controlling weeds can be crucial because of their short half-life in soil, their high specificity and their potentially benign toxicological profile. Plant natural compounds often have highly diverse chemical structures with novel and multiple modes of action, representing a real alternative to the repeated use of conventional herbicides. Although there are numerous marketed pesticides from natural compounds, development and marketing of herbicides from plant secondary metabolites is still in an initial phase.
Harmaline (1-methyl-7-methoxy-3,4-dihydro-beta-carboline) is an indole alkaloid widely found in seeds of the species *Peganum harmala* that has been found to show antiparasitic activity against *Leishmania mexicana* and bioinsecticide activity on *Plodia interpunctella*. Tested on seedlings of *Arabidopsis thaliana*, harmaline showed strong inhibitory activity on root and shoot growth, inducing shortening and thickening roots with extraordinary increasing of root hairs. While root hairs emerged from the elongation zone in control roots, root hairs from harmaline-treated roots emerged along the whole root, also very close to the root tip, which could be due to the reduction of meristem length, specially after 7 days of treatment. IC_{50} and IC_{80} values (concentrations required for 50% or 80% inhibition of root growth) were as low as 14 and 29 μM, respectively, after harmaline treatment. Light and electron microscopy images showed also important aberrations on radicle development, as both, differentiation and transition zones showed strong tissue disorganization with cells of different sizes and shapes that did not maintain the characteristic organized pattern of control roots.
Based on these results, auxin content was measured in harmaline-treated DR5 mutant roots, obtaining strong differences among control and treated roots at so low concentrations as 10 to 20 μM harmaline. *In silico* studies of harmaline and auxin synthetic herbicides were done to test the potential function of our compund as an auxin-like bioherbicide.

Biosynthesis of piperamides in black pepper (Piper nigrum)

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This project addresses the biosynthetic pathway of piperine and piperamide conjugate formation in commercially used black pepper (Piper nigrum) fruits. Piperamides are a characteristic subtype of hydroxycinnamic acid amides responsible for the pungent taste of this widely used spice. By combining classical organic synthesis and enzymology with state of the art molecular tools, various critical, yet unknown and potentially unique steps of this metabolic grid, including chain elongation of hydroxycinnamic acids, critical conjugation of piperoyl CoA to piperidine, and formation of the methylenedioxy group will be investigated at the enzymatic and molecular level. Based on classical enzyme purification in the case of piperine synthase, database mining, and comparing RNA-seq profiles of fruits high in piperine with leaves and roots, both low in piperine, candidate genes will be selected and functionally expressed in heterologous systems. While the aromatic part of the piperine molecule is specifically addressed in this project, the molecular approach will also lead to the identification of candidate genes essential for the biosynthesis of the piperidine heterocycle present in important alkaloids, such as coniin and lobelin.

Overexpression of AmRosea1/AmDelila leads to synthesis and accumulation of anthocyanin in orange carrots

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Anthocyanins are phenolic compounds, which are produced in various parts of plants like fruits, flowers and in the tap roots of black carrots. Naturally, anthocyanins are involved in attracting pollinators and in protection against biotic and abiotic stress; however, they can also be used as natural food dyes. As compared to hydrocarbon based synthetic food , they do not have any side effects; rather they are found to be beneficial for humans because of their antioxidant and thus anti-carcinogenic properties. The anthocyanins are synthesized by a specific branch of the phenylpropanoid pathway, the anthocyanin pathway, thought to be controlled by a triad of R2R3 MYB, bHLH and WD40 transcription factors (TFs). We cloned *AmRosea1* and *AmDelila* transcription factors from snapdragon (*Antirrhinum majus*) which are known R2R3 MYB and bHLH TFs involved in promoting anthocyanin biosynthesis. Overexpression of these TFs under *35S*x2 promoter lead to the synthesis and accumulation of anthocyanins in the orange carrot cultivar Danvers 126. A differential comparative transcriptome and homology-based analysis of black carrot cultivars(purple and non-purple regions of the taproots) resulted in identification of TF enhancers and TF competitors promoting or reducing anthocyanin production in tap roots, respectively. Subsequent overexpression of enhancers lead to the accumulation of anthocyanins in orange carrots and increase in anthocyanin content of black carrots.

THE EFFECT OF VARIABLE LED LIGHT ON THE PHOTOMORPHOGENESIS AND STEVIOSIDES CONTENT IN STEVIA REBAUDIANA

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Studies on the plant secondary metabolite biosynthesis and enhanced production in controlled environment (*e.g.,* plant factory) are of an increasing interest because some of them are widely used. The object of our research is *Stevia rebaudiana* Bertoni (*Asteraceae*). Its leaves are rich with sweet glycosides, and steviosides appear to be the major and the most valuable substances among them. They are about 300 times sweeter than sucrose, however, they do not increase blood sugar level and generally are recognized as safe. Thereby they are commercially used as a low-calorie sweetener and sugar substitute product.
We studied the effects of light spectral composition on the stevioside biosynthesis in *Stevia rebaudiana* plants. Light-emitting diodes (LEDs) provide unique possibilities for manipulating plant photosynthesis, photomorphogenesis, and synthesis of secondary metabolites. Stevia plants were cultivated under various light conditions during 70 days. The unique LED modules were assembled using four types of high-performance narrow-band LEDs: short wave red (640 nm), long wave red (660 nm), far red (730 nm) and blue (460 nm). Photosynthetic photon flux density was 180 μmol/m2 * s, photoperiod 18 h. The control variant included all 4 types of diodes, in each of the other regimes one of them was excluded in order to elucidate the wavelengths that affect crop productivity.
The exclusion of far-red or blue regions caused a decrease in the leave number in comparison with the control light treatment. Without a short wave red plants produced larger leaves on the upper part of the shoot. The highest stevioside content was found in the light regime without blue part of the spectrum. This data reveal that red part of the spectrum is responsible for the secondary metabolite biosynthesis. Further studies on the optimal LED lighting regime design could result in the increased yield of targeted functional compounds.

Benzoate-activating CoA ligase from Hypericum calycinum cell cultures: From Gene discovery to Homology modelling

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Benzoate-CoA ligase (BZL) is involved in xanthone biosynthesis. Xanthones are polyketide scaffolds with immense pharmacological potency. They were identified in a number of higher plant families, mainly Hypericaceae and Gentianaceae [1]. A representative genus is *Hypericum*, including the well-known medicinal plant *H. perforatum*. Elicitor-treated cell cultures of *H. calycinum* accumulate prenylated xanthones [2]. Formation of the xanthone skeleton requires benzoyl-CoA, which is the product of benzoate-CoA ligase [3]. Like 4-coumarate-CoA ligase (4CL) and cinnamate-CoA ligase (CNL), BZL is an adenylate-forming enzyme catalyzing an ATP-dependent two-step activation, here of benzoic acid. All previous records on *BZL* genes were related to benzoate-degrading microorganisms [4,5]. Using publicly available transcriptomic resources, the first plant full-length BZL cDNA containing a 1647 bp coding sequence was cloned from yeast-extract-treated *H. calycinum* cell cultures. It encodes a 59.9 kDa dually localized protein encompassing a type 2 peroxisomal targeting signal. Luciferase-based substrate specificity assaying and kinetic characterization indicate that benzoic acid is the preferred physiological substrate followed by isobutyric acid. Homology modelling and docking of various substrates support the kinetic findings. Together with bioinformatic analysis, the docking experiments provide information on the key amino acid residues, which may account for the unique substrate specificity of BZL, compared to CNL and 4CL. Information on the first plant BZL garnered from this study may allow the expansion of the substrate permissivity by site-directed mutagenesis and metabolic engineering via de-novo assembly of polyketide scaffolds in heterologous hosts.

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Insight into the biosynthetic pathway of gramine in Hordeum vulgare

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Barley (*Hordeum vulgare*) is known to accumulate some secondary metabolites as defensive compounds. Certain barley cultivars contain gramine (*N,N*-dimethyl-3-aminomethylindole) as the anti-pathogen and anti-herbivore compound. 3-Aminomethylindole (AMI), which is considered to be derived from tryptophan (Trp), is the precursor of gramine and undergoes *N*-methylation twice by *N*-methyltransferase (NMT) whose gene has been isolated. The most momentous reaction in the AMI and gramine biosynthesis is that causes decrease in the number of carbon atoms between the indole ring and the amino group of Trp. The past experiments using radioisotope labeled Trp demonstrated that its indole ring and methylene side chain are incorporated into gramine. On the other hand, the origin of the amino group in AMI remains obscure. Identification of the atom provenance of gramine is indispensable to the elucidation of its biosynthetic pathway. In this study, we fed Trp labeled with stable isotopes at various position to barley seedlings and analyzed gramine and Trp using LC-MS. We first investigated gramine content in various barley cultivars to determine the one with high gramine concentration. Then the barley cultivar was grown with labeled Trp for 2–4 days. When [2-^{13}C-indole]-Trp and 3-^{13}C-Trp were fed, gramine that possesses higher molecular mass by 1 dalton was detected while C-2 labeled Trp did not affect the mass of gramine. Trp labeled at the amino group with ^{15}N also gave the gramine having one unit higher mass. These data clearly shows that the α-carbon as well as carboxyl group is eliminated and the amino group is retained during the biosynthesis, suggesting an unknown rearrangement mechanism for this step. Several genes whose expression levels correlate with gramine content were obtained by RNA-seq, and more detailed analyses are now under investigation.

Variation in volatile profiles from eucalypt leaves and flowers

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Eucalypts (genus *Eucalyptus*) are ecologically important trees dominating forest and woodland over an area of 92 million hectares throughout Australia, providing habitats for many forest dependent species. Further to this, more than 2.3 million hectares of dense eucalypt plantations exist globally for the primary purpose of timber and pulp for paper.
Eucalypts synthesize a plethora of specialized metabolites that play an important role in moderating interactions with the environment, such as attracting pollinators and protecting against herbivores. Many of these specialized metabolites are volatile. Studies on volatile organic compounds (VOCs) from eucalypts have mainly focused on isoprene and monoterpene emission. As a result, little is known about the quantity, complexity and variation of the full volatile emission profile from eucalypt leaves and flowers under natural conditions.
Here we present the results from the first in-depth study of the variation in volatile emission profiles from eucalypt leaves and flowers sampled from nine different species in their natural habitats. Volatile emissions were collected from mature trees in a non-invasive manner by a push-pull enclosure technique enabling volatile collection into adsorbent cartridges, which were analyzed by GC-MS following thermal desorption. Complex emission profiles of more than 100 different VOCs were detected, including isoprene, mono- and sesquiterpenes, and benzenoids. Total emissions and emission composition were highly variable between the different species sampled. For example, some species emitted significantly higher proportions of monoterpenes or benzenoids, whilst isoprene was the dominant VOC in other species. Moreover, high variability in VOCs was measured between leaves and flowers in all species tested. These results provide important knowledge about how eucalypts interact with their abiotic and biotic environment, and they have wider implications for predictions of carbon emission from eucalypt forests and plantations.

Potato defenses elicitation with oligosaccharides to control Phytophthora infestans

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The oomycete *Phytophthora infestans* is the major threat to the potato culture worldwide and possesses a vast array of effectors to bypass resistance (R) genes. The stimulation of basal immunity to fight the disease relies on the perception of conserved pathogen-associated molecular patterns (PAMP) by membrane receptors. COS-OGA is a new active substance that stimulates PTI and combines chitosan oligomers (COS) with pectin fragments (OGA), thereby mimicking fungal presence and cell wall degradation.
The efficacy of Fytosave® and FytoSol, two COS-OGA derivatives, against late blight was assessed on Bintje, a highly susceptible potato variety. The mode of action of both oligosaccharide compositions was studied through phytohormones quantifications, RTqPCR and completed by an RNAseq study to assess FytoSol effect on potato transcriptome w/without *P. infestans*.
Bioassays performed in controlled conditions showed that FytoSave® was able to slow down late blight on potato leaves while FytoSol completely controlled it. FytoSave® triggered salicylic acid (SA) accumulation in potato leaves as a function of the number of sprayings while FytoSol did not change SA content. Both oligosaccharide compositions did not have any effect on jasmonic acid (JA) and its derivatives. FytoSave® induced early expression of SA-related genes in absence of pathogens while FytoSol strongly repressed expression of downstream SA-, JA- and ethylene (ET)-related genes in the necrotrophic stage of the pathogen.
The RNAseq study performed on potato leaves preventively sprayed with FytoSol and collected 24 h after inoculation with *P. infestans* revealed a major accumulation of transcripts coding for peroxidases, glutathione S-transferases and pathogenesis-related proteins. FytoSol seemed to downregulate auxin and brassinosteroid pathways while enhancing up to variable levels those associated with abscisic acid, ET and oxylipins. Compared to other hormonal pathways, SA appeared only minimally regulated. FytoSol also induced the accumulation of several receptor-like kinases essential for PAMP perception.

Dandelion as a source and model organism for the synthesis of isoprenoid-derived plant lipids

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The Russian dandelion *Taraxacum koksaghyz* produces natural rubber (NR) - a unique high molecular mass biopolymer composed of more than 5,000 isoprene units. In specialized cells named laticifers, isopentenyl diphosphate (IPP) units are covalently linked to growing polyisoprene chains. The IPP molecule can also be converted to diverse triterpenes and triterpenoids that accumulate in the milky sap of the plant. Our current studies focus on the characterization of various enzymes that catalyze the formation of polyisoprenes, triterpenes and triterpenoids in the dandelion plant. Thereby, enzymatic activity of relevant enzymes is analysed by genetic engineering in dandelion as well as in heterologous expression systems like tobacco and yeast cells. Moreover, we optimize purification of the isoprenoid-derived plant lipids from Russian dandelion and from the yeast platform for a detailed analysis of their properties and potential applications.

The effect of storage on the profile of phenolic compounds in selected apple varieties

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Apples are considered as one of the most important fruit crop with excellent health benefits and extensive area of cultivation. Majority of their benefits is associated with a relatively high content of antioxidants including phenolic compounds that belong to the health-promoting phytochemicals. The aim of this work was to determine the concentration of phenolic acids in selected apple varieties originating from the Station of apple breeding of the IEB. We investigated three different varieties of apples (red, yellow and streaked) for their phenolic acid contents in peel, flesh and seeds immediately after the harvest and after 7 months of storage.
Detection and quantification of free, ester-bound, glycoside-bound and the cell wall-bound phenolic acids were carried out by HPLC/MS. According to the bioaccessibility of phenolic compounds we selected free and glycoside- bound phenolic acids to compare harvested and stored apples. Chlorogenic acid (by about 100 – 300 nmol g^{-1} DW), followed by *p*-hydroxybenzoic, protocatechuic, vanillic, *p*-coumaric, ferulic and caffeic acids (in much lower concentrations) represented the major free phenolic acids in peels, fleshes and seeds of all three apple varieties. Protocatechuic acids (300, 500 and 1000 nmol g^{-1} DW in peels) was the most abundant glycoside-bound phenolic acid. Free and bound form of *p*-hydroxybenzoic and vanillic acids were found in relative high concentrations in seeds. After the storage period the levels of phenolic acids increased. The concentration of free chlorogenic acid increased significantly in peels, fleshes and seeds, and free gallic acid was found in peels. In glycoside-bound fraction the enhancement of protocatechuic acid contents in peels and higher level of caffeic acid in fleshes were observed.

Targeted induction of plant secondary metabolism in horticultural plants by controlled stress applications

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Plants rely on morphological or biochemical mechanisms to protect themselves under challenging environmental conditions. This involves the accumulation of diverse bioactive secondary metabolites. Such secondary metabolites are recognized as valuable compounds for human health, as ingredients of cosmetics or for further industrial purposes, utilized after extraction of purified compounds or in plant extracts. The interdisciplinary projects InducTomE and TaReCa evaluate a novel process to make use of secondary metabolites in residual plant biomass of horticultural plants. In this process, stress treatments after the last fruit harvest are applied to increase the amount of valuable metabolites in the plant residuals. Thereby, the residual biomass can be used for the extraction of industrially relevant metabolites. In a pre-screen, young bell pepper and tomato plants were exposed to various abiotic stress treatments like water or nutrient deficiency, salt stress or cold. We identified suitable stress treatments that induced the accumulation of total phenolics, flavonoids and also valuable target metabolites, like the flavonoid rutin and the polyisoprenoid solanesol. A comparison of commercial lines and a wild relative in these experiments revealed differences in stress-induced metabolite accumulation and biosynthesis gene expression indicating genetic variability of responses of secondary metabolism to stress. According to the results of the pre-screen, stress treatment protocols for the induction of target metabolites will be developed for use in commercial greenhouses. In addition, phenotyping methods were applied to quantify plant stress responses and to monitor the intensity of the treatments. This will also help to develop easy-to-use tools to control the targeted stress application in commercial greenhouses. The proposed method for a targeted tailoring of the secondary metabolism in horticultural residuals demonstrates the valorization of underutilized by-products of horticultural food production. Thereby, it has thus the potential to generate added value and increase sustainability of the horticultural food production.

Custom-made bioreactors for production of anthocyanins in suspension cultures of novel tobacco cell lines.

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Anthocyanins are water-soluble pigments that colour the fruit and flowers of many plants. More than 635 different anthocyanins have been identified, distinguished by methylation, glycosylation and acylation with both aliphatic and aromatic groups. There is mounting evidence that consumption of anthocyanin-rich food promotes health, supported by many recent studies of anthocyanin-rich fruits such as blueberry, bilberry and cranberry, using crude extracts. In the ERA-IB project ANTHOcyanin production Platform Using Suspension Cultures (ANTHOPLUS), novel plant cell cultures have been developed for stable production of a wide variety of anthocyanins. Our cell cultures, uniquely, allow sustained, high level production of diverse anthocyanins in bioreactors. Enhanced supplies of pure anthocyanins will be highly valuable for the colourant industry to investigate the effects of decorations, co-pigments, pH on colour and stability to provide a robust scientific foundation for developing new plant sources of natural colourants and new formulations for natural colours.At NMBU, bioreactors specially designed for plant cells were used in order to produce high levels of anthocyanin rich cells. Our bioreactors allow for constant monitoring and easy alteration of factors such as temperature, oxygen, light conditions and pH. This allows for customization for cell lines with different needs, and optimization of stress factors to increase either cell growth or anthocyanin production. Our tissue culture system demonstrated the potential of plant cells as green factories for the stable production of natural plant product. Our results suggest that the bioreactors are able to produce a higher density of plant cells than comparable suspension cultures, while maintaining a high anthocyanin concentration.

Characterization of agmatine coumaroyltransferase involved in the chemical defence of Hordeum vulgare

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Some species in genus *Hordeum* produce hordatines or murinamides whose presence are restricted to the genus. These compounds are formed by dimerization of hydroxycinnamic acid amides (HCAAs) such as coumaroylagmatine and feruloylagmatine. The HCAAs in barley are biosynthesized by an *N*-acyltransferase, agmatine coumaroyltransferase (HvACT). Based on the activity and primary structure, HvACT belongs to the clade IV of BAHD superfamily of plant acyltransferases, and is the sole enzyme whose function is known in this clade. In this study, we analyzed the tissue localization of HvACT in barley and examined the substrate specificity to investigate its functions. Additionally, we also tried to crystallize HvACT to elucidate its molecular properties. The analyses of HCAA-accumulation and gene expression revealed that HvACT is mainly present in coleoptile and plays a role in the biosynthesis of coumaric acid amides. As a result of the analyses of the substrate specificity, HvACT was showed to have low selectivity for hydroxycinnamoyl-CoA as the acyl donors while has high specificity to agmatine as the acyl acceptor. We also screened the condition for its crystallization, and could obtain needle crystals under a single condition. After optimization of the condition, plate-like crystal was formed and subjected to X-ray diffraction. Although diffraction image was obtained, the resolution was not sufficient and the phase could not be determined by molecular replacement method. The information about the reaction and substrate recognition mechanism were not provided, but the reaction mechanism of HvACT is likely to be similar to already-known BAHD acyltransferases because the substitution of the histidine residue within HXXXD motif, one of the conserved sequences among this superfamily, caused significant decrease in the activity.

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Nutrient dynamics and Leaf litter decomposition of Maple (Acer velutinum Boiss.), Alder (Alnus subcordata C.A.Meyer) and Norway spruce (Picea abies (L.) Karst) in Caspian habitat

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Leaflitter decomposition and dynamics of nutrients is essential process in forest ecosystems and playes an important role in the transfer of energy and ecological sustainability of forests. The amount and composition of litter nutrients in biogeochemical cycles is necessary, and the nutrient sycles, Carbon dioxid releasing and nutrient mineralization are regulated by it. This research with purpose to evaluating of nutrient dynamics and leaf litter decomposition in both pure and mixed mode was performed in mixed stands of Maple, Alder and Norway spruce. For this purpose, litters were treated for 400 days using litterbag method in Lajim Region. In this study, litterbags (30×20 Cm and 2 mm mesh) were sampled with four replications during the period and the following factors were measured: decomposition rates, chemical components of leaf litters such as nitrogen, phosphorus, potassium, calcium, magnesium, manganese and lignin. The results showed that litter decomposition rates were different among leaf litters. Maple and Alder (40.36% and 44.45% respectively) compared with Norway spruce needles (28.45%) had higher decomposition rates. The advantages of adding broadleaves litter with different applications to Norway spruce needles had a positive effect on the needles decomposition so that decomposition rates in Norway spruce needles in combination with Maple and Alder litters had significant increases (30.92% and 35.7% respectively). In contrast, the adding of broad leaf litters to Norway spruce needles hadn’t a significantly effect on nutrient dynamics (Except for phosphorus and calcium). In view, point of releasing rate of nutrients it was observed that in Norway spruce needles expect for the concentration of lignin, the releasing rate of nitrogen, calcium and manganese was negative. So that in Maple and Alder leaf litters expect for the concentration of manganese, the releasing rate of nitrogen, calcium and lignin was positive.

Effects of Vermicompost Application on Physiological and Merphological Triats of Medicago rigidula L. , Medicago polymorpha L. and Onobrychis sativa L.

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The use of bio-fertilizers may due to heavy metals immobilization in the soil. This research has been conducted based on a factorial experiment in a completely randomized design with four replications in Malayer University. Pots contained a mixture of soil and different amounts (0 and 60% by weight) of vermicompost as bio fertilizer. Plants exposed to cadmium nitrate concentrations (0, 4 and 8 mmol per kg). The results showed that by increasing the concentration of cadmium root length and plant height significantly (P <0/05) decreased. The effect of Cadmium ion vermicompost fertilizer on root to shoot ratio was significant at the 5% level. The highest value of root to shoot ratio was 69 seen in Cd1V1 treatment. The maximum and minimum numerical value of total protein and survival capacity were seen Cd1V2 and Cd3V1 treatments. Translocation Factor (TF) significantly decreased by use of vermicompost fertilizer. The highest root concentration factor (RCF) of Cd was found in V1×Cd2 as compared to the other treatment levels. Generally, tolerance index (TI) values of all studied plants were significantly higher in the lower lead concentrations. Also compare the performance of three studied plants showed that MR compared to other species had higher survival capacity, tolerance, Translocation Factor, protein content and higher growth capacity in the presence of cadmium ion and fertilizer.

Biostimulating properties of protein hydrolysates derived from waste products on cereals

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The reduction of negative environmental impact of agriculture is one of the greatest challenges worldwide. The use of environmentally friendly products such as plant biostimulants generated from waste is a promising solution for new sustainable crop cultivation. Moreover, the re-use of raw-materials is one of the key principles of the Circular Economy Package adopted by the EC.
Biostimulants are substances enhancing crop growth performance and/or stress resistance regardless of their nutrient content. These products with rapidly increasing market share are still insufficiently regulated by appropriate EU legislation. Proof of their beneficial effects is essential for establishment of a legal framework.
The biostimulating effects of foliar-applied protein hydrolysates (PHs) derived from organic waste material were tested on winter wheat and spring barley in a pot experiment. In addition, the influence of P nutrition and arbuscular mycorrhizal fungi (AMF) on the biostimulant efficacy was investigated.
The results showed that the efficacy of the PHs can be influenced by both growth conditions and plant nutrient supply. The outcome was dependent on crop species and P supply. In barley, total and aboveground biomass was significantly increased by biostimulant treatment, but only under low P supply. On the contrary, shoot dry weight of wheat was increased only in plants with standard P supply. In fact, the PHs' biostimulating properties are attributed to the N-containing compounds which can trigger distinct metabolic processes in plants, where also plant nutrient status may play role. No significant plant growth promotion by AMF was detected. In barley, the ratio of chlorophyll a/chlorophyll b was positively influenced by inoculation with AMF independently from the PH treatments or P supply.
In cultivations that require high fertilizer input, the PHs may be valuable complements contributing to the reduction of mineral fertilizer doses. However, the biostimulating properties must be further investigated under field conditions.

PEG-Induced Stress Effects on Soybean Germination Ratio and Root Elongation

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As a result of the global climatic changes, water deficiency is increasingly threatening food production for the growing population worldwide, especially drought-sensitive crops, to which soybean (*Glycine max* (L.) Merrill) belongs, by suppressing yield's potentials. Early drought stress can inhibit seed germination; furthermore, it can manipulate the root elongation of the germinated seeds.
A controlled two-stage experiment was conducted to investigate the drought stress, induced by PEG (Polyethylene Glycol), effects on the germination ratio (GR) and root elongation (RE) of two soybean cultivars, *'ES Mentor'* and *'Pedro'*, in Debrecen, Hungary in 2018. At the first stage, 5 PEG concentrations; 10, 15, 20, 25 and 30%, besides the control (0% of PEG), were applied to each cultivar in 3 replicates. The stage was considered completed when the control hypocotyl's length averaged 3 cm. The results showed that both cultivars could not germinate when 25 and 30% of PEG were applied. Significant differences between PEG concentrations, cultivars and their interaction were recorded. Based on these results, the second stage consisted of 6 PEG concentrations; 2.5, 5, 7.5, 10, 12.5 and 15% (besides control), also in 3 replicates. For both cultivars, (GR) and (RE) significantly decreased as the PEG concentration increased. *'Pedro'* could maintain a higher (GR) than *'ES Mentor'* under all PEG concentrations except for 15%, whereas (RE) was significantly lower under all concentrations.
It was concluded that *'Pedro'* can germinate better under different water-deficiency levels; however, germinated seeds of *'ES Mentor'* can tolerate water deficiency better, as the roots could elongate deeper searching for available water.
Based on these results, a future controlled experiment will be conducted to study the effects of PEG-induced drought stress during the early vegetative stages on some physiology traits of both cultivars.

A REVIEW ON WATERLOGGING AND ITS EFFECTS IN MULBERRY.

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The success of sericulture lies on the production of quality mulberry leaves. Mulberry *(Morus* sp.L.) forms the basis of commercial sericulture since mulberry leaf is the natural and only source of food for silkworm (*Bombyx mori* L.). Mulberry being a perennial crop, consistently good yield of quality leaf can be obtained continuously for many years after establishment of the plantation. Mulberry cultivation in India are restricted in certain places where production suffers heavy losses due to environmental stresses naturally inflicting in recurrent manner. In lower and middle Gangetic plane, mulberry cultivation suffers significant loss due to certain water-regime reasons, among which flooding is an important one. It is obvious that reduction in leaf yield and leaf quality directly affect silk worm production and maintenance. Usually the high yielding varieties are susceptible to water stagnation. If water logging stress resistant genotypes are raised through conventional and molecular marker assisted breeding and the physiological, biochemical and molecular basis behind the abiotic stress, i.e; waterlogging is understood, then the extent of loss inflicted upon the farmers would be minimized. In this article, waterlogged condition has been reported to set the mulberry plants on a war footage with endogenous supply of many phytochemical such as free radicals, singlet oxygen's and other by products of normal physiobiochemical cycles. Glycolytic pathways are preferred since the soil suffers from less oxygen content, hypoxia and ultimately anoxia in rhizosphere lead to decreased oxygen uptake and also there is prevalence of shunt mechanisms to produce energy molecules. Free radicals lead to damage in lipid protein backbone of the membrane and solute leakage has been reported in certain cases. Biochemical stress is reflected on the structural deformities also.

Structure of the leaf blades in some valuable horticultural crops culturedin vitro

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Methods of plant tissue culture *in vitro* are widely used in modern biology. One of the main topics is investigation of *in vitro* plant assimilating organs structure,which is associated with their successful adaptation *in vivo*. Histological analyzes of the leaf bladesin a number of valuable essential oil, fruit and ornamental plants were carried out by us.Plants were cultured for 12 months on modified MS and PMb media in a climatic chamber at 24±1°C, 16-hour photoperiod and light intensity 37.5 μmol $m^{-2}s^{-1}$. The leaf thickness varied from 91 μm to 235 μm. There was thin cuticle on epidermis surface (3-7 μm) in *Rosa* × *damascene* Mill., *Rosa chinensis*, *Lavandula angustifolia* Mill., *Lavandula hybrida* Rev. and *Ficus carica* L. Multiple simple and glandular trichomes (81-303 μm) on a leaf blades surfaces in *L. angustifolia*, *L. hybrida*, *F. carica*, *Chrysanthemum* × *hortorum* Bailey and *Diospyros kaki* Thunb were noted. Amphistomatic leaveswere in *Canna* × *hybrida* hort. ex Backerand *C.* × *hortorum*, in the other species–hypostomatic. The number of stomatal apparatus per 1 mm^2 varied from 15 to 265 ones. Mechanical and vascular tissues were developed in all the cultivars. Differentiated palisade and spongy tissues were characteristics of *F. carica*, *R.* × *damascene*, *R. chinensis*, *L. anustustifolia*, *L. hybrida*, some *D. kaki* and *Clematis* L. cultivars. Palisade index varied from 0.32 to 0.58. Homogeneous mesophylls with large intercellular spaces was noted in *C.* × *hybrida*, most cultivars of *D. kaki*, *C.* × *hortorum* and *Clematis*. Thus, it has been identified a number of cultures which under *in vitro* propagation demonstrated structural features typical for *ex situ* plants, providing their better adaptation *ex vitro*. This study was funded by the research grant N 14-50-00079 of the Russian Science Foundation.

Epigenetic modifications levels in roots treated with auxin are significantly changed in Hordeum vulgare

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Auxin is a plant hormone that orchestrates almost every aspect of plant growth and development. The cellular levels of auxin contribute to the regulation of the gene expression that defines cell fate and disruptions of auxin movement dramatically impacts root patterning. Auxin treated roots actually show a major reorientation of the axes of cell divisions from the longitudinal to the radial directions. Additionally, Ishida et al. (2010) showed that the auxin-mediated modulation of the mitotic-to-endocycle switch is closely coupled with the developmental transition from cell proliferation to cell differentiation in the root meristem. Most importantly, it was suggested that part of the auxin-dependent cell cycle control might rely on their posttranscriptional modification. However, how direct this regulation is in relation to the existing auxin-signaling pathway has not yet been established. Apical meristem activity, crucial for plant development, is strictly controlled by the numerous and interconnected pathways involving transcriptional regulation, phytohormones, and epigenetic gene regulation. Our results, published in 2013 (Braszewska-Zalewska at al., 2013), indicated that the levels of modified histones and DNA vary between various tissues within the barley root apical meristem. This epigenetic turnover was evident both along the longitudinal (from the root cap to the elongation zone cells) and across the transversal (from the epidermis to the metaxylem cells) axis of the meristem. Treatment of barley seedlings with IAA caused the global increase of histones modification levels in root meristematic cells. The most significant increase was observed for histone H3 and H4 acetylation (H3K18ac and H4K5ac) after 50 µM of IAA treatment. Additionally we noticed that the mitotic index did not change significantly in roots treated with auxin, whereas the number of metaxylem cells were higher in these roots.

Identification of flowering time genes associated with vernalization in Chinese cabbage and their differential expression in radish

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Flowering time (Ft) is an economically important characteristic of Chinese cabbage crops with high leaf yields and late flowering are favorable traits. There have been few reports on genes involved in Ft and the flowering mechanism in Chinese cabbage. Here, we conducted genome-wide transcriptome analysis using the inbred Chinese cabbage line, '4004' in response to vernalization. A total of 1,657 differentially-expressed genes (DEGs) were identified with and without vernalization. Transcriptome analysis identified 223 homologs of *Arabidopsis* Ft genes in Chinese cabbage, and 50 of these genes responded to vernalization. Real time-quantitative PCR (RT-qPCR) analysis of major Ft genes showed that the majority of flowering enhancers were upregulated in response to vernalization, whereas most flowering repressors were downregulated in response to vernalization. Among the major Ft genes, the expression of *BrCOL1-2, BrFT1/2*, *BrSOC1/2/3*, *BrFLC1/2/3/5*, and *BrMAF* was strongly affected by vernalization. Comparative analysis of Ft gene expression in Chinese cabbage and radish (*Raphanus sativus* L.) indicated that some genes had significantly different expression; *BrELF4*, *BrSPA3*, *BrGAI*, and *BrGID1A* expression in Chinese cabbage was the inverse of that in radish. Some major Ft genes, including. *RsFRI* and *RsLFY* were not expressed in radish, whereas most of the major Ft genes were expressed well in Chinese cabbage. These results provide new insight into the functional divergence in flowering pathways and regulatory mechanisms between the two Brassicaceae crops. Further analysis of the major integrator genes between early and late-flowering inbred lines facilitates understanding flowering trait variation and underlying molecular basis of flowering in Chinese cabbage.

A method for the isolation of intact vacuoles from Petunia petals

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Vacuoles are the largest compartment of plant cells, occupying more than 80% of the cell volume. A myriad of amino acids, sugars, lignin monomers and other metabolites are stored within these organelles. Flowers are known to produce specialized metabolites, e.g. pigments and volatile compounds, that are tissue/cell specific. To better understand the metabolism and intracellular fate of these compounds, it is crucial generate tools to analyze the content of floral-cell vacuoles.
We present here a comprehensive method for isolating pure, intact vacuoles from petal protoplasts of *Petunia* x *hybrida*, an important model plant for the study of floral traits. Briefly, to allow the separation of vacuoles, protoplasts are first isolated by incubating pierced floral limbs with hydrolytic enzymes, followed by their purification. Then, concentrated protoplasts are thermally and chemically lysed and the vacuoles are extracted by using a ficoll step gradient. At this point, a purified and enriched fraction of vacuoles is achieved. We demonstrate the use of this preparation for further uses such as GC-MS analysis for the discovery of sequestered volatile compounds and Western blot. This method is not time consuming and negates the need of using special equipment; yields pure and intact vacuoles for a range of downstream analytical purposes such as metabolomics, proteomics, lipidopmics and more.

Technology transfer and capacity building in Biotechnology and Biosafety for a sustainable and intensified agriculture in Africa

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The IPBO (International Plant Biotechnology Outreach) promotes access to scientific and technological innovations as ways of enhancing food security and promoting a sustainable intensification of agriculture in Africa. Innovations in biotechnology hold massive opportunities for developing a more sustainable agriculture. However, converting these opportunities into practice in emerging economies requires a concerted effort in training in--and access to--the latest technological developments and the design of effective biosafety and regulatory mechanisms.
IPBO is an active cell within the VIB (Flemish Institute for Biotechnology), Belgium, created by Prof. Marc van Montagu in 2000. Its mission is threefold: (1) improve understanding and create awareness about the importance of green biotechnology applications for sustainable development (communication), (2) empower plant biotechnologists and plant breeders from developing countries and emerging economies through training and capacity building in plant biotechnology and biosafety (training), (3) act as a focal platform for green biotechnology in Europe and leverage outreach to developing countries and emerging economies (networking). This network is being used to work out projects linking Flanders and European scientists or companies at one side and African partners at the other side. The final goal is to build a bridge to enable better transfer of technologies for development of agriculture and agribusiness sectors.

Cladophora glomerata L. as indicator of chemical pollution in coastal areas of Farahabad Region, Iran

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Abstract
Apart from key ecological role, some algal species have emerged as important indicators of chemical pollutants. Among such algal species, *Cladophora glomerata* L. which is a prevalent filamentous green alga found along the southern coast of Caspian Sea has emerged as important indicator of some metal pollution. The current study reports the analysis of *Cladophora* samples during summer of 2016 for selected metals and other nutrients. The antioxidant activity was also investigated in collected algal samples from various sampling points along the Caspian Sea with peak tourists' activity. The detection of heavy metals in *Cladophora glomerata* was found higher than normal background levels for studied metals (Cu, Zn, Mn, and Fe). Such higher values of metals in this indicator species is alarming and show a threat to recreational value of Coasts of Farah Abad region along Caspian Sea. It is urged that policy makers and stakeholders need to devise strategies to protect coastal areas of Caspian Sea so retain their recreational value and natural ecological phenomenon.
Key words: *Cladophora glomerata*; bioindicator; Caspian Sea; Farahabad; metal pollution, tourism

Monitoring rain-fed alfalfa crops through remote Sentinel-2A satellite and drone-adapted sequoia images

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The ”Bardenas Reales Natural Park” in the semi-arid Ebro basin (SE Spain) contains a unique and fragile landscape, highly sensitive to environmental changes because of the combination of highly erodible bedrock and semi-permanent water stress conditions that limit the vegetation cover on slopes. Traditional agriculture and animal husbandry are the main human activities in this region. Although alfalfa is successfully cultivated in this area under non-irrigated conditions, cereal crops prevail in this area in the last years. Currently, rain-fed alfalfa crops are being promoted to improve the forage offer for cattle grazing while favoring sustainability and biodiversity of the area. This study aims to assess the utility of Sentinel-2A and drone-adapted sequoia images for estimating rain-fed alfalfa density. Sentinel-2A remote sensing European Space Agency (ESA) program provide 10 and 20 meters spatial resolution multispectral images, which are one of the most developed technologies to manage medium extension crops. Sequoia multispectral camera spatial resolution could be less than 10 cm^2 per pixel. First, vegetation Indexes (VI) were calculated from Sentinel-2A and Sequoia images at the time that in-situ alfalfa density (1 m^2 plots) was determined for five rain-fed alfalfa fields. The correlations between in situ densities and remote VI derived from Sentinel-2A and Sequoia were calculated. Both comparisons showed high correlation coefficients (R^2=0.935) that prove that Sentinel-2A is suitable to monitor rain-fed alfalfa crops in Bardenas Reales. Among others, the NDVI index showed the highest correlation coefficient. Secondly, average NDVI value for a collection of 28 rain-fed alfalfa fields was compared with the NDVI estimated for one irrigated alfalfa plot from July 2016 to August 2017. The difference between NDVI values of rain-fed and irrigated fields was high, but in both cases, Sentinel-2A allow to follow the alfalfa crop development and the changes of crop coverage occurring throughout the year.

Revealing the role of protein phosphorylation in the progamic phase of tobacco male gametophyte - from phosphoproteome to particular candidate genes

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Angiosperm mature pollen represents an extremely desiccated, metabolically quiescent structure surrounded by a tough cell wall. Upon re-hydration it becomes metabolically active and later on pollen tube growth starts. These changes are accompanied by *de novo* protein synthesis from the stored transcripts together with changes in phosphorylation of the existing proteins.
The combination of phosphoprotein enrichment by aluminium hydroxide coupled with conventional 2D gel-based techniques, and phosphopeptide enrichment by titanium dioxide followed by gel-free approach was applied in our phosphoproteomic studies of tobacco mature pollen, and two stages representing progamic phase (5-min, and 30-min activated pollen). From these stages, we collectively identified 471 phosphopeptides that were assigned to 301 phosphoproteins, which belonged into 13 functional groups; the majority of them was put into these categories: transcription, protein synthesis, protein destination and storage, and signal transduction.
From the acquired phosphoproteomic data set, several candidate genes were selected, nascent polypeptide associated complex (NAC) amongst others. The double homozygous mutants of the genes coding for its beta subunit were acquired by a conventional cross of two available T-DNA insertion lines of *Arabidopsis thaliana.* These double homozygous mutants showed defects in male gametophyte, flowers and siliques; in particular their flowers were composed of abnormal number of flower organs compared to the wild type Columbia-0 plants, and their shorter-than-normal siliques carried a lower number of seeds per silique. A detailed functional analysis of these genes has been started, for instance transmission efficiency of the mutant alleles was compared to the wild type ones, and the mutant phenotype was complemented by inserting the functional copy of the gene to the mutants. Moreover, protein localization, and ectopic overexpression were also performed.
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Protein complexes in regulation of biological processes

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Six MYB and three MYC bHLH transcription factors (TFs) control the levels of glucosinolate (GLS) defense compounds in *Arabidopsis thaliana.* The bHLH TFs MYC2*,* MYC3 and MYC4 regulate all classes of GLS (Schweizer et al., 2013), while MYB28*,* MYB29 and MYB76 regulate aliphatic GLS levels (Sønderby et al., 2007, 2010) and MYB34*,* MYB51 and MYB122 control indolic GLS (Frerigmann and Gigolashvili, 2014). Both MYCs and MYBs are strictly required together for GLS production. Direct interaction between the MYBs and MYCs may play a role in the regulation of GLS as the three MYCs interact with all six MYBs (Schweizer et al., 2013).

If MYB-MYC complexes control transcription, then what controls complex formation? Two small glycine rich proteins associated with endo-membranes are co-expressed with enzymes of the GLS biosynthetic pathway and *MYB28.*

Split-ubiquitin assays show both proteins interact with MYB29, Bimolecular Fluorescence Complementation (BiFC) experiments show that they co-localize with MYB29 in *Nicotiana benthamiana,* and Flourescence Recovery After Photobleaching (FRAP) show that at least one of the MYB29 interactors increases the recovery time of MYB29 fluorescence in the nucleus. Together, these results suggest that the two novel MYB interactors play a role in GLS regulation. Further studies of their role in GLS regulation through their interaction with the MYB TFs. This will give new insight into the role of small proteins in protein interaction and complexes, and what part this plays in the regulation of biological processes.

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The spatio-temporal expression patterns of microRNA in response to high temperature stress in rice.

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Rice production is negatively affected by high temperature and other environmental stress. MicroRNAs (miRNAs) are a novel class of endogenous, non-coding small RNAs that have been established as ubiquitous regulators of post-transcriptional gene regulation via degradation or translational repression of the cognate mRNA targets. They also play an important role in mediating the stress responses in plants. Thus, understanding the miRNA mediated regulatory schema for high temperature stress tolerance is necessary to raise novel crop varieties that can withstand or avoid stresses imposed by fluctuations in temperature. In the present study, we analyzed the NGS datasets (IIlumina, GA) of control and heat stressed libraries generated from 15 day old seedling leaves, flag leaf and Panicles of heat sensitive Pusa Basmati1 rice variety. After comparative analysis, we identified several known and novel miRNAs that displayed a spectrum of response ranging from stable to highly variable between tissues. In depth profiling of selected miRNAs was performed under various heat stress durations. To understand the functional implications of the miRNAs, their targets were identified. The predicted targets include transcription factors, protein kinases, ATP-binding proteins, HSPs, HSFs, Growth-regulating Factors, oxidoreductases, antioxidants etc. that are linked with various metabolic & cellular processes thereby regulating the stress responses. Future studies of these miRNAs may provide better understanding of the molecular links behind these regulatory networks and may deliver fresh application routes for rice improvement during heat stress.
This work was supported by a grant from Department of Biotechnology, Government of India.
Key words: Rice, heat stress, microRNA, expression patterns, panicles

Development of a transient expression system in white teak (Gmelina arborea Roxb) and cocoa (Theobroma cacao L) mediated by transcriptional fusions transfected by Agrobacterium tumefaciens.

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Transformation mediated by *Agrobacterium tumefaciens* is one of the most used tools for developing genetic modifications in plants, however in forest plants like white teak (*Gmelina arborea* Roxb.) and cocoa (*Theobroma cacao* L.) this procedure is limited by intrinsic features associated to their physiology, for that reason, it is necessary to develop tools that allow inverse genetic applications in this field. Thus, in this work we propose *A. tumefaciens* transient expression as a potential tool to generate this kind of determinations, for that we evaluated the effect of Silwet-L77 (surfactant) and acetosyringone (inductor), also, we evaluated three methods of infiltration. In white teak's case we found that concentrations of 0,002% and 100µM of Silwet-L77 and acetosyringone respectively, infiltrated by co-cultivation give a satisfactory response in terms of the reporter gene (GUS), in the other hand, for cocoa we evaluated in the genotype EMC-67 the infiltration by vacuum, syringe injection and co-cultivation and found that by the three methods infiltration is satisfactory. In conclusion we found that under the conditions tested transient expression was reached, nevertheless, it is necessary to optimize this procedure, assessing other features like *A. tumefaciens* strains, reporter genes and plant tissues.

LOB-domain transcription factor gene family of Brachypodium distachyon

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Brachypodium distachyon became widely accepted model plant for grass species including important crop plants like wheat barley and rye. We study LOB-domain transcription factors in this model. These transcription factors are plant specific proteins involved in diverse developmental processes from floral development to emergence of lateral roots therefore important regulators of plant architecture.
In *Brachypodium* genome twenty-eight genes code for this protein family. Based on their sequence similarities, unified nomenclature for this gene family was proposed. Various plant part/organ specificities were found in the expression of these genes, one of them possesses exceptionally high root-specificity. Ectopic expression of two of them in *Brachypodium* lines resulted in severe developmental phenotypes including altered inflorescence development and reduced fertility.
Early Y2H results indicated that some of these proteins may interact with cyclins, components of the central regulatory complex of the cell cycle and the analysis of the protein sequence of LOB-domain proteins revealed several putative serine-threonine phosphorylation sites. In vitro phosphorylation confirmed that at least two members of the LOB-domain protein family can be substrate of cdk-cyclin complexes.
We hypothesize that some members of this transcription factor family may play key role in the decision of the plant cells ”to divide or to differentiate”. Root development is studied because root architecture is base on the delicate balance of cell division and differentiation and a strong and efficient root system is also extremely important for the survival of our crop plants under stressful environmental conditions. Root specific LOB-domain protein coding genes are compared in selected lines representing the six major groups of Brachypodium ecotypes in terms of root development under limiting water supply.

The level and localization of Arabidopsis nitrate reductases are regulated by ammonium and E3 SUMO ligase AtSIZ1

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Nitrate reduction is an important step for providing sufficient nitrogen in plants, which is occurred by the action of nitrate reductases. Previously, we reported that NR activity was regulated by sumoylation through the E3 ligase activity of AtSIZ1. However, it is not clear identified how nitrate reductases interact with AtSIZ1 in the cell, or how nitrogen sources affect the levels of nitrate reductases and their cellular localization. Here, we show that the subcellular localization of nitrate reductases is modulated by the E3 SUMO ligase AtSIZ1 and that the levels of nitrate reductases are regulated by nitrogen sources. Transient expression analysis using green fluorescence protein showed that the nitrate reductases NIA1 and NIA2 localize to the cytoplasmic membrane and that AtSIZ1 localizes to the nucleoplasm, including nuclear bodies, when expressed separately, whereas nitrate reductases and AtSIZ1 localize to the nucleus when co-expressed. Transcription analysis showed that nitrate did not affect the subcellular localization of the nitrate reductases and they were not detected in ammonium-treated cells. Immunoblot analysis revealed that the levels of nitrate reductases increased in response to nitrate but decreased in response to ammonium. In addition, the levels of nitrate reductases increased in *cop1-4* and DN-COP1-overexpressing transgenic plants, and the degradation of nitrate reductases was delayed in *cop1-4* than in the wild-type. These data indicate that AtSIZ1 controls nuclear localization of nitrate reductases, and ammonium negatively regulates their levels, and also suggest that the function and stability of nitrate reductases might be post-translationally modulated by ubiquitination through the activity of E3 ubiquitin ligases including COP1.
This work was supported by a grant from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center no. **PJ01327601**), Rural Development Administration, Republic of Korea.

Ubiquitination and sumoylation of E3 SUMO ligase AtSIZ1 are specifically modulated by heat and drought stresses

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Sumoylation regulates numerous cellular functions including the localization, level and stability of various proteins in plants as well as aimal systems. However, the regulatory mechanisms controlling E3 SUMO ligase are poorly understood. Here, sumoylation and ubiquitination of the Arabidopsis E3 SUMO ligase AtSIZ1 was specifically regulated by abiotic stresses. AtSIZ1 ubiquitination was induced by exposure to heat stress in transgenic plants overexpressing the E3 ubiquitin ligase COP1. In addition, AtSIZ1 ubiquitination was strongly enhanced in transgenic plants overexpressing SUMO isopeptidase ESD4 under heat stress. By contrast, drought stress induced sumoylation rather than ubiquitination of AtSIZ1 and sumoylated forms of AtSIZ1 accumulated in *esd4* and *cop1-4* mutants. Moreover, *siz1* mutants were found to be tolerant to heat and drought stresses. Our data indicate that ubiquitination and sumoylation of AtSIZ1 in response to abiotic stresses depend on the activities of COP1 and ESD4, and that the activity and stability of AtSIZ1 can be specifically controlled by different abiotic stresses.
This work was supported by a grant from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center no. **PJ01327601**), Rural Development Administration, Republic of Korea.

Solanum lycopersicum (tomato) possesses multiple lipoyl synthases capable of increasing lipoylation levels in vivo

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Lipoic acid (LA) is a functional metabolite with powerful antioxidant capacities present in eukaryotic and prokaryotic organisms. LA is both lipid- and water-soluble, and is the prosthetic group of several key multi-subunit enzyme complexes, including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase of the TCA cycle. LA biosynthesis and incorporation into these target proteins (lipoylation) proceeds *de novo* or via a salvage pathway. During *de novo* synthesis, octanoyl transferase (LIP2) uses octanoyl groups linked to an acyl carrier protein to transoctanylate target proteins. Subsequently, lipoyl synthase (LIP1) catalyses the final step by inserting two sulphur atoms into the prosthetic group. Whilst a number of the enzymes have been functionally-characterised in *Arabidopsis thaliana*, the aim of the current work is to identify and evaluate the role of this pathway in a fruit-bearing species. Towards this aim, we identified two proteins in tomato (*Solanum lycopersicum*) with the molecular characteristics of LIP1. We call these proteins SlLIP1 and SlLIP1p, which possess 78% and 84% amino acid identity with AtLIP1 and AtLIP1p, respectively. Confirming bioinformatic predictions, SlLIP1 has a mitochondrial localisation whereas SlLIP1p is plastidial, as shown by confocal microscopy after transient transformation of tobacco leaves with GFP fusion proteins. Furthermore, both proteins rescue carbon source requirements and lipoylation levels of an *Escherichia coli* lipoyl synthase mutant (*lipA*), and thus act as lipoyl synthases in this heterologous system. Additionally, stable over-expression of these genes in tomato produces transcriptional alterations in genes encoding proteins involved in LA metabolism, and target proteins of the TCA cycle, which in turn correlate with developmental differences and increased levels of lipoylation measured in several over-expressing lines. Funding: Fondecyt 1181198.

Implication of hybrid necrosis in Petunia speciation

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By impeding genetic exchange between populations, reproductive isolating barriers promote their divergence and are the origin of new species. In sympatry, plant species can be well-isolated from each other solely through high pollinator preference towards one species. This is due to particular combinations of floral traits such as scent, color and morphology (termed pollination syndromes) that are adapted to attract specific animal pollinators. Using two distinct Petunia species co-occuring in a region of Brazil, we set out to unravel the genetic basis of floral diversity through quantitative genetic approaches. *Petunia exserta* has a hummingbird pollination syndrome with red, UV-reflecting, non-scented petals and exerted reproductive organs, whereas the hawkmoth-pollinated *P. axillaris* has white, UV-absorbing, volatile-producing petals and non-exerted reproductive organs. Surprisingly, most of the QTLs controlling those traits are tightly linked in a large region located on chromosome two where recombination is very low, allowing a fixed combination of floral traits. We recently found that this region also triggers necrosis when it comes from *P. axillaris* in an otherwise *P. exserta* genetic background. Hybrid necrosis is a known postzygotic isolating barriers affecting plant fitness. To study the evolution of hybrid necrosis and its potential relation to the prezygotic isolating barriers, we need to identify the genes underlying this trait. Using quantitative genetics and genomics, I am elucidating the genetic basis underlying hybrid necrosis. The genetic data obtained so far strongly indicate a system of two or more interacting loci. While the locus on chromosome two is nearly resolved at the gene level, interacting loci are currently being mapped. Ongoing experiments to characterize and identify the genes underlying hybrid necrosis will be presented and hypotheses about the potential role of necrosis in Petunia speciation discussed.

Coordination of starch biosynthesis in cereal amyloplasts

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A number of different starch synthases, starch trimming enzymes and co-factors interacting with these enzymes, coordinates biosynthesis of storage starch granules. By changing expression levels of the different enzymes and factors coordination among them is changed with a subsequent effect on the structure and chemical characteristics of the storage starch. We have used different means to study starch biosynthesis and how the orchestration of the starch biosynthesis machinery can be changed to direct starch and grain quality in a direction of new functionalities and carbohydrate types. Expression of transgenic enzymes in amyloplasts is a way to modify starches and may act as an alternative to chemical, physical and enzymatic modification of native starch. Expression of transgenic factors with strong binding affinity for starch competitively interacts with the starch biosynthetic machinery. We also applied new breeding techniques to generate cereal lines with previously uncharacterized carbohydrates in the grain. We used fluorescent tagging of starch synthases to image their action in and around starch granules in developing cereal grains. By using fluorescence resonance energy transfer (FRET) we have observed direct interaction among different starch synthases and starch trimming enzymes in developing amyloplasts and how they are coordinated during cereal grain development.

Kinetin derivatives with UVA and UVB photoprotective effect on human skin cells

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6-Furfurylaminopurine (Kinetin, Kin), a compound that belongs into aromatic cytokinins, has been described as a multiactive molecule with various activities both in animal and plant cells. New Kin derivatives were synthesized to obtain biologically active compounds. Standard routes of preparation via nucleophilic substitution of 6-chloropurine and 2,6-dichloropurine by appropriate amines were modified to obtain compounds in high purity and decent yields. Cytokinin activity of new derivatives was tested in tobacco callus, detached wheat leaf chlorophyll retention bioassay and *Amaranthus* bioassay. Cytokinin activity of prepared derivatives was confirmed. Selected compounds were subsequently tested on normal human dermal fibroblasts (NHDF) and keratinocyte cell lines (HaCaT) to exclude possible phototoxic effects and, on the other hand, to reveal possible UVA and UVB photoprotective activity. Naturally occurring antioxidant Rosmarinic acid and structurally similar well known compound 6-furfurylamino-9-(tetrahydrofuran-2-yl)purine (Kin-THF) were used as positive controls. The protective antioxidant activity of prepared cytokinin derivatives together with Kin-THF was further studied using induced oxidative stress (OS) on nematode *Caenorhabditis elegans* damaged by 5-hydroxy-1,4-naphthoquinone (juglone), a generator of reactive oxygen species. The observed biological activity was interpreted in relation to the structure of the prepared derivatives. The most potent compounds were able to significantly protect human skin cells against UVA radiation *in vitro* and to shield *C. elegans* against juglone induced OS *in vivo*. Finally, the ORAC assay showed that the compounds did not act as direct antioxidants as they were unable to directly scavenge oxygen radicals. These data suggested that the mechanism of oxidative stress protection is indirect and triggers other mechanisms than direct interaction with ROS and therefore there must exist an alternative mode of action worth study caused by these compounds.

Improving the Agricultural Traits of Crops by Sound Wave

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As the quality of life has improved, interest in secondary metabolites such as vitamin C, flavonoid and anthocyanin *etc*. We investigated whether sound treatment could increase the vitamin C and flavonoid of sprouts vegetables. We treated to alfalfa, broccoli and lettuce, with various sound wave frequency, treatment time, and treatment period. Sound wave treated sprout vegetables had higher flavonoid content than non-treated sprout vegetables, and total flavonoid content of the alfalfa, broccoli and lettuce sprout vegetables were increased by about 300, 35, and 30%, respectively. And the activity level of superoxide dismutase (SOD) was higher in the treated than non-treated sprouts vegetables, suggesting that sound waves affect antioxidant activity in plants. To investigate how sound wave affects to increase the total flavonoid, we analyzed the expression of ethylene-related genes by qRT-PCR analysis.
We previously reported that sound wave delays tomato fruit ripening by altering the expression of gene in ethylene biosynthesis. We measured the transcript levels of Arabidopsis homologs of tomato ethylene biosynthesis genes in protoplasts transiently transfected with RIN and HB-1, which are major transcription factors. Compared to untreated conditions, RIN or HB-1 induced expression of AtACS and AtACO gene was decrease. To confirm these results, we performed transient assays in *N. tabacum*, which produced results similar to those observed in Arabidopsis. These results indicated that the expression of ethylene biosynthesis related genes is controlled through the modulation of RIN and HB-1 function by sound wave treatment. This study provided a foundation for further investigating sound wave-mediated responses in plants, also suggest that acoustic biology can also be used in agriculture to help increase product quality.

An incoherent feed-forward loop switches the Arabidopsis clock rapidly between two hysteretic states

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Circadian clock plays important roles as an endogenous timekeeper that anticipates the daily changes of earth. In many eukaryotes, the core structures are composed of direct activators and repressors that form coupled negative and positive feedback loops. However, in the higher plants (ex: *Arabidopsis thaliana*), the core oscillator is mostly governed by repression process and lacks of direct activators. With a series of simplified models, we studied the underlying mechanism and found that the *Arabidopsis* clock consists of type-2 incoherent feed-forward loops (IFFLs), one of them creating a pulse-like expression in *PRR9/7*. The double-negative feedback loop between *CCA1/LHY* and *PRR5/TOC1* generates a bistable, hysteretic behavior in the *Arabidopsis* circadian clock. We found that the IFFL involving *PRR9/7* breaks the bistability and moves the system forward with a rapid pulse in the daytime, and the evening complex (*EC*) breaks it in the evening. With this illustration, we can intuitively explain the behavior of the clock under mutant conditions. Thus, our results provide new insights into the underlying network structures of the Arabidopsis core oscillator.

Genome-Wide Screening of Abiotic Stress-Responsive Long Noncoding RNAs in Rice

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Recently, long noncoding RNAs (lncRNAs) have emerged as important regulatory factors of diverse biological processes in both plants and animals. However, the number and functional roles of lncRNAs in crops remain largely unknown. In particular, systematic examination of rice lncRNAs involved in abiotic stress responses has not been performed. In this study, we re-analyzed the expression profile of lncRNAs in publicly available rice transcriptome datasets derived from abiotic stress treatments to unveil the potential roles of rice lncRNAs in abiotic stress responses. Overall, we identified 10,831 rice lncRNAs that were significantly altered in shoot and/or root tissues under four different abiotic stresses, including ABA, drought, cold, and high salt. Out of them, 5,516 and 5,315 rice lncRNAs were upregulated or downregulated, respectively, in a highly stress-specific or tissue-specific manner. These stress-responsive lncRNAs were classified into 15 groups from their stress-specific expression patterns. Based on Venn diagram analysis, we observed strong crosstalks between different stress signaling pathways, showing transcriptional regulatory networks underlying lncRNA expression changes in response to abiotic stresses. Lastly, qRT-PCR validation confirmed the differential expression patterns of these lncRNAs under various conditions. This study shows the first comprehensive identification of a group of rice lncRNAs that are involved in abiotic stress responses. The results suggest that rice lncRNAs may play crucial roles in abiotic stress tolerance mechanisms.

Towards a more realistic leaf shape model for virtual cucumber

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Leaf shape plays a major role in plant performance and, particularly, in the plant's light harvest. The effect of an environmental factor such as light on the interplay of canopy architecture and physiological functioning can be estimated using functional-structural plant models (FSPMs or virtual plant models). However, leaf models used in FSPMs are often greatly simplified in order to reduce model complexity. Such leaf models neglect, e.g., the realistic three-dimensional shape of the leaf. The aim of this study was to improve the prototype model of leaf shape used in the functional-structural plant model *L-Cucumber*. This model for dynamic growth and development of greenhouse cucumber plants is sensitive to light and temperature. The leaf shape model of *L-Cucumber*, which is used for communication with the coupled light model, allows for size adaptation, but the three-dimensional shape is quite simple: It is symmetric to the midrib and leaf area is equally distributed on four equilateral triangles, where both leaf halves are slightly tilted down. For leaf prototype improvement, three-dimensional data of more than 1000 digitized cucumber leaves of a greenhouse experiment, each measured by 17 characteristic points (landmarks), were used for comparisons of the leaf shape model and measurements. Robust Bayesian comparison of groups was used to assess statistical differences between leaf halves while respecting fluctuating asymmetries. Results detected systematic deviations shared by both halves. This information was included in an improved leaf prototype. Comparative simulations with *L-Cucumber* using either the original or the novel leaf model revealed a slight improvement in plant internode development against independent data from two further greenhouse experiments using the novel leaf shape model. Future studies could now focus on the development of a dynamic, environment-sensitive leaf shape model.

Expression and characterization of plant-derived recombinant TGF-beta families

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Activins, members of the transforming growth factor-β (TGF-β) superfamily, are secreted growth factors to regulate pituitary follicle stimulating hormone production. They also have important roles in the regulation of bone and muscle mass, liver regeneration and wound repair. Inhibin A and inhibin B, the constituents of activins, are dimeric glycoproteins composed of α-subunit and β(A/B)-subunit. Among activins, activin A is a 27.4 kDa disulfide-linked homo-dimeric protein composed of βA-subunit (inhibin A). As interest in biosimilar and molecular farming research has increased, mass production studies using plant-based expression system have been conducted. In here, Activin A is introduced in transgenic plant by *Agrobacterium*-mediated transformation, and produced with high expression level.

ACC oxidase expression during abscission in tomato leaves and flowers

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The gaseous plant hormone ethylene plays an important role in regulation of abscission. The abscission is a controlled separation of cells in the abscission zone (AZ) that is usually located at the base of an organ to be removed. The enzyme 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase or ACO) catalyzes the last step in ethylene biosynthesis, which is conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene. In tomato, there are seven ACO genes. We have analyzed the expression of two genes with contrasting patterns of expression, *SlACO1* and *SlACO4*. *SlACO1* is induced with abscission and the expression increases after induction, while *SlACO4* expression is high before abscission and decreases following abscission induction. Both genes are expressed in the tissue near the AZ. ACO1 protein was localized using immunolocalization on light microscopy and ultrastructural level, where ACO was found primarily in vascular tissue in the phloem companion cells. The specificity of the ACO antibodies was tested using Western blot of flower pedicel protein extracts. The ethylene produced by ACO in the vascular tissue may be related to the diffusible signal responsible for abscission induction.

Wheat breeding in the Canadian Prairies

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Canada is the fifth largest producer of wheat in the world. Majority of this wheat is the premium quality Canada Western Red Spring (CWRS) bread wheat produced in western Canada. Total Canadian wheat production is estimated at 25.5 million tonnes, which is 19.5% lower compared to 2016. In 2017, Canada exported approximately 20 million tonnes of wheat contributing approximately $4.5 billion dollars to the Canadian economy. Spring hexaploid wheat accounts for 69% of the total wheat production followed by durum wheat (23%) and winter wheat (8%). Approximately 96% of the wheat is grown in the prairie provinces of Alberta, Saskatchewan and Manitoba. The three Prairie Provinces vary in their growing conditions with Alberta and Saskatchewan being drier and fewer diseases whereas due to warmer summers and higher rainfall, Manitoba has some of the highest wheat grain yields in western Canada. Manitoba is a hotspot for major wheat diseases such as Fusarium head blight (FHB), leaf, stem and stripe rusts. Wheat blossom midge is also a problem in the Prairies. The registration of CWRS cultivars in Canada requires support for registration from recommending committee comprising of agronomy, disease and quality sub-committees and the Canadian Food Inspection Agency administers the variety registration process and protection of plant breeder's rights. The poster presentation focus on breeding methodology, major diseases and their sources of resistance and a breeding flowchart outlining key steps involved in wheat breeding for Canadian Prairies.

Characterization of early salinity-stress responses in FL478 rice (Oryza sativa) variety

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Climate change is reducing the quantity and quality of agricultural land due to soil and water salinization. This will directly affect crops production, such as rice which is one of the most economically and nutritionally important food for more than half of the world's population. Furthermore, rice is the most salt-sensitive cereal, specially at the early stages. Salinity stress in rice triggers several adaptive responses at the molecular, cellular, metabolic, and physiological levels to cope with the osmotic and ionic stress that salt excess implies. These are mainly involved with ion homeostasis but also with antioxidant metabolism activation and protein modifications. A major QTL present in rice for salinity tolerance is *Saltol*, which is located on chromosome 1 and present on landraces such as Pokkali and Nona Bokra. The gene conferring the high salinity tolerance encodes for an HKT-type transporter identified as OSHKT1;5. In this study, we characterized the early responses to salinity (0 and 100 mM NaCl; 6, 24 and 48h treatment) in shoots and roots at 14 days after germination in hydroponic cultures using FL478, an inbred rice line harbouring Pokkali's *Saltol* region. It was observed that shoot length, shoot and root weight were significantly reduced after 48h of salinity exposure whereas root length significantly increased. Moreover, Na^+ and K^+ displayed contrasting patterns, being significantly increased and reduced in the salinity condition respectively. Moreover, through a principal component analysis it was observed that the differentially expressed proteins in root are grouped in treatments and controls in contrasting manner, whereas shoot protein grouping was not treatment-dependent. Moreover, protein expression of antioxidant and protein protection enzymes was increased during salinity in roots. In conclusion, plantlets show adaptive responses which are more drastic in roots, which suggest that this tissue has a critical role in the salinity stress tolerance.

Integrated Crop Production - bringing together stakeholders in the research and innovation cycle

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Europe and the world are facing major challenges in terms of food and nutritional security and safety, whilst mitigating impact of climate change, preserving human and environmental health and establishing a sustainable food system. The dimension of these challenges requires transition of the sector from a linear disciplinary approach to an integrated networking approach.
The way forward is to establish a common language, arrive at joint understandings, learn from each other, mobilize resources, and develop positive externalities. In such a ”system” knowledge is co-produced by all actors in the network and smartly established feedback loops should ensure that the outcomes of collaboration are usefully fed back into the research and innovation process.
Integrating stakeholders in the research and innovation cycle offers a number of benefits. It may deliver innovations fit for purpose, higher novelty, higher success rates, reduced time-to-market, and lower costs, etc. The intrinsic value of the collaborations is that stakeholders innovate beyond their resources and make best use of internal and external ideas. Furthermore, engaging with consumers can increase the ability of the sector to develop a more agile approach to innovate and create value, as it allows to better understand customers’ needs and behaviours and their willingness to adopt products and their development accordingly.
There are different non-exclusive options for implementation of such a concept, including: contractual public-private partnership under an EU Research and Innovation Framework Programme, self-funded network or joint stakeholder document. Depending on the mode of implementation a different level of integration and reach will be achievable from weak and fragmented joint stakeholder document to strong and fully integrated cPPP.

Bioinformatic prediction and experimental validation of miRNAs and target genes in Amaranthus hypochondriacus

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The miRNAs are a class of small single-stranded and non-coding RNAs of approximately 21-25 nucleotides in length, which have been identified as important regulators of gene expression by the specific interaction with white mRNAs in multiple organisms such as plants, animals, and some viruses. In plants, the accumulation of miRNAs is spatially and temporally regulated in different organs and tissues and in different stages of development where they play a key role in various metabolic and biological processes. In several species of plants, recent research around miRNAs has been based on two main strategies: 1) identification of miRNAs by bioinformatic tools and 2) molecular validation of identified miRNAs. Following both strategies, in the present research work, a bioinformatic prediction was made that allowed the identification and validation of 16 miRNAs in the non-coding transcriptome of *Amaranthus hypochondriacus*, which predicts the possibility of elucidating the gene regulatory networks that are activated by different metabolic and biological processes in this crop.

A novel genotype-independent technique for successful somatic embryogenesis of adult plants of Jatropha curcas: Thin Cell Layer (TCL) from Petioles.

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Obtaining somatic embryos in *Jatropha curcas* has been achieved by several authors using different explants (Jha et al. 2007, Cai et al. 2011, among others), however none of these protocols have been developed using adult plants as mother plant material. Furthermore, overcoming the limitations on morphogenesis successfully and plant regeneration of adult plants of *Jatropha curcas* has been a big challenge. In this study, the main objective was to develop a somatic embryogenesis protocol for adult plants of *Jatropha curcas*. In this protocol, nine accessions of *J. curcas* of various ages (3-10 yrs) have been tested and show the protocol to be potentially genotype-independent. We have tested five different types of explants from young leaves and petioles: a) leaf discs and segments of petioles and b) variations of Thin Cell Layer technique (TCL) techniques; longitudinally (lTCL) of petioles, transverse (tTCL) of leaves and tTCL of petioles. Two different basal media (MS and Y3) in full and half strength, using four different PGRs (2,4-D, IBA, BAP and KIN), alone and in combinations and at different concentrations were tested. The preliminary results show 100 % response in inducing callus of tTCL of petioles from young leaves of *J. curcas* and pro-embryos structures (85% in the combination 0,5 mg/l of 2,4-D + 1,0 mg/l of BAP), independent of the age of the mother plant, the accession or the concentration of hormones. Therefore, the fate of the cells obtained from this type of explant can undergo different morphogenesis processes depending on the type of hormone and their concentration. However, once the bottleneck of obtaining pro-embryos is passed, we will further optimize media for maturation and regeneration of obtained embryos into fully formed plants. This study demonstrates an approach to obtain somatic embryos of mature elite plants of *J. curcas* through somatic embryogenesis.

Did the Pyrrolizidine Alkaloid Biosynthesis evolve two times independently within one tribe of Legumes?

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Within the Fabaceae the genistoid clade is in particular known for the occurrence of quinolizidine alkaloid (QA) producing taxa. Most of the genistoids produce either QA or no alkaloids but a few exceptions belonging to the Crotalarieae tribe contain complex hepatotoxic pyrrolizidine alkaloids (PA) instead. PA producing taxa are *Crotalaria* and *Lotononis* that are no direct sister lineages within the Crotalarieae suggesting an independent evolution of this trait. Therefore, we study homospermidine synthase (HSS), the key enzyme of PA biosynthesis, as a marker for the evolution of PA biosynthesis in the genistoids. It was shown that the *hss* gene evolved from a gene duplicate of the deoxyhypusine synthase (*dhs*), a gene encoding for an essential enzyme for eukaryotic translation. Hence, HSS- and DHS-coding sequences identified from several genistoid taxa are used for phylogenetic analysis. First results suggest more than one duplication event that are discussed to be the origin for the sporadic PA occurrence in this genistoid lineage. Notably, not only the origin of the *hss* gene may differ between the main PA taxa *Crotalaria* and *Lotononis* but also the organ of *hss* expression. In *Crotalaria spectabilis* the *hss* is exclusively expressed in the root nodules after inoculation with a *Bradyrhizobium* strain whereas in *Lotononis galpinii* the potential *hss* gene seems to be expressed in the roots uncoupled from rhizobial infection. Although it is known for several years that the PA biosynthesis evolved independently in different plant families this could be the first case of independent *hss* evolution within two genera of the same tribe.

Can the two carboxypeptidase inhibitors of tomato act as signalling peptides during fruit development?

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The tomato *TCMP-1* and *TCMP-2* genes code for metallocarboxypeptidase inhibitors, a subclass of the cystine-knot peptides family. The two transcripts display a sequential expression during flower/fruit development, with *TCMP-1* highly expressed in flower buds before anthesis and *TCMP-2* in ripe fruits. The alteration of their endogenous levels by expressing the *TCMP-1* coding sequence under the control of the *TCMP-2* promoter revealed that their relative levels are crucial for the fruit set timing (Molesini et al., 2018). The two mature TCMPs are 32% identical and highly similar in structure to the potato carboxypeptidase inhibitor (PCI). Both TCMPs and PCI share structural homology with some mammalian growth factors such as the epidermal growth factor (EGF) and vascular epidermal growth factor (VEGF), and are bioactive in mammalian cells. PCI competes with *EGF* for the *binding* to EGF receptor, *inhibiting its activation* (Blanco-Aparicio et al., 1998); TCMPs inhibit angiogenesis both *in vitro* in human umbilical vascular cells and *in vivo* in zebrafish by affecting the VEGF receptor activation (Cavallini et al., 2011; Treggiari et al., 2015). Thus, TCMPs can interfere with growth factor signalling pathways in animal cells. We can speculate that TCMPs could exert a similar activity also in plant cells. By sequence comparison we searched for plant receptors containing extracellular EGF-like domains that could represent good candidates for TCMPs recognition. EGF repeats in plants are found in receptor-like kinases of the wall-associated kinases-type (WAKs-type) and S-locus receptor kinases-type (SRKs-type). WAK and SRK members are shown to be involved in stress responses but also in growth and development.

Transport of cadmium and lead in soil as affected by organic matter (vermicompost)

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Heavy metals are important environmental pollutants, particularly in areas with high anthropogenic sources. This study was conducted to evaluate changes of chemical properties of Pb and Cd enriched municipal wastewater after passing through soil columns with different levels of vermicompost. Polyethylene columns filled with a clay loam soil. Enriched municipal wastewater with Pb (40 mgl-1) and Cd (20 mgl-1) was added to soil columns during 8 periods of 10 days followed by the measurement of chemical properties of drainage water in each stage. Experimental treatments consisted of 3 levels of vermicompost comprising control (V1), 2% (V2) and 4% wt (V3) and time in 8 levels with 3 replications. The results indicated that vermicompost and time have significant effects on chemical properties of drainage water. Treatment V3 showed a significant effect on pH, EC, cations and anions concentrations, P, N, total organic carbon and the amount of Pb and Cd in drainage water. All measured properties except for pH showed decreasing trends with time. Nitrate and chlorine concentrations exhibited a great increase in drainage water of the two last stages. A decreasing trend was observed in drainage pH until the sixth stage followed by an increase. Cd concentration in drainage water was larger in the first two stages of experiment compared to Pb. The results of the soil analyses showed that soil depth has significant effect on soil chemical properties. The effect of vermicompost on the amounts of Na, K, Ca, Mg, Cl, and HCO3-, pH (Pb column), P, organic carbon and Cd was significant. Significant increase of Na and K, Cl, nitrate and phosphate, organic matter, Cd and Pb was observed at surface depth of 0-20 cm compared to lower depth of 40-60 cm. Larger contents of pH, Cl and $HCO3^-$ was measured in depth of 40-60 cm.

Changes in pectin esterification and AGPs indicate the cell wall remodelling during somatic embryogenesis of Quercus suber

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Modifications in cell wall components and pectin residues have been reported as being crucial for initiating cell responses in relation to cell fate and development. Recent reports have indicated that changes in cell wall mechanics controlled by the esterification/deesterification status of pectins, mediated by pectin methyl esterases (PME) and pectin methyl esterase inhibitors (PMEI) underlie organogenesis initiation, early embryo growth and embryogenesis progression. Arabinogalactan proteins (AGPs) are highly glycosylated hydroxyproline-rich proteins that are present in cell walls, plasma membranes and extracellular secretions and play a key role in several plant developmental processes.
In this study, we have investigated, in *Quercus suber*, changes in pectin esterification and AGPs during induction and progression of somatic embryogenesis, which would suggest the cell wall remodeling during the process. Expression analysis of PMEI and AGP genes by qPCR, immuno-dot-blot assays, immunofluorescence and confocal analysis were performed at specific developmental stages of SE, by using a battery of monoclonal antibodies to specific epitopes of AGPs and high- and low-methylesterified pectins (LM6, LM2, LM19, LM20, JIM7, JIM5).
The results showed high levels of esterified pectins and endogenous AGPs in early embryo cells and embryogenic cell masses. Pectin methylesterase inhibitor genes *Qs*PMEI and *Qs*PMEI2 showed expression in proliferating embryogenic masses, while they were down-regulated with somatic embryogenesis progression, correlating with high pectin esterification in embryogenic masses and increasing pectin de-esterification in differentiating embryo cells. Arabinogalactan proteins were detected in cell walls of developing somatic embryos, and the AGP genes *Qs*AGP and *Qs*AGP-Lys-rich were up-regulated during somatic embryogenesis induction and progression.
The results indicated the cell wall remodelling, associated with cell proliferation and differentiation, during somatic embryogenesis (SE) of *Quercus suber*, a woody species, in which information on cellular processes underlying SE in still scarce.
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Study of the vacuolar and secreted cytokinin dehydrogenases of Arabidopsis thaliana, their influence on the cytokinin distribution in vacuoles and on the root system architecture

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Plant hormones cytokinins regulate numerous processes related to the cell division and development. Intracellular cytokinin homeostasis is maintained primarily by metabolic inactivation of the hormone, which is catalyzed by cytokinin oxidase/dehydrogenase (CKX). Arabidopsis CKX gene family is comprised of seven members, which differ in the subcellular localization of their protein products. CKX2, CKX4, CKX5, and CKX6 are processed by the plant secretory pathway, while CKX1 and CKX3 proteins are targeted to the vacuole and only CKX7 isoform is localized to the cytosol. Enhanced expression of *CKX* genes causes increased root growth phenotype. Transgenic *Arabidopsis* plants overexpressing *CKX1*, *CKX2* and *CKX3* and T-DNA knock-out lines *ckx2* and *ckx3* were used in this work as a tool for mapping the associated root architecture and characterizations of the total intracellular vs vacuolar pool of all cytokinin forms. Specifically, primary root length, number of lateral roots, and gravitropic set-point angles were measured in transgenic and control plants. The results showed that *CKX2* overexpressing plants produced the greatest number of lateral roots as well as the largest primary roots. In contrast, the cytokinin-deficient plants were characterized by a shift to a near vertical gravitropic set-point angle (GSA) of their lateral roots as compared to WT. The total quantities of the intracellular and vacuolar content of cytokinins from *ckx2* and *ckx3* loss of function mutants, *CKX* overexpressing line, and Col-0 control were determined by UHPLC-MS/MS analysis. The results confirmed prevalence of the cytokinin storage forms (both *O*-glucosides and *N*-glucosides) in vacuoles. Interestingly, significant changes in the vacuolar pool of several cytokinin forms were observed in CKX3 but not in CKX1 lines. Our results confirm CKX3 as the main vacuolar isoform and contribute to better understanding of the mechanism of the cytokinin transport and storage in vacuoles.
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Synthesis and perception of fluorescently labeled N6-isopentenyladenine derivatives

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Cytokinins play many important roles in the regulation of plant growth and development. To study cytokinin receptor properties in plants, we designed and prepared fluorescent derivatives of 6-[(3-methylbut-2-en-1-yl)amino]purine (N^6-isopentenyladenine, iP) with several fluorescent labels attached to the C2 or N9 atom of the purine moiety *via* a 2- or 6-carbon linker. The fluorescent labels included dansyl, fluorescein, 7-nitrobenzofurazan, rhodamine B, coumarin, 7-(diethylamino)coumarin and cyanine 5 dye . All prepared compounds were screened for affinity for the *Arabidopsis thaliana* cytokinin receptor (CRE1/AHK4). Although the attachment of the fluorescent labels to iP *via* the linkers mostly disrupted binding to the receptor, several fluorescent derivatives interacted well. For this reason, three derivatives, two rhodamine B and one 4-chloro-7-nitrobenzofurazan labeled iP were tested for their interaction with CRE1/AHK4 and *Zea mays* cytokinin receptors in detail. We further showed that several derivatives were able to activate transcription of cytokinin response regulator *ARR5* in Arabidopsis seedlings. The activity of fluorescently labeled cytokinins was compared with corresponding 6-dimethylaminopurine fluorescently labeled negative controls. Selected rhodamine B C2-labeled isopentenyladenines with short and long linkers and 4-chloro-7-nitrobenzofurazan N9-labeled compound and their respective negative controls were used for *in planta* staining experiments in *Arabidopsis thaliana* cell suspension culture using live cell confocal microscopy. Staining of Arabidopsis cells with fluorescent cytokinin was fast, showed clear intracellular signal distribution represented by typical mesh-like structures at the cell cortex suggestive of ER after approximately 10 min of a continuous treatment with 5 µM fluorescent probe while negative fluorescent control used at the same concentration showed only weak cytoplasmic fluorescent signal.

Exogenous abscisc acid impacts on the development of isolated endosperm in bread wheat

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The storage tissue called an endosperm plays crucial role for embryo development. In some plants, like cereals the endosperm is a part of mature seeds which are valuable source of key substances for humankind and animals. There is common known that tissue culture conditions can alter a developmental pathway of plant cells, and the abscisic acid (ABA) is a key plant growth regulator for a proper seed development. Our previous studies revealed that isolated immature endosperm of bread wheat explants could actively grow in absence of ABA. The analysis of internucleosomal fragmentation of DNA suggested that the induction of PCD as well as DNA degradation typical for necrosis in explants were detected. The positive impact after addition of the exogenous ABA on size and ultrastructural features of outer cells dedicated aleurone and sub-alurone layers were found. The content of starch in cultured endosperm did not significantly differ in comparison to caryopsis at the same age, in contrast to soluble carbohydrates. Average diameter of A-type starch granules was larger in caryopsis, while size of B-type granules were similar. Percentage proportions of isolated A and B-type granules measured at 30 days age were inverted than in caryopsis. Storage proteins analyses showed the amount of gliadins and glutenins in explants was lower than in caryopsis at the same age. Interestingly, the amount of albumins and globulins were similar or even higher for some fractions of low molecular weight after supplementation with ABA. RNA-Seq method revealed that transcriptions level of starch and storage proteins related genes in the cultured endosperm heavily depends on addition of plant growth regulators, especially the ABA.
The present works are financially supported by National Science Centre, Poland (grant no. 2012/07/B/NZ9/01325), Hungarian Scientific Research Fund (grant no. K100881) and, in part, from the Institute of Botany at the Jagiellonian University.

The role of hydrogen peroxide and its detoxification in acclimation to supplemental UV-B

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Experiments in growth chamber have shown that an efficient hydrogen peroxide (H_2O_2) detoxification is a key factor in the acclimation to supplemental moderate UV-B doses. UV-inducible oxidative stress is mainly avoided due to the increased activity of peroxidase enzymes (Czégény et al. 2016), although non-enzymatic antioxidants, such as flavonoids, are also expected to contribute (Csepregi & Hideg 2017). The aim of our work was to study whether marked increases in class-III peroxidase (POD) isoforms upon UV-B treatment (Rácz et al. 2018) are also brought about by H_2O_2 directly and whether a pre-treatment of model plants with H_2O_2 affected UV-B responses. Four weeks old tobacco plants were grown under 200 mmol m^{-2} s^{-1} PAR in growth chambers and treated with H_2O_2 for three days before exposure to daily supplementary UV-B treatment (6.9 kJ m^{-2} d^{-1} b.e.) for four days. Leaf H_2O_2 contents were determined according to Mátai & Hideg (2017); POD activities were measured using ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and isoforms were visualized on native gels (Rácz et al. 2018). Non-invasive pigment and photosynthesis measurements were carried out with a Dualex Scientific™ optical sensor and the MAXI-version of the Imaging PAM, respectively. Plants acclimated to UV-B or to the applied exogenous H_2O_2 without significant loss in leaf photochemistry and avoided oxidative stress via increasing peroxidase activities. Leaf flavonoid concentrations were increased in UV-B-exposed leaves but not upon the H_2O_2 treatment. Pre-application of H_2O_2 modified responses to successive a UV-B treatment, especially the induction of peroxidases.

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splitTALE - a TALE based two-component system in planta

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Transcription activator-like effectors (TALEs) are bacterial proteins that act as transcriptional activators *in planta*. Due to their modular DNA-binding domain TALEs can be designed to target any desired DNA sequence and therefore can be used to activate any target gene. In order to broaden the applicability of TALEs we generated a splitTALE two-component system that is composed of the TALE-DNA-binding (DB) domain and the TALE activation domain (AD). In an initial AND gate system the TALE BD and AD are fused to interacting protein domains allowing the reconstitution of the TALE and induction of target genes. By using ligand-dependent interacting domains the sTALE system can be used for dosage-dependent transcriptional induction. Here, an optimized sTALE system and its potential applications will be presented.

Shoot glucosinolate distribution determines Spodoptera littoralis feeding pattern

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Glucosinolates are specialized metabolites of *Brassicales* involved in defense against chewing herbivores. Myrosinase enzymes hydrolyze glucosinolates upon tissue damage yielding a range of degradation products that negatively affect herbivore fitness and performance. Larvae of the generalist herbivore *Spodoptera littoralis* have been shown to perform better on glucosinolate-deficient mutants of *Arabidopsis thaliana* compared to wild-type plants. Larvae further prefer feeding on mature over juvenile leaves, indicating a non-uniform distribution of nutrients and/or defenses in the wild-type.
Here we use glucosinolate transporter and synthesis mutants to study whether changes in glucosinolate distribution and content have an influence on the insect feeding behavior. The double mutant of *NITRATE PEPTIDE TRANSPORTER FAMILY 2.10 (AtNPF2.10/GTR1)* and *2.11* (*AtNPF2.11/GTR2)* is known to alter glucosinolate distribution within leaf tissue. Analysis of the glucosinolate content by LC-MS/MS showed that concentrations are highest in juvenile leaves and gradually decrease with leaf age in a GTR-dependent manner. The pattern of leaf damage was inversed in the transporter mutant, correlating with levels of Met-derived glucosinolates. Interestingly, this feeding pattern is consistent with that observed on glucosinolate-deficient mutants. The mass of larvae fed on glucosinolate-deficient plants was significantly increased compared to wild type and transporter mutant, indicating that glucosinolate content, but not distribution, has a significant effect on larvae performance.
In conclusion, the preformed GTR-mediated glucosinolate distribution determines the feeding pattern of *Spodoptera.* If it is disturbed, the *Spodoptera* prefers young tissue with fatal consequences for the plant, but without increasing herbivore performance

Identifying the biosynthetic genes of specialized metabolic pathways and characterizing their function in response to aphid attack

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Plants in nature are continuously challenged by insect herbivores. To deter their attackers, minimize pest damage, and preserve their fitness, plants produce a range of specialized defense metabolites. One such class of defense metabolites is benzoxazinoids, that has been detected in economically and nutritionally important crops for humans and livestock. Despite the tremendous importance of biosynthesis of specialized defense metabolites such as benzoxazinoids, the majority of the enzymes involved in this pathway and their regulation remain uncharacterized in certain major crops.
The overall goal of our research is to reveal the yet unknown genes of specialized metabolite pathways in wheat as a response to herbivore feeding. We focus on two traits: metabolic diversity and insect resistance and characterize them in two selected wheat species: the wild tetraploid emmer wheat Zavitan, and the durum cultivar, Svevo. Our preliminary results showed major insect resistance differences between the two wheats. While the Zavitan was susceptible, the cultivated wheat Svevo was resistant to *Rhopalosiphum padi* aphids. In addition, the metabolic analysis showed a massive metabolic modification in response to aphid attack of both central and defense metabolites.
Our next step is to screen the Zavitan x Svevo recombinant inbred line (RIL) population to expose new alleles and genes that involve in plant defense mechanisms. This discovery will add to the fundamental understanding of plant fitness to biotic stresses and the responses to insect damage in one of the most important food crops in the world.

Variability in phenophases of Norway maple in urban and natural habitats

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Norway maple is an adaptable species, resilient to environmental changes. Determining the variation of Norway maple's offset, duration and ending of phenophases, in relation to different environmental conditions and through 5-year period enables strategic use of the species and prediction of its future prospects.
During the research, vegetative period of 400 Norway maple individuals lasted on average 224 days in natural habitats and 240 days in urban population. Phenophases started the earliest, lasted the longest and ended the latest in urban population. Flowering lasted on average 2 days longer, leafing was 12 days longer and fruiting was 10 days longer in urban population. Relation of beginning, duration and end of phenophases to climatic factors, especially air temperature, was determined. The phenophases in 2014. stand out from others years, while 2013. and 2012. are the most similar. The earliest offset of phenophases was registered in 2015. - the warmest year ever according to the world meteorological organization. Phenophases succeeded in the same order each year, despite the climatic and other environmental changes throughout the years, with the earliest offset in Belgrade and the latest in natural habitat Rudnik 1. Significant differences in beginning, duration and the end of phenophases (flowering, leafing and fruiting) were noted among all 4 populations. The duration and changes in phenophases during this period did not influence phenotypic and morphological characteristics of Norway maple.
According to the results of this study and in accordance to previous researches, maple is the species with the large variations, easily adjustable to urban environments and evolutionary favored for survival in conditions of climate change. The sanitary and aesthetic functions of Norway maple remain unchanged through different environmental conditions. This species has a good perspective for use in urban areas and large variability will enable good reproducibility and survival in changed environments.

Induced coiled branch (cbr) mutant in Arabidopsis by overexpression of a novel RING-type E3 ubiquitin ligase gene

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To investigate the molecular mechanism of development contributing to coiled morphology, screening was carried out from Arabidopsis activation tagging lines obtained by activation T-DNA treatment that have curly/wavy morphology. The mutant named *cbr*, was found to have a wavy and curly morphology with *c*oiling *br*anches. Plasmid rescue and genomic southern blot analysis revealed the site of T-DNA insertion in the genome. RT-PCR was performed to monitor expression levels of the genes adjacent to the T-DNA integration site and showed the activation of an E3 ubiquitin ligase gene. Database search revealed that the protein with the C3HC4 type RING domain belongs to a family of E3 ubiquitin ligases. Complementation test by overexpression and RNA interference of the gene showed that activation of the novel gene caused the *cbr* mutant phenotypes. Ubiquitylation has been linked virtually to every cellular process including plant development. E3 ubiquitin ligase has been reported to recognize target proteins that are to be ubiquinated for further degradation by the proteasome complex. Therefore, we are performing 2-DE and Y2H experiments to find specific substrate(s) of the novel E3 ubiquitin ligase gene.

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Sugar toxicity in Arabidopsis - what Arabinokinase tells us

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Ara1-1 is a mutant in a sugar kinase, strongly impaired in it's activity. The mutant accumulates arabinose indicating that plant cell wall polymers and glycoproteins are constantly turned over. The released sugars are normally subsequently recycled to nucleotide sugars. Ara1-1 mutants are largely unable to bring arabinose into the recycling pathway. Instead, ara1-1 dies within 3 days when fed with low mM concentrations of arabinose.
We will explain the biochemical problem of the mutant enzyme and provide data that the gene regulation in ara1-1 follows a starvation pattern though the plants possess more than enough sugars.
The ara1-1 mutant and similar mutants in other sugar kinases clearly demonstrates that sugar recycling is the rule in plants and not the exception.

Transcriptomic and metabolomics studies of Arabidopsis response to glucosinolate hydrolysis product

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Plants are sessile organisms and are exposed to a vast range of biotic stress. Like how plants respond to pathogen/microbe-associated molecular patterns (PAMPs/MAMPs), they can also recognize endogenous damage-associated molecular patterns (DAMPs) through pattern recognition receptors (PRRs), intracellular calcium and oxidative burst, mitogen-activated protein kinase (MPK) cascade activation, transcription factors activation, and defense-related genes regulation. Glucosinolate hydrolysis products (GHPs), which are volatiles released upon disruption of plant tissue, may act as DAMPs and trigger plant innate immune system. Our project aims to decipher how plants respond to GHP and activate signaling pathways. We used the Arabidopsis T-DNA insertion mutants in different hormone-related signal pathways to study their responses to GHP treatment. We also measured the metabolic profile to indicate the plasticity of how Arabidopsis respond to GHP. To identify putative regulators involved in the GHP-response signaling pathway, we performed microarray to study the gene expression profile with and without GHP treatment. As a result, GHP causes yellow chlorosis leaves in Arabidopsis wildtype plants, but not in Mut2. Interestingly, monosaccharides in Mut2 are more abundant than those in wildtype. Our results show that GHP treatment induces several biological processes in Arabidopsis leaves.

Understanding the molecular pathways underlying periderm formation

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Periderm is an outward layer that replaces the epidermis in older stems and roots during plant development, providing protection against biotic and abiotic stresses. Periderm formation results from the activity of the lateral meristem phellogen, which has the ability to differentiate into phelloderm (inwards) and phellem/cork (outwards), resembling the functioning of the vascular cambium. Unlike most species, where the phellogen has a limited lifespan, in *Quercus suber* the phellogen is active throughout the entire life of the tree, producing a continuous and thick layer of cork. Most studies on the *Q. suber* periderm have focused on the cellular structure and chemical composition of cork, while scarce information is available regarding phellogen activity and cork differentiation. To identify specific aspects of the cork formation process, as compared to wood, we have performed a transcriptomic study of *Q. suber* secondary tissues, using HiSeq Illumina technology. Phellogen/phellem and xylem tissues of *Q. suber* were harvested during the growing season, and RNASeq libraries were prepared for sequencing. The analysis of the transcriptomic data identified a total of 5968 differentially expressed genes (DEGs). Following a functional enrichment analysis of the identified genes, several biological process, molecular function and cellular component categories have been highlighted as putatively relevant for cork formation. These include fatty acid biosynthetic process, response to oxidative stress, response to auxin and membrane transporters (up-regulated in phellogen/phellem samples). Transcripts of specific genes and gene families have been identified for the first time in periderm tissues, including members of the SAUR family and transcriptional regulators previously described in other meristems, namely the vascular cambium. The over-representation of the SAUR family and transcription factors/regulators ARF and AUX/IAA in cork tissues, emphasizes the central role of auxins in periderm formation.

Class XI myosins contribute to polar auxin transport and senescence-induced programmed cell death in Arabidopsis

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The integrity and dynamics of actomyosin cytoskeleton is crucial not only for plant cell architecture, but also for modulating polar auxin transport (PAT), programmed cell death (PCD), and other processes regulating plant development. Depletion of actin binding proteins, including myosin motors, affects actin cytoskeleton and causes defective plant growth and morphology, altered lifespan and reduced fertility. Overlapping phenotypic features of mutant plants where myosin cytoskeleton or auxin signalling pathways are affected imply possible link between actomyosin function and auxin signalling.
To investigate the contributions of the actomyosin cytoskeleton to the PAT and PCD in Arabidopsis, we used triple gene knockout mutant xi-1 xi-2 xi-k (3KO), in which three highly expressed class XI myosins were eliminated. We show that activity of the auxin dependent DR5 promoter is decreased during early vegetative and generative development of the 3KO plants. In addition, stable expression of the auxin efflux carrier PIN1 tagged with green fluorescent protein (PIN1-GFP) enhances the semi-sterile phenotype described earlier in 3KO plants. Analysis of a genetically rescued line xi-1 xi-2 xi-k XI-K:YFP (3KOR) revealed partial overlap in the expression patterns of XI-K and PIN1 throughout floral development.
We further found that the early yellowing and premature leaf senescence of 3KO vegetative shoots is accompanied by up-regulation of the early senescence-associated gene SAG13 and massive loss of chlorophyll. Impaired auxin distribution and premature leaf senescence also correlated with accumulation of anthocyanins in the tissues of 3KO plants. The abnormalities in flower development, premature senescence and anthocyanin accumulation observed in 3KO plants were genetically rescued by the stable expression of myosin XI-K:YFP, indicating a myosin-dependent nature of these defects.
Our results provide genetic evidence supporting functional contributions of class XI myosins to PAT and senescence-induced PCD in Arabidopsis.

Validation and selection of internal reference genes for real-time quantitative RT-PCR in Amaranthus hypocondriacus

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There are several processes, including plant development, differentiation or responses to different types of stress involving different genes, for which an appropriate normalization is critical in the interpretation of gene expression. Currently, reverse transcription quantitative real-time PCR (RT-qPCR) is a high-throughput, sensitive and widely used method of gene expression analysis. RT-qPCR requires a precise normalization of data to avoid the misinterpretation of experimental data. For various plants, including amaranth, there are no validated reference genes for the normalization of RT-qPCR data. The aim of this study was to determine the most stable reference genes in amaranth for the normalization of gene expression analysis using RT-qPCR. A set of seven housekeeping genes were analyzed. The transcript stability and gene expression level of candidate reference genes were analyzed in different tissues, at different developmental stages, and under different types of stress in amaranth. The results were compared using the GeNorm, NormFinder, and Bestkeeper statistical methods. The reference genes optimum for the normalization of data varied respect the treatment. The results indicate that use *ahyMDH, ahyGAPDH, ahyEF-1α and ahyACT* would be optimum for the normalization of data for the accurate normalization of experimental data, when all the treatment are analyzed in the same experiment. Finally, this study provides useful information about various reference genes for use by RT-qPCR studies of amaranth, which will contribute significantly to future gene studies of amaranth.

Water and nitrogen effects on ABA signaling, leaf gas exchange and water use efficiency of tomato plants under nitrogen fertigation

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Fertigation has been widely used in a large range of crop species in many regions of the world, and it has been demonstrated to be able to improve water and nutrient use efficiencies.The physiological mechanisms underlying the improved water use effiency (WUE), however, remain largely elusive. Therefore, the aim of the present study was to investigate the water and nitrogen (N) effects on ABA signaling, leaf gas exchange and water use efficneicy of tomato plants under N fertigation. The results showed that when analyzed across the N treatments, moderatley and severely-water stressed water treatments significantly decreased leaf and root water potential (LWP and RWP). The reduced irrigation water quantity significantly increased root and leaf ABA concentration ($[ABA]_{leaf}$ and $[ABA]_{root}$), resulting in the decreased stomatal conductance and thereby signifiantly greater intrinsic water use efficiency (WUE_i). Nonetheless, across the water treatment, N treatment showed no significant impact on physiologcial attributes. $\delta^{13}C$ gives a time-integrated photosynthesis-weighted integration of CO_2 supply and demand, and variation in $\delta^{13}C$ could be due to changing stomatal conductance or photosynthetic capacity, or both. Since $\delta^{18}O$ is affected by variation in stomatal conductance but is not affected by changes in photosynthetic capacity, $\delta^{18}O$ can be used to identify if differences in $\delta^{13}C$ are primarily a result of changes in photosynthetic capacity or stomatal conductance. Our results indicated that reduced irrigation quantity resulted in significantly greater $\delta^{13}C$ and $\delta^{18}O$ compared with those under high soil water treatment. The significant positive linear correlation between $\delta^{13}C$ and $\delta^{18}O$ indicated that stomatal control was the main determinant for improved water use efficiency under N fertigation.

Root proliferation in response to neighbors in wheat (Triticum aestivum)

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A pot experiment with vs. without root dividers between pairs of soybean (*Glycine max*) plants showed root proliferation and reduced aboveground growth in response to the presence of roots of a neighboring plant, but this result has been criticized as a possible artifact resulting from differences in soil volume available to roots in the two treatment. We grew pairs of spring wheat (*Triticum aestivum*) plants with two types of root dividers: (a) film, which completely divides the soil into two volumes, and (b) fine mesh, through which roots cannot grow but chemical signals can move. Thus, the soil volume available for roots was the same in both treatments. The experiment was performed on an old land race and a modern cultivar of wheat to compare their responses. Plants grown with mesh dividers had significantly more root biomass and significant less shoot biomass than pairs with film dividers. Spring wheat plants ”overproduce” roots in response to the roots of neighboring plants, resulting in a so-called ”tragedy of the commons”. The size of these effects was smaller in the modern cultivar than in the old land race, suggesting that root proliferation in response to neighboring roots has decreased over the course of plant breeding, due to inadvertent ”group selection” by plant breeders.

How do defence compounds accumulate in phloem-cap cells for vascular protection?

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Plant vascular tissues, the conduits of water, nutrients, and small molecules, are pivotal for plant develop and growth. Extensive studies have led to the underlying mechanisms of cell specification and patterning of plant vascular tissues, whereas the molecular mechanism of vascular protection remains largely unknown. In *Arabidopsis*, defence compounds glucosinolates (GLS) are accumulated in sulphur-rich cells (S-cells) that contain above 100mM GLS in the vacuole. S-cells and the adjacent myosin idioblast are ideally localized in the phloem-cap of inflorescence stem for defence of vasculature from pest attack. Obvious questions raised e.g.: How do GLS accumulate to such high levels in the S- cells? Is the accumulation of defence compounds in phloem-cap cells a Brassica specific phenomenon?
One typical character of S-cells is their enormous higher turgor pressure, which enable the collection of single-cell sap with micropipetting based methods using a capillary. Similarly, single-cell sampling of developing flower stalk in *Lotus japonica* and *Nicotiana Benthamiana* discovered highly enrichment of defence compounds e.g. cyanogenic glucosides and nicotine respectively. Using GLS accumulation in S-cell as a model system, we implemented single-cell metabolomics and proteomics to investigate mechanism of accumulation of defense compounds in phloem-cap region. We found inter- and intra-cellular transport but not de-novo synthesis is essential in translocation of GLS from biosynthetic cells to S-cells. Loss of function of plasma membrane GLS importers GTR1, GTR2 and GTR3 cause approximately 80% reduction of aliphatic GLS concentration in the developing young S-cells, suggests the essential role of transcellular transport route. Grafting experiment between biosynthetic mutants with transport mutants showed that the transcellular transport route of different type of GLS were largely dependent on their synthetic sites. Charactering the vacuolar transporter candidates from single cell proteomics will underline the molecular mechanism of cell type specific accumulation of defence compounds for vascular protection.

Gibberellin biosynthesis pathway - transcriptomic comparison between dwarf and high triticale plants derived from [(Jana x Tempo) x Jana] x Aegilops juvenalis cross combination and Dw1-containing triticale cultivars

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Gibberellin biosynthesis regulation is of fundamental importance to plant growth and development. Therefore, expression of genes encoding for the GA-biosynthetic enzymes have been the focus of many studies. Here we aim to analyze expression of genes involved in early steps of GA-biosynthetic pathway - that is *ent*-copalyl diphosphate synthase gene (CPS), *ent*-kaurene synthase gene (KS) and *ent*-kaurene oxidase gene (KO) - along with major genes involved in bioactive gibberellins formation (GA-20 oxidase and GA-3 oxidase genes) and their inactivation (GA 2-oxidase gene). The goal of present research was to investigate the GA biosynthesis regulation mechanisms that occur at transcriptomic level in dwarf and high triticale plants derived from [(Jana × Tempo) × Jana] × *Aegilops juvenalis* cross combination as well as in *Dw1*-containing triticale cultivars (Fidelio, Magnat, Woltario, Zorro). Plant material, which consisted of 7-day old leaves of chosen forms, was used for RNA extraction that was carried out using TRIzol-based procedure. RNA, after electrophoretic and spectrophotometric assessment, was reverse transcribed with iScript™ cDNA Synthesis Kit (BIO-RAD). Obtained cDNA was used as template in the qPCR analysis. The qPCR reactions were performed with TaqMan Gene Expression Master Mix (Applied Biosystems) on QuantStudio 3 System apparatus. Performed analyses answer the question whether or not genes encoding for the GA-biosynthetic enzymes are expressed in a similar manner in dwarf triticale plants derived from [(Jana × Tempo) × Jana] × *Aegilops juvenalis* cross combination and selected triticale cultivars containing *Dw1* gene.

Acknowledgement
The results of the study were obtained within the framework of the project funded by Polish Ministry of Agriculture and Rural Development entitled ‘Development of new triticale genetic sources by wide crosses’.

INVITED SPEAKERS ABSTRACTS

Theme 1: Phenotyping on Different Scales

Integrating multi-scale phenotypic information into prediction models for genotype by environment interaction by a synthesis of statistical-genetic and physiological models

Fred van Eeuwijk, Daniela Bustos-Korts, Emilie Millet, Marcos Malosetti, Willem Kruijer & Martin Boer
Biometris, Wageningen University and Research, The Netherlands

New phenotyping techniques at multiple scales of biological organisation will allow biologists to monitor plant growth and development at high temporal and spatial resolutions. Now that technical problems on how to record such types of phenotypic information increasingly are solved, a greater challenge presents itself in how to make use of this information. A major question in plant biology and genetics concerns understanding and predicting genotype by environment interactions (GxE), which includes questions on adaptation, stability and resilience. In plant breeding and genetics, for the prediction of target traits like yield (say fitness) across environmental ranges in the face of GxE two main approaches are followed. The most common approach uses statistical genetic models in which a target phenotypic trait (yield) is predicted from a linear function that has as inputs DNA polymorphisms and low resolution environmental characterizations (quantitative and qualitative). As an alternative, crop growth models can be used, where the inputs typically contain multiple physiological parameters and component traits together with detailed and high resolution environmental information. New types of phenotypic information require a reconsideration of traditional quantitative methodology to predict and understand GxE in target phenotypes. A synthesis of statistical genetic and crop growth modelling approaches can be shown to produce improved accuracy for the prediction of GxE. We will describe the outlines of a modelling framework and give illustrations in maize and wheat.

PBE 2018

Chrisitan Sig Jensen:

Advanced phenotyping in a breeding setup

Entering the post-genomics area, many breeders have a acquired a number of molecular tools, which allows them to predict genotype performance, identify major QTLs for certain traits, and introduce breeding short-cuts. However, the development of these tool has also revealed a clear demand for higher trialing precision. At the same time there is a strong request from all markets to improve plant robustness to meet future climate challenges; of course without compromising yield or quality. The academic- and industrial response to this situation has been to invest heavily in highly controlled phenotyping facilities, either based on indoor systems, where single plants are monitored constantly from germination to maturity, or outdoor systems, where crop phenotypes can be monitored at plot level. Each of these systems have their strength and weaknesses. While indoor systems are made to magnify genetic differences and in some cases also the genetic interaction with certain environmental cues, they seldom account for plant-to-plant competition. Outdoor systems do take this factor into account, but if not scaled properly may fall short of statistical power to detect genetic differences and interactions with the environment. Crop breeding companies hold a century-long record of efficient field trialing with phenotyping based on both destructive measurements and visual assessment. This talk will discuss some of the new phenotyping methods as seen from a breeder's perspective. Emphasis will be laid on practical results in grasses, but several of the benefits (and short-comings) is applicable to any crop.

Revealing photosynthetic regulatory paradigms using natural variations and massive field measurements

David M. Kramer
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Photosynthetic organisms must tightly regulate their (often competing) needs for efficient collection of solar energy with the avoidance of toxic side products that can be produced by the photosynthetic machinery when energy input and use is unbalanced. To test these modes of regulation under real world conditions, we developed a series of phenotyping platforms that aim to bring the lab to the field (PhotosynQ.org) and the field to the lab (Dynamic Environmental Phenotype Imagers). For example, it was recently proposed that photosynthetic efficiency is limited by the slow rate of onset and decay of photoprotective nonphotochemical quenching (NPQ). Combining results from over 1M PhotosynQ experiments and over 5M DEPI data sets led us to a similar conclusion but for very different reasons. We found that photosynthesis is often strongly limited by the effects of the thylakoid electric field ($\Delta\psi$) that is generated during the initial events of photosynthesis, particularly under rapidly fluctuating light conditions, and results in a process that we call Field Recombination Induced Photodamage (FRIP), where large "spikes" in $\Delta\psi$ induce photosystem II recombination reactions that produce damaging singlet oxygen ($^1O_2$). We also show that FRIP is directly linked to the thylakoid proton motive force (*pmf*), and in particular the slow kinetics of partitioning *pmf* into its ΔpH and $\Delta\psi$ components that in turn are controlled by transmembrane ion movements. We then explored the possibilities and pitfalls of efforts to improve plant productivity by modifying this process. Finally, I will describe how community-based platforms, such as PhotosynQ.org can enable scientific data gathering and analyses to assess the limitations and modes of regulation of photosynthesis in different species and environments.

Theme 2: Photosynthetic diversity

Strategies for improving C_4 photosynthesis

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Recent activities to improve photosynthetic performance in crop plants have focused primarily on C_3 photosynthesis where there are clear identified targets such as improving Rubisco kinetics, installation of a CO_2 concentrating mechanism and alleviating limitations in chloroplast electron transport. However, C_4 plants that utilise the C_4 photosynthetic pathway also play a key role in world agriculture. For example, C_4 crops such as maize and sorghum are major contributors to both first and third world food production and the C_4 grasses sugarcane; miscanthus and switch grass are major plant sources of bioenergy. Strategies to manipulate and enhance C_4 photosynthesis thus have potential for major agricultural impacts.

The C_4 photosynthetic pathway is a biochemical CO_2 concentrating mechanism that requires the coordinated functioning of mesophyll and bundle sheath cells of leaves and species have evolved a complex blend of anatomy and biochemistry to achieve this. The limitations to photosynthetic flux are not as well studied in C_4 plants, but our work with transgenic *Flaveria bidentis*, a transformable model C_4 dicot, has provided gene candidates for improvement of carbon metabolism. Chloroplast electron transport in C_4 plants is shared between the two cell types, providing opportunities not only to alleviate limitations to flux through intersystem electron transport by targeting nuclear encoded proteins in the cytochrome (Cyt) b_6/f complex, but in better sharing the harvesting of light energy between mesophyll and bundle sheath chloroplasts.

We are using the model monocot C_4 species *Setaria viridis* (green foxtail millet) to generate transgenic plants with altered C_4 photosynthetic metabolism to address these questions and will report on our recent findings.

Photosynthetic diversity: are we concentrating too much on carbon?

Great progress is being made in understanding C4, CAM or the biophysical Carbon Concentrating Mechanism in algae and cyanobacteria. There have been recent tremendous new insights identifying key molecular components, their cell-specific targetting, and potential operation in alternative higher plant hosts. Using these advances as a starting point, we will then go on to consider the extent that other environmental drivers, such as nitrogen availability and recovery, as well as water supply and hydraulic conductance, could also have provided coherent selective pressures for the evolution of CCMs in the past, and resilience that will be required under future climatic conditions. For instance, to what extent is CAM and adaptation to rapid recharge and rehydration, rather than gradual metering of water use? To what extent did the availability of nitrogen, energetics of light use and hydraulic conductance drive C4 diversification? What might be the unintended consequences of introducing a CCM into higher plants for nitrogen use, resource partitioning and grain quality? Finally, from an overall food security perspective, we should perhaps consider the extent that food and clothing imports steal water, rather than carbon, from those parts of the world least able to waste such a limited resource.

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Redirecting photosynthetic reducing power

Poul Erik Jensen

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Photosynthesis drives the production of ATP and NADPH mainly used to fix CO_2. Surplus of redox power can be exploited for biotechnology such as for production of high-value compounds. Important natural products are often synthesized in low quantities by their host organism and can be difficult and expensive to produce by chemical synthesis because of their complex structures. The cytochromes P450 (P450s) situated in the endoplasmic reticulum play key roles in natural product biosynthesis, and are powered by electron transfers from NADPH. We have shown that plant P450s can be expressed in chloroplasts and cyanobacteria can be directed to the thylakoid membrane and that photosynthetic electron transport will support P450 catalytic activity independent of NADPH and dedicated reductases. In order to route reducing power more efficiently to P450s, we have fused them with ferredoxin (Fd) or flavodoxin-like FMN domains. These fusions allow the P450s to obtain electrons for catalysis directly from the photosynthetic electron transport chain by interacting with photosystem I and make them competitive with all the natural occurring ferredoxin requiring enzymes in the chloroplast. Further dedicated redirection of reducing power can be obtained by scaffolding all the enzymes of a pathway on the thylakoid membrane. In a novel strategy, we have fused enzymes with transmembrane domains of TatB and TatC from the chloroplast twin arginine translocation system. This reduced the accumulation of unwanted intermediates and side products and increased the accumulation of the end product fivefold. This work shows that photosynthetic organisms have many attractive features for metabolic engineering, and suggests much unexplored potential for engineering of photosynthetic electron transfer chains to accommodate heterologous enzymes.

Theme 3: Genome Based Breeding – New breeding technologies

Genome editing with programmable nucleases in crop plants

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Genome editing can accelerate plant breeding by allowing the introduction of precise and predictable modifications directly in an elite background. The most promising utilization of both the CRISPR/Cas9 system and TALENs can be used to generate targeted genome modifications including mutations, insertions, replacements and chromosome rearrangements. We developed simple and efficient genome editing approaches in which wheat plants are regenerated from callus cells transiently expressed with CRISPR/Cas9 reagents introduced as DNA, RNA or RNP. We also established a plant base editing protocol suitable for introducing targeted point mutations to wheat, rice and maize genomes. This approach will not only technologically advance plant genome engineering, but may also provide better solution for social acceptance of genome-edited crops as they do not require a donor DNA template or chromosomal cleavage. These approaches may be widely applicable for producing genome edited crop plants and has a good prospect of being commercialized.

Genome Editing in Cereal and Brassica Crops – Progress and Prospects

Wendy Harwood

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Genome editing using RNA-guided Cas9 (CRISPR / Cas9) is changing the way we approach functional genomics and offers valuable opportunities for the development of improved crops. The production of targeted gene knock-outs using CRISPR / Cas9 is now routine in a range of crops. Examples will be described for wheat, barley and *Brassica oleracea* that demonstrate the efficiency of the technology and show how it has contributed to diverse research projects. The occurrence of 'off-target' mutations in addition to 'on-target' mutations will also be considered. Barley has proved to be an excellent model crop in which to advance genome editing technologies; being a diploid cereal with a very efficient transformation protocol. Recent work to extend the use of CRISPR / Cas9 to create 'knock-ins' or targeted insertions will also be described and prospects for the future use of this technology in crops will be considered.

Theme 4: Seeds for the future

Ionome to Genome: Tales of Gene Discovery

Mary Lou Guerinot

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Understanding how seeds, often the edible portion of the plant, obtain and store nutrients is key to developing crops with higher agronomic and nutritional value. Most of our work has been focused on the essential micronutrients iron, manganese and zinc. Combining genetics, high throughput elemental analysis via ICP-MS and high resolution imaging via synchrotron X-ray fluorescence, we have identified and characterized a number of Arabidopsis mutants that have increased tolerance to iron deficient growth conditions and have increased iron accumulation relative to wild type plants. One of these, Ig14, has a similar metal content to wild type when grown on normal soil, but thrives on alkaline soil, accumulating significantly more iron in its shoot and seeds. A triple mutant of three closely related negative regulators of the iron deficiency response (*bts-1 btsl1 btsl2*) has increased tolerance to iron deficient growth conditions and increased iron accumulation without resulting toxicity. We have also uncovered unique patterns of iron and manganese localization in seeds and have now shown that the vacuolar transporters VIT1 and MTP8 are responsible for setting up these patterns, allowing us to determine whether the patterns are biologically significant and, ultimately, whether they can be altered in support of biofortification of staple crops.

We are also taking similar approaches to determine how arsenic, a non-threshold, Class 1 human carcinogen, accumulates in plants. Rice, a staple food for over half the world's population, represents a significant dietary source of arsenic. It is imperative that strategies to reduce grain arsenic are developed, and identifying the mechanisms that enable arsenic to reach and accumulate within the rice grain is key to this endeavor.

How do seeds prepare for their future?

Wim Soppe
Rijk Zwaan Breeding B.V., De Lier, The Netherlands

Seeds represent a crucial link between generations and enable plants to survive unfavourable conditions. Accurate timing of seed germination determines fitness in nature and yield in agriculture. Germination timing is controlled by dormancy, which is defined as the temporal inability of a viable seed to germinate under favourable conditions. In nature seeds select the best moment of the year to germinate but at the same time variation exists within seed batches to spread mortality risks. Seeds in agriculture should germinate immediately and uniformly after sowing.
Germination speed and dormancy are regulated both by intrinsic genetic factors and by environmental conditions during seed development and storage. Important environmental factors are temperature, light and nitrate, which give information about the time of the year and the vicinity of competing plants. Intrinsic factors are plant hormones and specific dormancy and germination genes. Abscisic acid inhibits germination and promotes dormancy whereas gibberellin has the opposite effect. Environmental factors act on intrinsic factors to influence germination.
In my talk I will give two examples of dormancy genes and their regulation by the environment. The gene *DELAY OF GERMINATION 1* has an essential role in dormancy in Arabidopsis. I will present how it controls germination by acting on the PP2C phosphatases of the abscisic acid signalling pathway. In the second example I will discuss a lettuce gene that influences germination at high temperatures.

Safeguarding genome integrity in germination and seed longevity

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Successful germination is important for agriculture and plant survival in natural ecosystems. Deterioration in seed quality is associated with the accumulation of cellular damage to macromolecules including DNA. Striking levels of DNA damage are further increased in seeds exposed to environmental stresses associated with seed ageing. Genome integrity is crucial for cellular survival and the faithful transmission of genetic information and repair of damage in early germination is essential to minimise growth inhibition and mutation of the genome. We have discovered that maintenance of germination vigour and viability requires several distinct repair pathways specific for particular forms of DNA damage. In particular, mutants deficient in repair of highly cytotoxic chromosomal breaks are hypersensitive to seed ageing, and repair of DNA double-strand breaks (DSBs) is rate-limiting for germination. The crucial link between genome integrity and germination was further supported by our findings that the DNA damage signalling kinases ATAXIA TELANGIECTASIA MUTATED (ATM) and ATM AND RAD3-RELATED control seed vigour and viability. In response to ageing, ATM delays germination, whereas *atm* mutant seeds germinate in the presence of DNA damage, resulting in extensive chromosomal abnormalities. Mechanistically, ATM functions through control of DNA replication in imbibing seeds which is mediated by transcriptional control of the cell cycle inhibitor SIAMESE-RELATED 5. Our current research is revealing how DNA damage signalling integrates repair, cell cycle control and cell death in seeds. Collectively, our findings provide insight into the roles of genome maintenance mechanisms and DNA damage response networks in regulating germination, a process critical for plant survival in the natural environment and crop production. Understanding the mechanistic basis of seed vigour and viability will underpin the directed improvement of crop varieties with enhanced germination resilience and longevity, and support preservation of genetic resources in seedbanks.

Theme 5: Niche crops

Amazonia biodiversity, a source of fine cocoa flavour niches

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The cocoa market is divided into two categories, "standard" cocoa and "aromatic fine flavour" cocoa, for which the consumer demand is growing despite a rare and low production. Two main old varieties were at the origin of Fine cocoa flavour : "Criollo" from Central America, domesticated for 3800 years by Olmec and Maya people, and "Nacional" from Ecuador present in the pacific coast at least five centuries ago. Both varieties were introduced in their main growing areas from a few genotypes with a narrow and highly homozygous genetic base, before being mixed more recently with newly introduced cocoa genotypes, constituting heterogeneous hybrid populations.

To better understand the *T. cacao* diversity, and the domestication history of these two old varieties, genetic studies were carried out, allowing to recover representatives of the ancestral varieties and to localize their origin in Upper Amazonia despite their current growing area.

In the case of Nacional variety, caracterised by floral taste, the Ecuadorian South Amazonia was identified as their domestication area of origin. Collect of genetic resources native to this region were organised to enlarge the genetic base of aromatic cocoa trees suitable for the selection of productive and aromatic new varieties adapted to Amazonia. Agricultural colleges and local indian populations were closely associated to this project, facilitating the recover of native cocoa trees and its further use to produce new aromatic varieties adapted to Amazonia.

An integrated genetic and biochemical approach was conducted to caracterize this material. It confirmed their large diversity and the richness of volatile compounds they display, offering new opportunities to develop fine flavour cacao niches and potential new incomes for small cocoa farmers of Amazonia.

GWAS (Genome Wide Association Studies), conducted on hybrid Nacional and native cocoa populations were engaged to analyse the genetic and biochemical determinants of the quality traits of Ecuadorian cocoa and identify diagnostic markers or new strategies to facilitate fine flavour cocoa breeding.

Faba bean: from gene to plate and back again

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ABSTRACT

Faba bean is doubly an orphan crop: it is both relatively neglected, and lacks a known wild progenitor. Our international collaboration is sequencing the gene space of its large diploid genome, 13 Gbp, and will eventually cover the whole genome. Stress responses and phenology limit the growing of the crop by farmers, and anti-nutritional factors limit use of the crop for food and feed, so these are among the current targets for improvement where genomic information would be valuable for accelerating breeding.
Faba bean is a traditional food in the Mediterranean basin, western Asia and China, and can return to the dining table elsewhere. It has higher yields than pea in 10 EU countries and higher protein yields in 16, because of its higher protein content (world average 29% in faba bean, 23% in pea). The high solubility of its protein makes faba bean a suitable alternative to soybean for people allergic to soy products, and for cool climates where soybean does not thrive.
We have identified sources of large root systems that explore root-zone water effectively, along with sources of stomatal control that limit water loss in different circumstances, and new sources of earliness of flowering and maturity. Vicine and convicine are the main anti-nutritional factors limiting food and feed use, and we have developed markers closer and closer to, and finally within, the currently known gene that causes a 90-95% reduction in their concentration. Further exploration of their biosynthetic pathway should allow their eventual elimination. Novel food processing methods allow elimination of vicine, convicine and their aglycones in a food matrix, and showed the importance of lowering lipoxygenase activity in order to maintain palatability of food products. Our programme encourages dialogue between genomics and application, accessing the gene information that is most needed for impact.

Theme 6: Plant Microbiome

The role of plant host rhizosphere signalling in root microbiome recruitment

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Plants secrete a variety of organic compounds and chemical signaling molecules into the rhizosphere, but we are only just beginning to understand the complexity and plasticity of these root exudates, let alone their biological significance. An intriguing example of this are the strigolactones, which were initially identified in the root exudates of just a few plant species and shown to be germination stimulants for root parasitic plants. Now we know that the strigolactones are present in all plants/plant root exudates and play an essential role as plant hormone and in the symbiotic interaction of plants with AM fungi. In my group, we study the biological relevance of the strigolactones and other metabolites exuded by plants into the soil. Our hypothesis is that many more of the molecules that plants secrete have a signaling function to other rhizosphere organisms, including micro-organisms. So far the latter has been demonstrated for a small number of molecules only, which we expect to represent only the tip of the iceberg. Indeed, it seems that belowground a chemical arms race is going on in which plants evolve specific signaling relationships with beneficial organisms, on which plant enemies listen in. This is illustrated by the large structural diversity in strigolactones. Potentially this also allows plants to use one chemical class in signaling relationships with several different organisms. Also this is perhaps illustrated by the strigolactones, for which it was recently shown that different types of strigolactones recruit different root microbiomes in sorghum. We use a number of targeted and untargeted approaches to further unravel signaling relationships between plants and rhizosphere organisms. This will increase our understanding about the role of the plant and plant signaling in root microbiome recruitment and should result in tools that will help to optimize the use of beneficial micro-organisms in agriculture.

Lotus japonicus and rhizobia interactions; from simple to complex associations

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Legume-rhizobia interactions are controlled by protein-carbohydrate recognition events that take place at the epidermal-soil interface. Legumes use LysM proteins to recognize carbohydrates produced by pathogens or symbionts. This suggests that an ancient recognition process has been used in legumes for evolution of elaborated mechanisms for various carbohydrate perceptions.

In *Lotus japonicus* two LysM receptor kinases, NFR1 and NFR5, initiate root nodule symbiosis after perception of Nod-factors secreted by *M. loti*, while EPR3 scrutinizes rhizobial exopolysaccharides controlling the elongation of infection threads. *Lotus* encodes several additional LysM receptors, and we have used reverse genetics coupled with *in planta* functional studies to study their role in *Lotus*. Our studies based on binary interactions identified novel components involved in carbohydrate signaling that contribute to the ability of *Lotus* to distinguish symbiotic and pathogenic microbes.

Recent analyses of bacterial taxa associated with roots of soil-grown *Lotus* wild-type and symbiotic mutant plants identified a previously unsuspected role of the nodulation pathway in the establishment of distinctive bacterial assemblages in root and rhizosphere. However, the role of soil microbiota on legume-*Rhizobium* symbiosis is currently unknown. We have employed specific members of a newly established culture collection to investigate the complex *Lotus*-*Rhizobium*-soil bacteria interactions in tailored microcosms. Our findings from these investigations based on plant and bacterial mutants will be presented.

Abstract PBE2018

A central role of multivesicular bodies in plant immunity

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Membrane trafficking is responsible for transport of membrane vesicles and their cargo between intracellular compartments and the plasma membrane. An important compartment is the multivesicular body (MVB), also named the late endosome. It contains intraluminal vesicles, which are generated by the highly complex ESCRT system on its surface. When MVBs fuse with the vacuolar membrane, their cargo including the intraluminal vesicles become degraded. When MVBs fuse with the plasma membrane, the cargo is secreted and the intraluminal vesicles become extracellular vesicles, which we call exosomes.

In recent years, we have found MVBs to be involved in a number of processes related to plant immunity. From studies of plants interacting with the powdery mildew fungus, we have found that pre-invasive immunity involves exosome secretion, and we have uncovered endosome and plasma membrane components involved in this process, including PEN1 and GNOM. This indicated to us that MVBs are involved. In a detailed study of well-known MVB components, we surprisingly found them not to be involved in this pre-invasive immunity mechanism. Rather, we found that these components have a major contribution to post-invasive, encasement-based immunity. This suggests existence of more than one MVB pathway in immunity.

In a separate study, we have revealed that an MVB-associated de-ubiquitinase is required for effector-triggered immunity (ETI) mediated by a subset of CC-NB-LRR-type resistance (R-) proteins, but not by TIR-NB-LRR-type R-proteins. Evidence suggests that this de-ubiquitinase requirement is due to a role of MVBs in these specific ETIs. The same study has exposed that a double edged sword is present here: The de-ubiquitinase is on one hand positively required for PTI mediated by CC-NB-LRR. However, on the other hand it appears to be a negative regulator of PTI mediated by TIR-NB-LRRs. The latter observation suggests that MVB components are monitored by one or more TIR-NB-LRRs, which may be the reason that ESCRT complex mutations occasionally are lethal.

Theme 7: Environmental resilience

Global change and the questions for which we have no answer (yet).

Hendrik Poorter, Plant Sciences (IBG-2), Forschungszentrum Jülich, Germany.

Thousands of experiments have been done to analyse how plants respond to their abiotic environment, and how this will affect our food security and natural ecosystems in the future. However, we as a community are currently not able to provide quantitative answers to even some of the basic questions about plant responses to global change. In this talk I will discuss a method to derive quantitative dose response curves (DRCs) from a wide range of independent experiments where a given environmental factor was manipulated. By deriving these DRCs we will be able to much better integrate a multitude of data into ecophysiological knowledge. This allows not only to better forecast what happens to plants in the future, but will also show where the major knowledge gaps are. It also allows for a more efficient teaching of new generations of researchers. In this talk I will present a range of dose-response curves to light, CO_2 , temperature and water and use them as a starting point to discuss several questions related to global change which have not received sufficient attention so far, including the supra-cellular side of systems biology and the transfer of knowledge from lab to field.
For more information see www.metaphenomics.org.

Functional biodiversity and biogeochemistry: linking plant traits and processes in a globally changing environment

The traits of organisms result from evolutionary and physiological processes, and reflect variation in ecological strategies. They provide a lens through which those ecological strategies, and their consequences, can be compared among taxa that co-occur locally, as well as across climate zones and vegetation types worldwide. For example, most taxa have leaf, stem and root traits that reside somewhere along a continuum from a 'slow' to a 'fast' return on investment design strategy. Thus, traits influence whole-plant function, and the dynamics, structure, and function of communities and ecosystems, including feedbacks to belowground processes and biogeochemical cycling. Such links are relevant to both the historical ecological landscape of the past and to the dynamic and rapidly changing world of the 21st century, replete with its changing climate, chemistry and biota. Using data ranging from to ecosystem-scale experiments with global change factors such as CO_2, temperature, rainfall and biodiversity to cross-continental observations to global earth system modeling, I provide an overview of the connections across *some* of these ecological strands.

Peter B. Reich
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Genetic characterization of salinity tolerance traits to increase salinity tolerance in crops

Mark Tester, *King Abdullah University of Science & Technology, Saudi Arabia*

One-third of the world's food is produced under irrigation and this is directly threatened by over-exploitation of water resources and global environmental change. This talk will focus on the use of forward genetics to discover genes affecting salinity tolerance in barley, rice, and tomatoes, along with some recent genomics in quinoa, a partially domesticated crop with high salinity tolerance. Rather than studying salinity tolerance as a trait in itself, we dissect salinity tolerance into a series of components that are hypothesised to contribute to overall salinity tolerance.

For barley, two consecutive years of field trials were conducted at a site with sandy soil and very low precipitation. Drip irrigation systems allowed the control of salinity. A barley nested association mapping (NAM) population developed by Klaus Pillen has been used to dissect physiologically and genetically complex traits in response to salt stress. Ten traits related to yield and yield components (e.g., days to flowering, harvest index, 100-seed mass) were recorded and we identified two significant loci located on the long arms of chromosomes 1H and 5H, which are both associated with several traits contributing to salinity tolerance - days to flowering, days to maturity, harvest index, and yield.

For tomatoes, the focus is on the genetics of tolerance in wild tomatoes, specifically *Solanum galapagense, Solanum cheesmaniae*, and *Solanum pimpinellifolium*. An association genetic approach is being taken. High-quality genome sequences have been made and genotyping-by-sequencing undertaken. Tomatoes have been phenotyped in The Plant Accelerator® and in the field, and analyses are currently in progress.

The application of this approach provides opportunities to significantly increase abiotic stress tolerance in crops and thus contribute to increasing agricultural production in many regions.

Theme 8: Genome evolution

The genetic basis for *Lotus japonicus* cold adaptation and colonization of Japan

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During colonization of new regions, sessile plants face physical barriers limiting dispersal and environmental variation challenging phenotypic plasticity, but the genetics underpinning adaptation and colonization are poorly understood. Based on re-sequencing 136 wild *Lotus japonicus* accessions collected throughout Japan, we found that *Lotus* is a relatively recent addition to the flora of Japan. It likely migrated to southern Japan 50-60,000 years ago and reached northern Japan only a few thousand years ago, while differentiating into three distinct subpopulations and losing genetic diversity along the way. Colonizing northern Japan required adaptation to severe winter conditions, and using genome-wide association analysis we identified SNPs strongly associated with winter hardiness under field conditions. Remarkably, the same SNPs also clearly distinguished the central and northern subpopulations. This identifies winter hardiness as one of the traits that has driven population differentiation, and at the same time points to candidate genes and genomic regions associated with this specific trait. Our results suggest that phenotype-independent analysis of population differentiation coupled with in-field transplantation experiments represents a powerful approach for dissecting plant evolution and adaptation.

Title: Adaptive evolution of meiosis in Arabidopsis arenosa

By Kirsten Bomblies

Abstract: Meiosis is essential for fertility of sexual eukaryotes and its core structures and progression are conserved across kingdoms. This system has been fine-tuned by evolution over the eons to separate pairs of homologous chromosomes. Polyploids, however, have more than two copies of every chromosome. In neopolyploids this presents a challenge, with multiple chromosomes often pairing, recombining and forming so-called multivalents that can cause chromosome segregation problems. Multivalents are associated with reduced fertility, and thus it is not suprising that evolved polyploids rarely if ever make multivalents. This shows that polyploids can evolve solutions to the problems they face initially, but how meiotic stability evolves in polyploids has remained largely mysterious. In a genome scan for adaptation to whole genome duplication in *Arabidopsis arenosa*, we previously found evidence that eight interacting meiotic proteins were under strong selection in the polyploid lineage. The proteins encoded by these genes are critical for axis formation, recombination and synapsis, in other words, some of the most central structural processes in meiosis. We hypothesize that modifications of these proteins stabilize polyploid meiosis by directly altering crossover number and/or the strength of crossover interference, with the outcome that there are fewer multivalent associations among the available chromosome copies. Our work provides insights not only into polyploid stabilization, but also more generally how modified recombination rates can evolve and what pleiotropic effects this might have.

Oscillating long non-coding RNAs are novel regulators of circadian-mediated processes

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Abstract

Long non-coding RNAs (lncRNAs) have emerged as new players in transcriptional and post-transcriptional regulation able to modulate various biological processes. Although widely studied in animals, only few plant lncRNAs have been characterized in detail.
Using an *Arabidopsis thaliana* lncRNA custom-made array, we identified several circadian-regulated lncRNAs that are expressed antisense to protein-coding genes. Many of these lncRNAs display an oscillatory pattern of expression that is antiphasic to their antisense partners.

We functionally characterized the natural antisense pair comprising *CDF5* (*CYCLING DOF FACTOR 5*) and *FLORE* (*CDF5 LONG NONCODING RNA*) and found that their antiphasic behavior reflects a mutual inhibition, which is required for their proper oscillation. Moreover, *CDF5* and *FLORE* oppositely regulate photoperiod-dependent flowering by modulating *FT* (*FLOWERING LOCUS T*) expression. *CDF*s encode transcriptional regulators involved in flowering time control and abiotic stress responses. Considering that other members of the *CDF* family have antisense lncRNAs, it is likely that these antiphasic modules could perform different biological functions.

Moreover, these regulators are conserved in both in dicot (*Arabidopsis*) and monocot (rice) species. Therefore, we anticipate they would perform relevant biological functions, acting as fine-tuning modulators of different circadian-mediated processes, and thus contribute to improve plant fitness.

Theme 9: Natural products

The place to be for metabolic engineering : the ER

Alain Goossens

Abstract:

Across the plant kingdom, hormones such as jasmonate steer the delicate balance between growth and the activation of defense programs, including the production of bioactive specialized metabolites. Plant cells are capable of producing an overwhelming variety of (specialized) metabolites, both in terms of complexity and quantity. These small organic molecules allow plants to cope with various types of stresses and often also have biological activities of high interest to human. Yet, this impressive metabolic machinery is still hardly exploited, mainly because of the limited molecular insight into plant (specialized) metabolism.
By using cutting-edge functional genomics tools, in combination with reverse genetics screenings, we aim to characterize the molecular mechanisms driving plant natural product biosynthesis in crop, medicinal and model plants. Extensive gene collections are generated that allow increasing our fundamental understanding of the central mechanisms that steer hormone signalling in the context of plant growth and metabolism and that serve as a novel resource for metabolic engineering tools that will facilitate the sustainable production of bioactive plant-derived molecules. I will highlight the central role of the endoplasmatic reticulum in the organisation and regulation of plant specialized metabolism and present data on organelle-specific metabolic engineering in synthetic biology and gene discovery programs.

Production of high value natural plant products: from the laboratory bench to the drugstore shelf

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Plants have always been a source of nourishment and care for living beings. Their dual task as producers of nutrients and drugs played a fundamental role in the evolution (and co-evolution) of herbivorous and omnivorous organisms. The so-called secondary (or special) metabolites are molecules with well-defined functional roles. The complexity of the molecular structures produced by plants is only equal to their versatility and biodiversity, while the harmonious interweaving of the biosynthetic and metabolic pathways offers a perfect picture of the adaptive plasticity of plants to changing environmental conditions. In this lecture I will briefly discuss the concepts of biodiversity, sustainability and the functional role of bioactive natural products, exploring the sites of synthesis and accumulation, the strategies adopted by plants to defend themselves from stress and the use of bioactive molecules as food supplements and as a source for natural medicines to combat diseases. After illustrating the main biosynthetic pathways leading to the synthesis of natural products I will focus on plant biotechnology applications for the production of bioactive natural products both *in vivo* and *in vitro*. I will also provide some examples of technology transfer to industrial process and the advantages of experienced laboratories to create competitive spin offs for the production of standardized and highly qualified bioactive natural products for the pharmaceutical and nutraceutical industries.

AWARD TALKS

FESPB Award Talks

EPSO Award Talks

A molecular switch for regulation of photosynthetic light use efficiency in mosses and green algae, named LHCSR

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Light is the energy source for photosynthetic organisms and yet it causes photoinhibition when in excess because unquenched chlorophyll (Chl) excited states yield reactive oxygen species when reacting with O_2 (Barber and Andersson, 1992; Miller et al., 2008; Takahashi and Badger, 2011). A major photoprotective mechanism is Non-Photochemical Quenching (NPQ) rapidly dissipating Chl excited states as heat, which prevents photodamage. In vascular plants the fast NPQ component is qE (Energy quenching). It requires PSBS protein (Li et al., 2000) and the two xanthophylls lutein (Lut) (Pogson et al., 1998) and zeaxanthin (Zea) (Niyogi et al., 1998). In the green algae as *C. reinhardtii* and in lower plants, qE activation relies on the LHCSR proteins (Peers et al., 2009; Alboresi et al., 2010). LHCSR is a chlorophyll (Chl) *a*-xanthophyll-binding protein binding 8 Chls and 4 xanthophylls per polypeptide. Two Xanthophyll binding sites have strong selectivity for, respectively, Lut (site L1) and violaxanthin (Viola), site L2. Two additional sites, N1 and V1 bind Viola (Pinnola et al., 2017; Bonente et al., 2011). Moss LHCSR is constitutively expressed in *P. patens* and its activity is controlled by the xanthophyll cycle. The form binding Viola has low activity while is switched to an highly active form upon binding Zea in high light (Pinnola et al., 2013). LHCSR is expressed in low amount and difficult to purify. We obtained sufficient amounts of this pigment-protein complex by overexpressing and purifying a "tagged" version of *P. patens* LHCSR (PpLHCSR) from transgenic tobacco plants (Pinnola et al., 2015). The availability of the protein allowed for the first time the systematic and comparative study of the inactive and active form by spectroscopic methods and hypothetical models of activation and catalysis of quenching reactions could be verified. In its lowly active form LHCSR has a fluorescence lifetime similar any other LHC protein thus acting as a light harvesting antenna and transferring energy to Reaction Centers (RC) to fuel charge separation. Under excess light conditions thylakoid lumen becomes acidic, leading to two effects on LHCSR: first, Zea is produced from pre-existing Viola and binds to sites L2 and V1; second, the protein is protonated on three acidic residues exposed to the lumen. Together, these events cause LHCSR to switch from light harvesting (3.7 ns) into its quenched (80 ps) conformations, thus effectively competing for excitons with PSII RC and dissipating energy into heat. This model was supported by data from Fluorescence Lifetime analysis in detergent solution, by transient absorption spectroscopy and Single Molecule Spectroscopy (Pinnola et al., 2016, 2017; Kondo et al., 2017).
Full understanding of this molecular switch requires high resolution structural data for the two conformations. At present we are pursuing crystallization for X-ray diffraction analysis and isolation of supramolecular complexes including LHCSR for cryo-electron microscopy. Because of its unique property of including both the protein domains responsible for pH detection and those catalyzing quenching reactions, LHCSR appears to be the best model for elucidation of one of the most enigmatic and elusive aspects of photosynthesis despite the process has been studied for some 50 years already.

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Plant vulnerability to cavitation: recent advances and perspectives in one of the key mechanisms of vegetation shifts and forest decline.

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The continuous water column that connects the soil and the upper portions of the plants through the xylem is exposed to tensions generated by water evaporation at the leaf surface. Under drought conditions, these tensions increase and induce the appearance of cavitation events in the xylem conduits that reduce the water transport capacity of the of the plants. Cavitation, and its associated xylem hydraulic failure, is now considered to be the principal mechanism of drought-induced plant mortality. Under the actual climate change scenario, evaluating the mechanisms of plant resistance to drought is therefore crucial for predicting the effects of the expected increment in drought frequency and severity on the plant species distribution worldwide. During the las years, important advances have been made in this research field that have provided relevant information for addressing one of the main actual questions in Plant Ecophysiology and Ecology: why some plants survive while others succumb to drought? This talk aims to provide an overview of the last advances, knowledge gaps, perspectives and future challenges in the study of the physiological mechanisms of plant survival to drought, with an especial focus on plant vulnerability to cavitation.

A new tool for sensitive detection of phosphorus deficiency in plants under field conditions

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Phosphorus (P) is essential for optimal crop yields, however, it is estimated that about 30% of the world's arable land is suffering from P deficiency. Estimation of crop P requirement is usually performed by classic soil extraction analysis. However, they are very uncertain and are often not able to reflect the plant available P in the soil. I will present a unique analytical principle based on chlorophyll *a* fluorescence, that allows rapid, non-destructive, onsite assessment of plant P status by recording the so-called OJIP transient of a dark-adapted leaf.

Both mono- and dicots have been cultivated in hydroponics with decreasing P availability to ensure a wide range of P tissue concentrations to test and improve the coverage of the prediction model. Re-supply experiments were further performed to test P dynamics and flexibility. In addition, field experiments have been performed in low-P soils, to test the method under natural conditions.

Chlorophyll *a* fluorescence (also known as OJIP curves) is known to reflect the status of the photosynthetic electron transport chain. The plant P status is influencing the photosynthetic performance, especially by decreasing the rate of ATP synthase, causing an acidification of the thylakoid lumen, which will result in a changed flow of electrons between the two photosystems. These specific changes are revealed by the shape of the OJIP curve, which can be correlated with the bioactive pool of P in plants. A mathematical model has been developed which estimates the plant P status from both mono- and dicots. The model has been integrated into a new handheld P-tester which allows estimations of crop P status directly in the field, based on the obtained chlorophyll a fluorescence transients. With the new P-tester, it is possible to detect P deficiency much earlier and much more accurately than previously.

The Delivery of Cytoplasmic Effector in P. infestans Mediated by Secreted Exosomes

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Potato blight, a ravaging disease caused by the oomycete *Phytophthora infestans,* is a major threat to global food security. *P. infestans* secretes effector proteins that are delivered inside or outside plant cells to neutralise host immunity. Little is known about how and where effectors are delivered during infection. Here, two cytoplasmic effectors Pi04314 and Pi22926 C-terminally tagged a monomeric red fluorescent protein (mRFP) expressed in *P. infestans.* Confocal microscopy revealed that both effector fusions were secreted from haustoria, which form intimate interactions with plant cells, to accumulate at its sites of action in the host nucleus. The well-characterized apoplastic effector EPIC1 (a cysteine protease inhibitor), a pectinesterase (PE), a cell wall degrading enzyme, and INF4, a PAMP-like protein, was also secreted from haustoria. EPIC1, PE and INF4 secretion was inhibited by brefeldin A (BFA), demonstrating that they are delivered by conventional Golgi-mediated secretion. In contrast, the secretion of Pi04314 and Pi22926 was insensitive to BFA treatment, indicating that the cytoplasmic effector follows an alternative route for delivery into plant cells.
To further investigate the mechanism of the effector translocation from pathogen into host cells. Ultracentrafugation was performed to collect extracellular vesicles (EVs), which are secreted to facilitate intercellular and extracellular communication, to promote infection and evade host immune responses. These functions have been exploited by diverse organisms. Therefore, we hypothesised that EVs may play a key role in the dissemination of pathogen, also host-derived molecules during infection. Here, transmission electron microscopy has been performed to identify EVs, *in vitro* grown, *P. infestans*. Furthermore, immunoblotting shows that secreted EVs mediate cytoplasmic effector secretion but not for apoplastic effector. This is a major breakthrough in the plant pathology community. Providing more details for helping to understand weapons of pathogen, to develop a specific chemical to disrupt these processes and inhibit pathogen infection.

SCIENCE POLICY SESSION

Open Science

Niels GOETKE, Head of Division Danish Agency for Science and Higher Education, Member the European Bioeconomy Panel 2013-15, Chairman of FACCE-JPI 2013-16, Coordinator of ICT-AGRI ERA-NET, Vice-chair BBI-JU SRG, Danish representative in SCAR

Open Science – View on Open science and Open innovation. How can the Member States contribute? Giving examples from FACCE-JPI and SCAR.

Open Science has become a high priority on the research policy agenda. The Commission presented its vision for the European Open Science Cloud (EOSC) in its April 2016 Communication on the 'European Cloud Initiative as a part of the Digital Single Market Strategy. The objective of the EOSC is to give the Union a global lead in research data management and ensure that European scientists reap the full benefits of data-driven science.

We live in a unique time, where technologies, science and connectivity are all closely interrelated, and all have a wealth of data associated with them. Open data is needed to make better choices, within the agri-food area. This is recognised by SCAR and FACCE-JPI. In June 2017 FACCE-JPI (The Joint Programming Initiative with in Agriculture, Food Security and Climate Change) organised a workshop in Copenhagen with major stakeholders about the data challenges in FACCE's remit. On the basis of this workshop FACCE-JPI is now trying to operationalise interactions with key data initiatives and leverage existing resources for data sharing and appropriate centralisation of data. It is a great challenge to make the step from using the data-driven technologies in science to making them relevant and accessible to end users

SCAR (the STANDING COMMITTEE ON AGRICULTURAL RESEARCH) is following the development through its many initiatives, different H2020 projects e.g e-ROSA and ICT-AGRI ERA-NET. SCAR is also heavily in involved in the discussions of the Food 2030 policy framework.

Openness and connectivity go hand in hand

Open Science, Open Education and Open Innovation. This is quite a broad area on which to focus a talk lasting only a few short minutes. However, one important element of openness is to eliminate barriers to participation in public and public-private research and innovation programs and projects.

My organisation, the European Institute of Innovation & Technology, the EIT, is doing just that: eliminating barriers to participation.

By bringing together leading businesses, higher education institutions and research centres, in what we call the 'Knowledge Triangle', the EIT is removing obstacles to communication between these three essential actors. We create strong ecosystems for entrepreneurial innovation – the Innovation Communities, KICs – based upon diversity, mobility and connectivity. Don't underestimate how complicated this is.

This open connectivity has the effect of ensuring a free and effective exchange of information and knowhow. Were that not the case, our Innovation Communities would not be seeing the successes that they are, with accelerating numbers of start-ups, scale-ups, products and services created, and more than 1,100 partners attracted from every side of the Knowledge Triangle – and with much more to come.

In education, our six Innovation Communities are leading the way in providing entrepreneurial skills to a new generation. But more than that, they make increasingly use of MOOCs – Massive Open Online Courses. These courses are open to all.

The EIT's Regional Innovation Scheme is gathering all the experience and knowledge and good practice of the EIT Community and takes it to regions that are considered to be modest or moderate innovators, with the intention of raising their capacity to innovate.

All strong examples of eliminating barriers to openness – where strong connectivity plays a major role. But openness requires checks and accountability to ensure quality? For the EIT Community, this is carried out in a connected interplay between members of the Community and the EIT itself.

Peter Olesen

Pekka PESONEN, Secretary General CopaCogeca, BE

Open Science and access to technologies and solutions – the importance of access to agricultural technologies and of building partnerships between scientists and farmers.

We (Copa-Cogeca, European farmers, European agri-cooperatives) desperately need better, more sustainable solutions for farming (better genetic material for seeds and livestock, PPPs, veterinary pharmaceuticals, machinery, data processing capabilities, digital solutions, networks…). The European public opinion has had some reservations when it comes to the use of certain technologies in food production. However, farming community is struggling between the international market realities and the European consumer preferences. All too often, EU farmers face higher input costs that the consumer market is not able or willing to cover. However, we remain optimistic. For instance, New Breeding Techniques will provide some opportunities for the EU farming, provided that each of these technologies is assessed on their own merits. Furthermore, the use of modern machinery, equipped with GPS, supported by EU systems such as Galileo and Copernicus (to which farming community contributed with some funds) and BIG DATA can help farmers to achieve ambitions objectives. We support our European businesses and research bodies to develop new, innovative solutions for farming, in particular taking into account our European conditions. Europe has been the leading region in improving agricultural productivity by better genetic material. Plant breeders' open access to existing gene pool is essential. Survival of European agriculture requires superior knowledge and use of the latest innovative technologies, in line with consumer market expectations. It's therefore of European farmers interest that Europe remains a global leader in research and innovation to ensure farmers reap the benefits of new technologies and solutions adapted to our farmer's needs.

Annette SCHNEEGANS, Senior Expert; European Commission**,** DG AGRI

EU agricultural research: from open science to open innovation. Contributions from Horizon Europe (FP9) and the future Common Agricultural Policy.

Under the EU programme Horizon 2020, funding for agricultural research is mainly channelled through Societal Challenge 2 (SC2: Food Security, Sustainable Agriculture and Forestry, Marine, Maritime and Inland Water Research and the Bioeconomy). Calls for proposals under the two first Work Programmes covering the 2014/2015 and 2016/2017 periods have resulted in about 800m€ allocated to almost 150 projects related to agriculture. This includes research on crop and animal production, resource use, technologies, value chain organization, rural development and policy support.

The increased emphasis on innovation under Horizon 2020 as compared to its predecessors has resulted in increased attention given to Open Science and - in the area of agricultural research - to participatory research as vehicles for increasing the impact of research activities "on the ground". The multi-actor approach applied systematically under SC2 reflects this ambition.

The presentation will address the concept of Open Science from a broader perspective, i.e. go beyond open publications and open data to the notion of Open Innovation in the agricultural context. It will show how in-built links between Horizon 2020 agricultural research, the European Innovation Partnership EIP AGRI and the Common Agricultural Policy are used to effectively engage the agricultural sector in research and build a supportive framework for Open Innovation.

Discussions on the forthcoming European Research and Agricultural Policies provide opportunities for strengthening the current agricultural innovation framework and for embedding Open Science and Open Innovation more firmly into the design and implementation of research activities.

Alan H. Schulman

Natural Resources Institute Finland (Luke); and Institute of Biotechnology, University of Helsinki

Open Science – basis for science, innovation and our societies today and in future. How can plant scientists contribute

Open Science includes a range of ideas and approaches, which are all aimed at reducing barriers of access to the process and results of science as a basis for fostering understanding, collaboration, and progress. Much of Open Science is based in protocols and tools for remote sharing and collaboration within the process of carrying out scientific research, ranging from the development of a study design to the management of materials and data, and then to data analysis and report writing. Open Science includes the means for stable and long-term access to data and materials following publication, along with sufficient documentation that meta-analysis is possible. Long-term data storage and availability poses many challenges as the era of precision phenotyping grows to complement the nucleic acid databases derived from high-throughput and genomic sequencing. Lastly, timely and open access to scientific communications both before and after peer-review and publication is a critical component of Open Science. The goal is to democratize the development and exploitation of scientific knowledge for the benefit of all.

Plant Biology Europe Congress 2018, Copenhagen

Policy Session - Open Science (19 June 2018, 13:15 – 15:00)

Nils Stenseth, Member Science Council of the European Research Council (ERC): Open Science – how the ERC can contribute

Abstract:

The European Research Council (ERC) supports the principle of open access to research results as a fundamental part of its mission. This includes journal articles, monographs, book chapters and other types of publications, but also research data, software and code, and other research outputs. The ERC recognizes the importance of preprints as one optional way to demonstrate research achievements and encourages the use of researcher identifiers such as ORCID.

As an organization that is governed by scientists, the ERC considers it crucial that the transition to Open Science takes into account the important role of researchers in this process, be it as producers of knowledge, as actors in the review and publication process (within the established system or otherwise), or as users of other researchers' output. The vast diversity of situations across different research communities makes this a complex and challenging task.

With this backdrop, I will shortly outline the ERC's approach towards open access and research data management. I will then give a quick overview of some of the initiatives that the ERC has engaged in to support the researchers it funds in opening up their results, and provide a glimpse of some ideas for the future.