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Efficiency of Arbitrarily Amplified Dominant Markers (SCOT, ISSR and RAPD) for Diagnostic Fingerprinting in Tetraploid Potato

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Abstract Three molecular markering techniques: start codon targeted (SCOT), inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers were compared for fingerprinting of 24 varieties and a segregating population of tetraploid potato. The number of scoreable and polymorphic bands produced using the SCOT, ISSR and RAPD primers for varieties was more than that of genotypes. SCOTs markers were more informative, followed by ISSRs marker, than other markers for the assessment of varieties based on polymorphism information content (PIC). There were no significant differences among these markers in terms of the evaluation of genotypes. All marker techniques individually illustrated that Diversity Index and Marker Index for varieties were higher than that of genotypes, and SCOT had superiority to other markers. The resolving power (Rp) of the SCOT, ISSR and RAPD techniques was 71.25, 46.62 and 30.63 for varieties and 21.38, 18.83 and 18.87 for genotypes, respectively. Standard Jaccard's similarity coefficient of each marker technique revealed that similarity among varieties was less than that of the genotypes. Overall the Shannon index showed that relative genetic diversity of the varieties was high when SCOT markers were used but it was fairly similar when ISSR and RAPD markers were applied. The results of Analysis of Molecular Variance (AMOVA) revealed that variation within groups of varieties of a country was significantly higher than among groups. These results suggest that efficiency of SCOT, ISSR and RAPD markers was relatively the same in fingerprinting of genotypes but SCOT analysis is more effective in fingerprinting of potato varieties. Overall, our results indicate that SCOT, ISSR and RAPD fingerprinting could be used to detect polymorphism for genotypes and for varieties of potato.

Resumen Se compararon tres técnicas de marcadores moleculares para caracterizar molecularmente 24 variedades y a una población segregante de papa tetraploide: enfocado a codón de inicio (SCOT), repetición de secuencia inter-simple (ISSR) y amplificación al azar de ADN polimórfico (RAPD). El número de bandas polimórficas registrables producidas usando iniciadores para SCOT, ISSR y RAPD, fue mayor para las variedades que el de los genotipos. Los marcadores SCOT fueron más informativos, seguidos por los de ISSR, más que otros marcadores para el análisis de variedades con base al contenido de información del polimorfismo (PIC). No hubo diferencias significativas entre estos marcadores en términos de la evaluación de genotipos. Todas las técnicas de marcación ilustraron individualmente que el Índice de Diversidad y el índice de marcador para las variedades fue mayor que el de los genotipos, y SCOT tuvo superioridad sobre otros marcadores. El poder de resolución (Rp) de las técnicas SCOT, ISSR y RAPD fue de 71.25, 46.62 y 30.63 para las variedades y de 21.38, 18.83 y 18.87 para los genotipos,

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respectivamente. El coeficiente estándar de similitud de Jaccard de cada técnica de marcador reveló que la similitud entre variedades era menor que la de los genotipos. En general, el índice Shannon mostró que la diversidad genética relativa de las variedades era alta cuando se usaban los marcadores SCOT, pero era muy similar cuando se aplicaban los marcadores ISSR y RAPD. Los resultados del Análisis de Varianza Molecular (AMOVA) revelaron que la variación dentro de grupos de variedades de un país era significativamente más alta que entre grupos. Estos resultados sugieren que la eficiencia de los marcadores SCOT, ISSR y RAPD fue relativamente la misma en la caracterización de los genotipos, pero el análisis SCOT es más efectivo en la caracterización molecular de las variedades. En general, nuestros resultados indican que la caracterización SCOT, ISSR y RAPD puede usarse para detectar el polimorfismo para genotipos y para variedades de papa.

Keywords Tetraploid potato · Fingerprinting · SCOT · ISSR · RAPD

Introduction

DNA fingerprinting for cultivar or variety identification has become an important tool for genetic identification in plant breeding and germplasm management (Jondle 1992; Smith 1998). One of the most important decisions for DNA fingerprinting is the marker system and technique to be used. Various systems and their related techniques are currently available. Prevost and Wilkinson (1999) concluded that ISSR-PCR gives a quick, reliable and highly informative system for DNA fingerprinting of potato. Demeke et al. (1993) used RAPD markers to identify potato cultivars. They discovered that RAPD technique is suitable for the detection of polymorphism, regardless of tissue or environment factors and is a highly useful method to distinguish and identify potato cultivars and clonal variation of cultivars. Sosinski and Douches (1996) also used PCR-based DNA amplification to fingerprint North American potato cultivars. Their result showed that all cultivars were discriminated with as few as 10 primers and could distinguish the russet sport of Burbank from a white-skinned clone by one band. McGregor et al. (2000) compared RAPD, ISSR, AFLP and SSR markers for fingerprinting in tetraploid potato. Their results showed that all the techniques could individually identify all the cultivars, but the mean number of bands generated per primer (or primer pair) for each cultivar was different.

Collectively, techniques such as AFLP, ISSR and RAPD, have been termed as arbitrarily amplified dominant (AAD) markers (e.g. Karp et al. 1996; Wolfe and Liston 1998) and are increasingly used for diagnostic genomic fingerprinting, genetic and qualitative trait loci (QTL) mapping and population genetic studies. AAD markers are also a source for phylogenetic inference and systematic studies at various levels, using both distance- and parsimony-based approaches (Gupta et al. 1999; Bussell et al. 2005; Semagen et al. 2006; Mark et al. 2007).

In recent years, many new alternative and promising marker techniques have been developed in line with the rapid growth of genomic research (Gupta and Rustgi 2004). Due to the tremendous growth in public biological databases, the development of functional markers that are located in or near the candidate genes have become considerably easy (Andersen and Lubberstedt 2003). With initiating a trend away from random DNA markers towards gene-targeted markers, a novel marker system called SCOT (Collard and Mackill 2009) was developed based on the short conserved region flanking the ATG start codon in plant genes. SCOT markers are generally reproducible, and it is suggested that primer length and annealing temperature are not the sole factors determining reproducibility. They are dominant markers like RAPDs and could be used for genetic analysis, quantitative trait loci (QTL) mapping and bulk segregation analysis (Collard and Mackill 2009). In principle, SCOT is similar to RAPD and ISSR because the same single primer is used as the forward and reverse primer (Collard and Mackill 2009; Gupta et al. 1994).

The use of primer pairs would be possible to obtain additional amplified bands compared to single primers. Theoretically, four times more bands would be expected from a single amplification reaction using two primers, than by using each primer individually in separate reactions (Williams et al. 1993). Such applications would also enable the appearance of novel amplified products which are absent from single primer reactions, as it was previously suggested in the case of RAPD by Williams et al. (1993). The simultaneous use of different primers in a single reaction would also enable the development of several new combinations from the same primer sets and thus reducing research costs.

The aim of the present study was to determine the efficiency of AAD markers (RAPD and ISSR) and start codon targeted markers in diagnostic fingerprinting of tetraploid potato cultivars and genotypes.

Material and Methods

Plant Material and DNA Isolation

Twenty four varieties of potato have been released in seven different countries (Table 1) and 85 genotypes from a

tetraploid F_1 potato population were randomly selected and used in this study. All genotypes were developed in the Potato Research Center (University of Pannonia, Keszthely, Hungary) from a cross between White Lady as female and S440 as male parent. Genomic DNA was extracted from 0.1 g of a mixed leaves and stems of in-vitro plants using the procedure of Walbot and Warren (1988).

SCOT Analysis

Fifteen sets consisting of random combinations of 12 single SCOT primers without any initial screening were used for fingerprinting. The primer pair was preferred to a single primer as it produced more polymorphic bands in our previous experiments. The sequences and their combinations are listed in Table 2. PCR amplification was carried out in a total reaction solution of 12 μl in 384-well plates using Eppendorf Mastercycler ep 384. PCR reaction mixtures contained 40 ng DNA as template, 1.2 μl from each 10 μM 12-mer primer, 1.2 μl 2 mM dNTP (Fermentas, Lithuania), 1.5 μl 10 × PCR buffer (1 mM Tris-HCl, pH 8.8 at 25°C, 1.5 mM $MgCl_2$, 50 mM KCl and 0.1% Triton X-100) and 0.29U of DyNazyme II (Finnzymes, Finland) polymerase. Amplified PCR products were mixed with 5 μl BromPhenol Blue dye (99.5% deionsied formamide, 10 mM EDTA pH 8, 0.05% bromphenol-blue, xylene-cyanol dye solution, 1 μl pure sterile water) and separated on 1.5% agarose gels (Promega, USA) in 0.5 × TBA (Tris-HCl, Boric acid, EDTA). Standard PCR cycling parameters were used: an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 30 s at 94°C, 1 min at 50°C and 2 min at 72°C. The cycles were followed by 5 min at 72°C for final extensions. After electrophoresis, amplified bands were visualized by ethidium-bromide staining, and documented with GenGenius Bio Imaging System (Syngene, UK).

ISSR Analysis

Inter-simple sequence repeat assay was conducted using 15 single primers. Primer sequences are listed in Table 2. PCR reactions were carried out as described in SCOT analysis with the exception of primer volume (2.4 μl). The thermocycler program for PCR was set to 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 1 min at 50°C and 2 min at 72°. The final extension at 72°C was hold for 5 min. The annealing temperature for UBS835, UBS841, UBS842 and UBS844 primers was 55°C. All PCR amplification products were separated on 1.5% agarose gel in TBA, stained with ethidium-bromide and documented with a GenGenius Bio Imaging System (Syngene, UK).

RAPD Analysis

To choose appropriate RAPD primers, various combinations of 50 RAPD primers were tested in an initial screening using two parents and 6 selected genotypes of WL × S440 cross population (3 resistant and 3 susceptible to *Potato Potyvirus Y*). After the screening procedure, fifteen primer combinations (Table 2) were randomly selected from suitable combinations for further analysis. Each sample was amplified twice to verify reproducibility. Total volume and composition of reaction mixtures were the same as described in the case of SCOT analysis. The PCR profile was as follows: 4 min predenaturation at 94°C, followed by 35 cycles of 30 s denaturation at 94°C, 1 min annealing at 37°C and 2 min extension at 72°C. The cycles were followed by 5 min final extensions at 72°C. Detection of PCR products was the same like in the case of SCOT analysis.

Data Analysis

Amplified products were scored as present (1) or absent (0) to form a binary matrix. Obscure bands were discarded and distinct and unequivocal bands were scored only. It was presumed that co-migrating fragments had been amplified from analogous loci. Jaccard's similarity matrix, Shannon's information index based on *Log2,* variance (after Bowman et al. 1969), AMOVA and principal coordinate analysis (PCoA) were computed using FAMD 1.23β (Schluter and Harris 2006) program. Frequencies per data matrix for *I*

Table 1 Potato cultivars used in this study and their country of origin (G = Germany, HU = Hungary, NL = Netherlands, CA = Canada, P = Poland, AU = Austria, RU = Russia and USA = United States of America)

Cultivar	Cultivar	Cultivar	Cultivar
Gülbaba (HU)	Sante (NL)	Lorett (HU)	Panda (G)
Somogyi kifli (HU)	Snowden (USA)	S440 (USA)	Lvovjanka (RU)
Atlantic (USA)	Franzi (G)	WL (HU)	Kennebec (USA)
Desiree (NL)	Agria (G)	Irga (P)	Linzer delicate (AU)
Shepody (CA)	Rioja (HU)	Kondor (NL)	Saturna (NL)
Swiss (USA)	Katica (HU)	Cleopatra (NL)	Vénusz Gold (HU)

Table 2 Characteristics of SCOT, ISSR and RAPD banding profiles produced in tetraploid potato genotypes: (PIC) Polymorphic information content, (Rp) Resolving power

Primer code	Sequence (5′-3′)	Total No. of bands	No. of scoreable bands	No. of poly. bands	% of poly. bands	PIC values	RP
SCOT primers							
1	CAACAATGGCTACCACCA						
2	CAACAATGGCTACCACCC						
3	CAACAATGGCTACCACCG						
4	CAACAATGGCTACCACCT						
5	CAACAATGGCTACCACGA						
11	AAGCAATGGCTACCACCA						
12	ACGACATGGCGACCAACG						
13	ACGACATGGCGACCATCG						
14	ACGACATGGCGACCACGC						
16	ACCATGGCTACCACCGAC						
33	CCATGGCTACCACCGCAG						
36	GCAACAATGGCTACCACC						
SCOT (primer pairs)							
S01-02	Combination of primer 1 & 2	13	9	0	0	–	–
S01-36	Combination of primer 1 & 36	9	8	1	13	0.124	0.76
S02-03	Combination of primer 2 & 3	11	10	0	0	–	–
S04-05	Combination of primer 4 & 5	9	8	1	13	0.102	0.99
S04-11	Combination of primer 4 & 11	10	7	1	14	0.203	0.97
S04-12	Combination of primer 4 & 12	14	9	3	33	0.251	2.18
S04-16	Combination of primer 4 & 16	10	9	1	11	0.128	0.69
S04-36	Combination of primer 4 & 36	12	10	3	30	0.309	2.92
S05-11	Combination of primer 5 & 11	15	9	4	44	0.225	3.31
S11-16	Combination of primer 11 & 16	14	9	2	22	0.108	1.84
S13-14	Combination of primer 13 & 14	13	8	2	25	0.324	1.56
S13-16	Combination of primer 13 & 16	12	10	4	40	0.198	2.97
S14-16	Combination of primer 14 & 16	12	4	1	25	0.219	0.94
S16-36	Combination of primer 16 &36	11	10	2	20	0.090	1.33
S33-36	Combination of primer 33 & 36	14	10	1	10	0.074	0.92
	Total	179	130	26	–	–	21.38
	Average	11.9	8.7	1.7	20	0.181	
ISSR primers[a]							
Issr9	$(AC)_8$ YA	9	6	2	33	0.278	1.98
IssrL1	$(AG)_8$ C	9	8	0	0	–	–
IssrL3	$(AC)_8$ YG	11	9	4	44	0.339	3.84
IssrT2	DDCCACCACCAC CACCA	11	9	0	0	–	–
UBC807	$(AG)_8$ T	10	9	1	11	0.092	0.87
UBC808	$(AG)_8$ C	11	10	0	0	–	–
UBC809	$(AG)_8$ G	9	8	0	0	–	–
UBC810	$(GA)_8$ T	10	7	5	71	0.437	4.23
UBC812	$(GA)_8$ A	6	5	2	40	0.320	1.84
UBC813	$(CTC)_5$TT	12	9	1	11	0.099	0.97
UBC822	$(TC)_8$ A	8	5	1	20	0.167	0.99
UBC835	$(AG)_8$ YC	10	8	1	13	0.082	0.69
UBC841	$(GA)_8$ YC	12	8	1	13	0.133	0.85
UBC842	$(AG)_8$ YG	10	7	1	14	0.137	0.97
UBC844	$(CT)_8$ RG	9	7	2	29	0.228	1.61

Table 2 (continued)

Primer code	Sequence (5′-3′)	Total No. of bands	No. of scoreable bands	No. of poly. bands	% of poly. bands	PIC values	RP
	Total	147	115	21	–	–	18.83
	Average	8.6	6.8	1.4	20	0.210	
RAPD primer pairs							
R01-91	GTCAGTGCGGCA-GGCATGACCTGT	7	4	0	0	–	–
R05-52	AGGCATCCTGAG-AGATGCAGCCAG	13	8	1	13	0.138	0.80
R05-57	GGCTCGCATCTA-AGATGCAGCCAG	15	7	3	43	0.340	2.05
R05-77	CCCACTAGACTC-AGATGCAGCCAG	13	12	2	17	0.167	1.79
R06-67	CATACGGGCTAC-ACATGCCGTGAC	12	9	2	22	0.202	1.65
R06-77	CCCACTAGACTC-ACATGCCGTGAC	12	7	2	29	0.247	1.95
R08-79	GTCGTAGCGGAT-AATGCGGGAGTC	14	9	2	22	0.135	1.31
R15-59	CCCCCGTTAGAA-AGATGCGGGGTA	13	5	2	40	0.352	1.43
R15-87	GTGACCGAGTCG-AGATGCGGGGTA	12	8	3	38	0.298	2.69
R18-33	GTCATGCGACGA-CTGTCATGCCGA	12	9	2	22	0.201	1.79
R35-91	GTCAGTGCGGCA-GGCTTATGCCGT	13	9	1	11	0.126	0.78
R41-97	CGCGAATTCC-GGACCCTTACTG	13	10	0	0	–	–
R43-90	ACTCCAGCCAGG-CCATCGGAGGTC	11	7	1	14	0.137	0.97
R44-57	GGCTCGCATCTA-CCGTCCATCCAC	13	5	0	0	–	–
R45-67	CATACGGGCTAC-GTAAGGCGCATC	12	5	2	40	0.273	1.66
	Total	185	114	23	–	–	18.87
	Average	12.3	7.6	1.5	21	0.218	

[a] Y= (C,T); D= (A,G,T); R=(A,G)

was computed as follow: p (i) = presences (i)/presences (data matrix). Distance Matrices were subjected to Neighbor-Joining methods to generate a dendrogram. A Strict Consensus tree was calculated in order to estimate the structural stability of clusters and to evaluate the reliability of trees bootstrap, analysis of the data was carried out with 2000 replication using Splits tree4 (Huson and Bryant 2006). The band informativeness (I_b) estimated as $I_b = 1 - (2 \times |0.5 - p|)$ (Prevost and Wilkinson 1999), where p is the proportion of the varieties or genotypes containing the band. The resolving power of the primer (Rp) measured in accordance with $Rp = \sum I_b$. Polymorphic Information Content (*PIC*) was calculated according to, $PIC = 1\text{-}p^2 - q^2$ (Ghislain et al. 1999) where p is frequency of present band and q is frequency of absent band. Marker Index (*MI*) was computed as *EMR* × *DI*, where *EMR* (Effective Multiplex Ratio) was the number of polymorphic markers generated per assay and *DI* (Diversity Index) was the average PIC value.

Results

The oligonucleotide sequences of SCOT, ISSR and RAPD primers and the resultant multiple band patterns for genotypes and varieties were summarized in Tables 2 and 3 respectively. The data on computed Shannon's index, diversity index and marker index for SCOT, ISSR and RAPD markers were listed in Table 4.

SCOT Analysis

The PCR amplification using SCOT primer pairs resulted in generation of reproducible amplification products. Fifteen primer pairs amplified 130 clear and scorable bands for the genotypes and 187 for the varieties. Effective multiplex ratio for genotypes and varieties was 26 and 119, respectively. The average number of scorable bands revealed by each primer pair was 8.7 for genotypes and 12.5 for varieties. Average numbers of polymorphic band per primer pair for genotypes and varieties was 1.7 and 7.9, respectively. The mean of percentage polymorphism for each primer pair of genotypes was 20 and it was 61 for varieties. Out of SCOT primers, primer pairs S04-12, S05-11, S11-16 and S13-14 showed more than one allele at a given locus. Diversity index and marker index for genotypes were 0.181 and 4.710 and for varieties it was 0.4 and 47.6, respectively. The Rp of the SCOT for genotypes (21.38) was less than that of varieties (71.25). The maximum Rp (3.31) was belonging to primer pair S05-11 of genotypes and primer pair S04-12 (9.75) of varieties.

Table 3 Characteristics of SCOT, ISSR and RAPD banding profiles produced in varieties of tetraploid potato: (PIC) Polymorphic information content, (Rp) Resolving power

Primer code	Total No. of bands	No. of scoreable bands	No. of poly. bands	% of poly. bands	PIC values	RP
Combination of SCOT primers[b]						
S01-02	13	11	5	0	0.38	3.83
S01-36	12	10	6	13	0.43	3.92
S02-03	14	13	9	0	0.43	6.08
S04-05	11	10	3	13	0.29	2.50
S04-11	12	10	5	14	0.45	2.75
S04-12	19	18	15	33	0.50	9.75
S04-16	14	13	10	11	0.49	5.33
S04-36	12	11	4	30	0.37	1.58
S05-11	13	11	7	44	0.26	2.42
S11-16	16	15	13	22	0.49	8.83
S13-14	13	12	9	25	0.42	4.83
S13-16	14	13	10	40	0.47	5.58
S14-16	17	16	13	25	0.49	8.92
S16-36	13	12	5	20	0.30	2.42
S33-36	16	12	5	10	0.29	2.50
Total	209	187	119	–	–	71.25
Average	13.9	12.5	7.9	61	0.4	
ISSR primers[a,b]						
Issr9	11	10	7	70	0.39	3.83
IssrL1	9	8	2	25	0.15	1.41
IssrL3	13	12	5	42	0.36	3.83
IssrT2	11	10	0	–	–	–
UBC807	14	13	11	85	0.49	8.42
UBC808	11	10	3	30	0.15	1.67
UBC809	6	6	0	–	–	–
UBC810	12	10	4	40	0.30	2.25
UBC812	12	11	8	73	0.45	5.33
UBC813	9	7	2	29	0.20	1.67
UBC822	6	4	3	75	0.34	1.75
UBC835	10	9	5	56	0.38	3.33
UBC841	13	12	8	67	0.46	5.75
UBC842	12	11	6	55	0.42	3.25
UBC844	10	9	5	56	0.40	4.12
Total	159	142	69	–	–	46.62
Average	10.6	9.5	4.6	47	0.34	
R01-91	8	7			–	
R05-52	11	10	1	10	0.06	0.67
R05-57	13	11	5	45	0.33	1.92
R05-77	11	10	3	30	0.19	1.50
R06-67	12	11	5	45	0.29	2.3
R06-77	11	8	1	13	0.14	0.83
R08-79	13	9	4	44	0.36	2.25
R15-59	12	10	4	40	0.30	3.42
R15-87	12	10	8	80	0.50	4.50
R18-33	14	13	8	62	0.43	5.00
R35-91	12	8	2	25	0.13	1.08
R41-91	12	11		0	–	–

Table 3 (continued)

Primer code	Total No. of bands	No. of scoreable bands	No. of poly. bands	% of poly. bands	PIC values	RP
R43-90	11	9	2	22	0.22	1.58
R44-57	12	9			–	
R45-67	16	15	7	47	0.36	5.58
Total	180	151	50		–	30.63
Average	12.0	10.1	3.3	31	0.28	

[a] Y = (C,T); D = (A,G,T); R = (A,G)

[b] Sequence of primers are same as that mentioned in Table 1

The band informativeness of genotypes was high and generally more than that of varieties (data not shown) but the number of polymorphic bands produced by each primer for the genotypes was less than that of the varieties. The same results were obtained with ISSR and RAPD markers. Shannon's information index computed to identify genetic diversity between genotypes and varieties. The results of genotypes and varieties were 4.470 and 6.704, respectively. The AMOVA was carried out to estimate population differentiation directly from molecular data and test hypothesis about such differentiation. The result showed that variation within population (94.9%) was more than among population (5.1%). In order to estimate structural stability of clusters and reliability of trees, bootstrap analysis was conducted with 2000 replications after constructing the Consensus Tree using NJ genetic distances based on the Dice coefficient. The analysis grouped genotypes and varieties into 9 and 7 main clusters, respectively. The SCOT marker technique identified all cultivars and 85 genotypes out of 87. For varieties, Phylogram comprises Snowden, Atlantic, Swiss and S440 derived from USA in cluster D, Desiree, Cleopatra and Kondor from Netherlands in cluster F, Katica and Rioja from Hungary and Panda and Franzi from Germany in cluster G (Fig. 1a). To create a predictive model based on uncorrelated variables, related to the original correlated variables and comparing to clustering analysis, we performed principal coordinate analyses (PCoA). The result of PCoA was comparable to the cluster analysis (Fig. 2a). The first three most informative principle component explained 55.04% of the total variation.

ISSR Analysis

ISSR primers produced different numbers of DNA fragments, depending upon their simple sequence repeat motifs. ISSR9, ISSRL3, UBC810 showed polymorphic bands which were alleles of a single locus. For genotype, the 15 primers produced 147 fragments of which 115 were scoreable and 21 were polymorphic. For varieties, it produced 159 fragments of which 142 were scoreable and 69 were polymorphic. The mean number of scoreable bands for genotypes and varieties was 6.8 and 9.5, respectively. PIC calculated for ISSRs scaled from 0.08 to 0.44 in the genotypes and from 0.15 to 0.49 in the varieties. The average percentage polymorphism of each primer for genotypes and varieties was 20 and 47, respectively. Diversity index and marker index for genotypes were 0.21 and 4.41 whereas it was 0.34 and 23.46 for varieties. For genotypes and varieties, the Rp of the ISSR was 18.83 and 46.64, respectively. The result of AMOVA exposed that variation within population and among population was 96.78% and 3.22% respectively. Overall Shannon's index for genotypes and varieties was 4.40 and 5.88, respectively. Construction of tree and bootstrap analysis was performed as in the case of SCOT markers. The results displayed that genotypes and varieties segregated into 10 and 8 clusters, respectively (Fig. 1b). The ISSR marker technique identified 85 genotypes out of 87 and 22 cultivars out of 24. The result of principal coordinate analysis was comparable to the cluster analysis (Fig. 2b). The first three most informative principle components explained 56.24% of the total variation.

Table 4 Data of Shannon's index, diversity index and marker index computed for SCOT, ISSR and RAPD markers

Marker	Shannon's index		Diversity index		Marker index	
	Variety	Genotype	Variety	Genotype	Variety	Genotype
SCOT	6.70	4.47	0.40	0.18	47.60	4.71
ISSR	5.88	4.40	0.34	0.21	23.46	4.41
RAPD	5.32	4.35	0.28	0.24	14.00	5.00

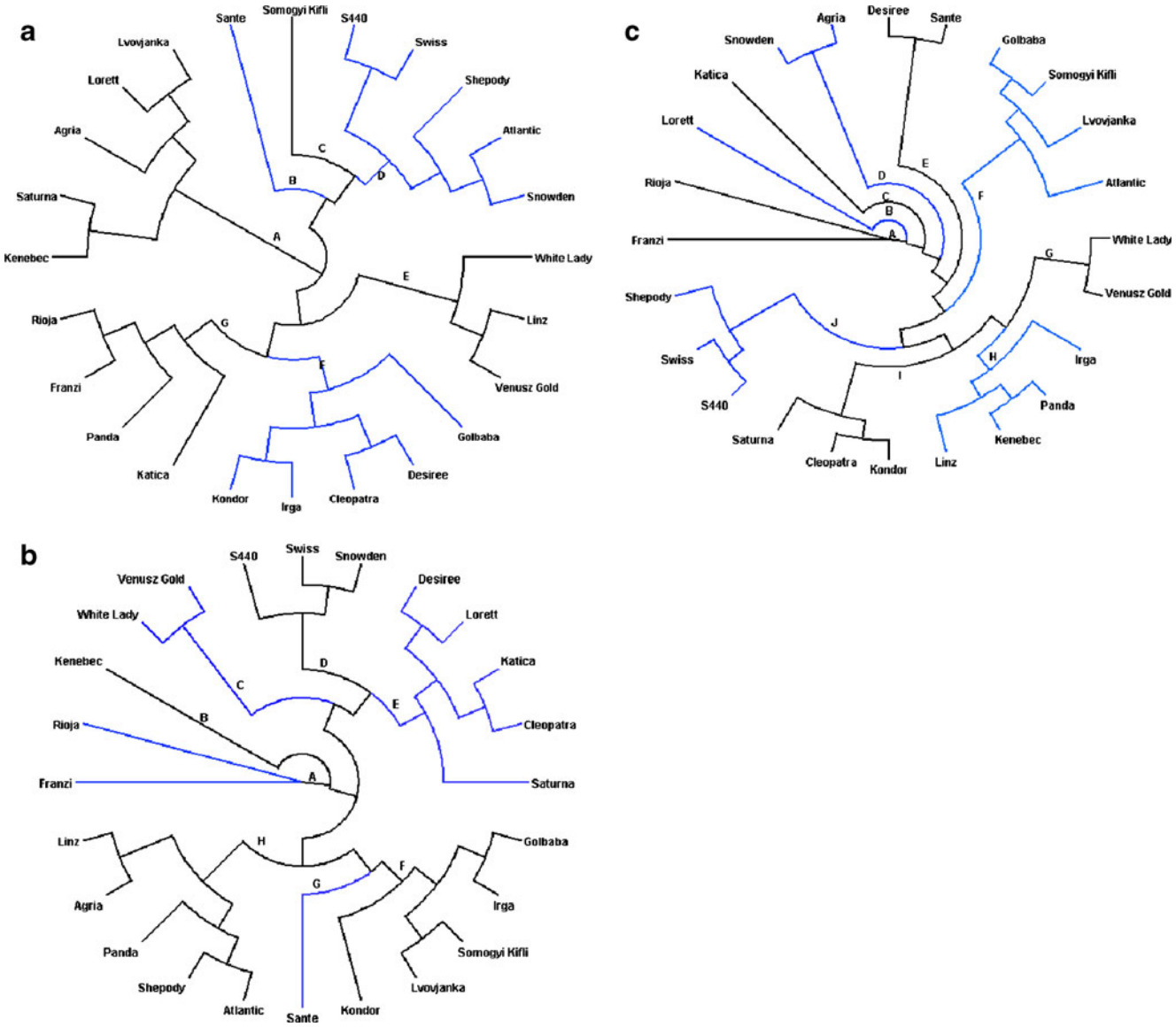

Fig. 1 Circular phylogram of consensus tree, using the genetic distance of NJ, based on SCOT (**a**), ISSR (**b**) and RAPD (**c**) markers; letters show clusters. **a.** SCOT **b.** ISSR **c**. RAPD

RAPD Analysis

PCR amplification of DNA, using 15 primer pairs of RAPD yielded 185 DNA fragments, of which 114 were scoreable and could be scored in all genotypes. Out of all RAPD primer combinations only combination of primer 57 with primer 5 (R05-57) showed amplified fragments from more than one allele of a given locus. Compared to SCOT primers, RAPDs generally produced less polymorphic and scorable bands per primer pair. For genotypes, the number of polymorphic and scorable bands per primer pair produced by RAPD primers was more than by ISSR primers. The number of polymorphic bands per primer pair produced with ISSRs was higher than by RAPDs using the varieties. PIC value ranged from 0.13 to 0.35, with diversity index of 0.22 for genotypes and from 0.06 to 50, with diversity index of 0.28 for varieties. The marker index for genotypes and varieties was 5.00 and 14, respectively. The Rp of the RAPD for genotypes and varieties was 18.87 and 30.63, respectively. Shannon's index for genotypes using RAPD markers were 4.35 and for varieties it was 5.32. For varieties, the result of AMOVA revealed that variation within groups (95.09%) was more than among groups (4.91%). In order to estimate the genetic distance among genotypes and varieties, the similarity matrix was computed with Jaccard's method. The results for SCOT, ISSR and RAPD discovered a high level of genetic diversity within the 87 genotypes (0.04 to 0.94) and

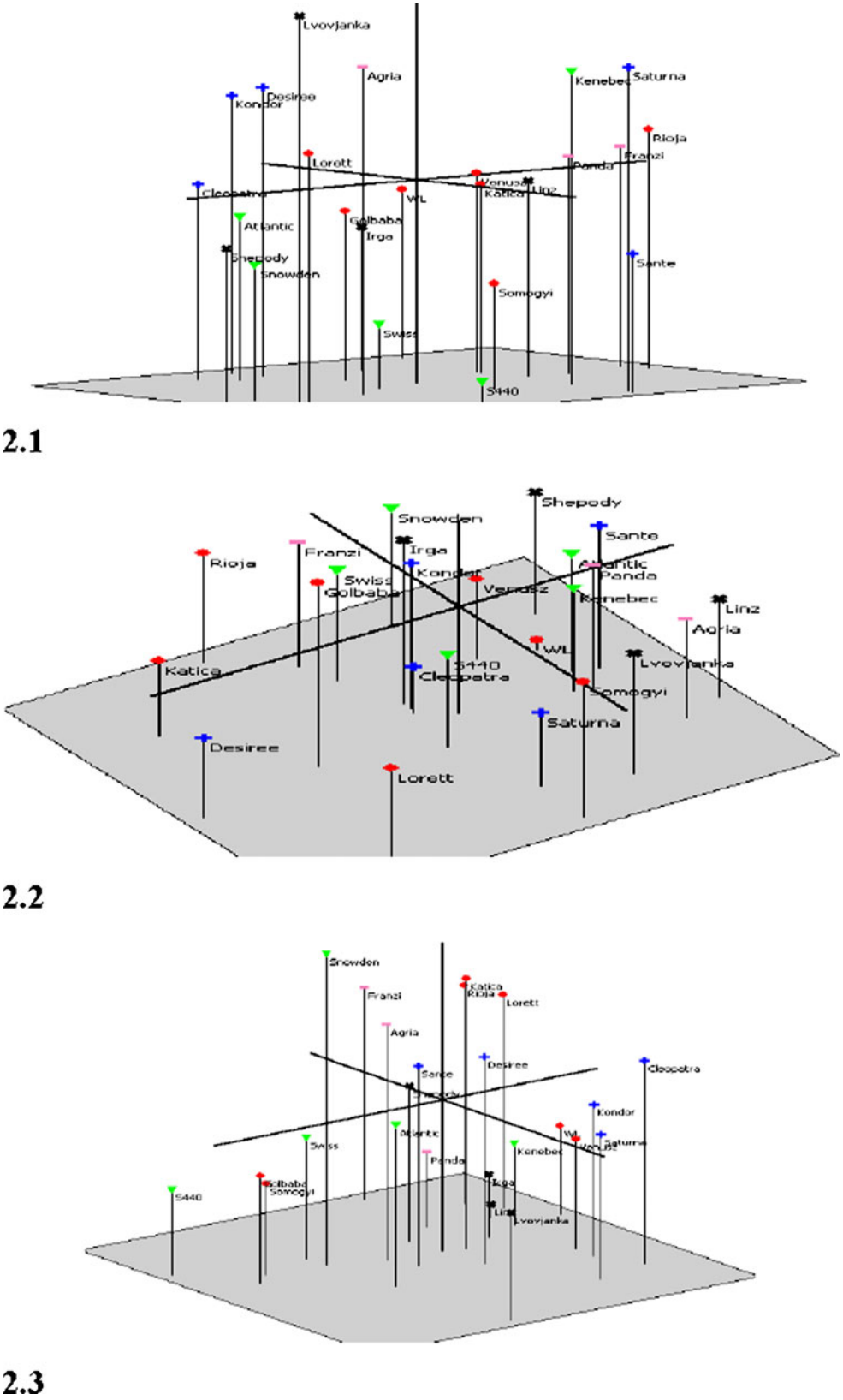

Fig. 2 Three-dimensional plot of principal coordinate analysis of 24 varieties using (**a**) SCOT, (**b**) ISSR and (**c**) RAPD analysis. WL: White Lady. The symbol represents origin of cultivars, (where, circle = Hungary, Triangle = USA, Cross up = Netherlands, Cross side = Canada, Poland, Russia and Australia, Rectangle = Germany). **a** SCOT **b** ISSR **c** RAPD

varieties (0.1 to 0.79). The rate of genetic diversity among genotypes, based on SCOT, ISSR and RAPD markers was nearly equal. The similarity of varieties that were assessed in the present study was generally low and less than that of the genotypes. The rate of genetic diversity among varieties, based on ISSR and RAPD markers was nearly equal and differed from SCOT markers (data not shown). Consensus tree with bootstrap analysis based on RAPD markers using NJ genetic distances showed diversity within the analyzed genotypes and varieties and grouped them into 12 and 10 clusters, respectively. The RAPD marker technique could identify 85 genotypes out of 87 and 22 cultivars out of 24 (Fig. 1c). The result of principal coordinate analysis was comparable to the cluster analysis (Fig. 2c). The first three most informative principle component explained 45.06% of the total variation. Comparison of phylograms created using SCOT, ISSR and RAPD markers demonstrated that only SCOT technique could distinguish all cultivars. ISSR and RAPD techniques can independently identify each cultivar except Rioja and Franzi. The clustering pattern obtained with each type of markers showed some common groups and clustered some of the varieties according to the location where they were released or according to their relationship. White Lady and Vénusz Gold successfully grouped into the same cluster based on all marker techniques (WL is the female parent of Vénusz Gold). Desiree and Cleopatra were also effectively included into the same group based on SCOT and ISSR data (Desiree is one of the parents of Cleopatra). S440 and Swiss of USA origin and Gülbaba and Irga of Hungarian and Polish origin incorporated into the same group based on all markers. S440, Swiss and Snowden from USA clusters in the same group based on SCOT and ISSR data while variety Sante forms independent clusters in SCOT-based and ISSR-based ones. Panda and Franzi originating from Germany was integrated into the same group based on SCOT data. Somogyi Kifli (HU) and Lvovjanka (RU) were classified in the same small cluster based on ISSR and RAPD data.

Discussion

The total number of amplification fragments per primer-template combinations depends on the size of the template genome, primer sequence, PCR conditions, competition between potential amplicons and base-mismatching between primer and template (Bussell et al. 2005; Williams et al. 1993; Smith and Williams 1994; Hallden et al. 1996). In the present study, the average number of scoreable bands produced per primer for genotypes using SCOTs, ISSRs and RAPDs were less than those produced for varieties. The average size of SCOT markers was larger than that of ISSR and RAPD markers. The average size of RAPD fragments was smaller than that of ISSRs (data not shown). This result is in agreement with the observation of Bussell et al. (2005). Some of the SCOT, ISSR and RAPD primers resulted in polymorphic bands which were amplified from different alleles of a given locus. As the shared absences represent the inverses of the shared presences for all individuals, we anticipate that they were different alleles of one locus and should not be scored as different characters. Scoring of different alleles of a given locus is problematic for dominant markers when plants having three or more alleles per locus are tested (Albert 2005; Strong and Lipscomb 1999).

The proportion of polymorphic markers is one of the methods for examining similarity. There is no simple relationship between similarity and taxonomic level (Bussell et al. 2005). Our results exhibited that percentage of average polymorphism within genotypes for SCOT, ISSR and RAPD primers was rather low and is on the same level (Tables 2 and 3). The percentage of average polymorphism among varieties was higher than that of genotypes and it was 61, 47 and 31 for SCOT, ISSR and RAPD, respectively. Wolfe and Liston (1998) compared 54 RAPD-based studies of genetic relationship and reported a mean of 18.6% polymorphic loci within population of cultivars (n=3) and 62% between populations or varieties of species (n=19). Another way to show the rate of similarity is Jaccard's coefficient of similarity. Our results demonstrate that high genetic diversity exists between the investigated genotypes and varieties. As the parents of genotypes are tetraploids and they very much differ in their pedigree, this range of diversity is normal. The rate of genetic diversity for genotypes was nearly the same using SCOT, ISSR and RAPD markers. Although the rate of diversity for the three marker techniques was approximately equal; we anticipate that the source of detected diversity is different, as each technique targets different regions of the genome. The rate of similarity between varieties is less than that of genotypes. The main reason for this is that the parents and origin of varieties which were used in this study are highly different. Overall Shannon index for ISSRs (4.41) was found to be similar to SCOTs (4.47) and RAPD (4.35). This indicates that the relative genetic diversity of the genotypes is similar when SCOT, ISSR and RAPD markers are used. For varieties, overall Shannon index of SCOTs (6.70) was more than ISSRs (5.87) and RAPDs (5.32). This suggested that the relative genetic diversity of the varieties is more when SCOT markers are used but it is fairly similar when ISSR and RAPD markers are used. The AMOVA analysis indicated that more than 90% of the total genetic diversity by SCOT, ISSR and RAPD markers is

distributed within groups and only a little of the diversity is attributed to differences between regions. This can be helpful during strategy development for variety collections and evaluations. This low variability between regions was reported in fig (Ikegami et al. 2009) and in Tunisian collections too (Salhi-Hannachi et al. 2005).

Many earlier reports manifested discrepancy between dendrograms when two different molecular marker techniques were used (Sonia and Gopalakrishna 2007; Arif et al. 2009). Discordance between dendograms or trees obtained using different marker types could be explained by the genetically inert nature of markers when compared to functionally active, different regions of the genome targeted by different markering techniques, level of polymorphism detected and the number of loci and their coverage of the overall genome (Souframanien and Gopalakrishna 2004). Our results promote the previous reports by clustering genotypes and varieties in different groups using different marker techniques. However, some common groups were identified between clustering patterns of each marker and similar results were reported in potato (McGregor et al. 2000; Norero et al. 2003), Shishma (Arif et al. 2009). Comparison of clustering patterns also revealed that location specificity of SCOT technique was higher than other markers because it discriminated a lot of varieties according to their relationship and location where they were released. PCoA analysis (3Dimension) from SCOT, ISSR and RAPD data showed similar results using cluster analysis (Fig. 2a, b and c).

Bootstrap values in general were low for the main clusters (data not shown). The number of markers and some biological factors which interfere with the DNA data could cause these results. Similar results were obtained for the species *Magnaporthe grisea* as well (Kumar et al. 1999; Sonia and Gopalakrishna 2007). Moreover, interpretation of bootstrap values has been a controversial topic and its reliable estimation could be obtained with sufficient polymorphic loci (Sanderson and Wojciechowski 2005; Kalinowski 2005).

In conclusion, SCOT, ISSR and RAPD generated high number of polymorphic markers which can be used in diagnostic fingerprinting studies of tetraploid potato. Based on the average percentage polymorphism, PIC, Rp, diversity index, marker index and overall Shannon index, the efficiency of SCOT for fingerprinting of varieties was more than other markers. In these terms ISSRs also had superiority over RAPD markers. The efficiency of SCOT, ISSR and RAPD markers for fingerprinting of genotypes is relatively the same. In general, these three techniques could be used in conjunction with each other for diagnostic fingerprinting of tetraploid potato. Similar results were reported by Prevost and Wilkinson (1999) and McGregor et al. (2000) in potato, Arif et al. (2009) in *Shisham* and Chen et al. (2006) in *Caldesia grandis*.

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