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# Molecular characterization of potato (*Solanum tuberosum* L.) genotypes using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers

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19 random amplified polymorphic DNA (RAPD) and five Inter-simple sequence repeat (ISSR) primers were used to characterize 35 potato accessions originating from Mexico, Europe and U.S.A, with the aim of generating information on the genetic diversity and relationship among the genotypes for better exploitation in breeding programs. A total of 222 and 82 *loci* were generated by the RAPD and ISSR primers, respectively. Both RAPD and ISSR primers revealed 81.45 and 82.98% polymorphism, respectively. Mantel test showed no correlation between RAPD and ISSR (0.27), RAPD and RAPD + ISSR (0.47) similarity matrices. However, a high level of correlation (0.74) was observed between ISSR and RAPD + ISSR similarity matrices. The ISSR marker was found to be more efficient compared to RAPD marker, thereby influencing more the consensus data. The use of consensus data generated more information related to genetic diversity in potato. RAPD and ISSR markers successfully differentiated between the potato accessions and subgrouped the genotypes based on origin. Information on genetic diversity and relationships will be useful in the selection of parents and mapping studies.

**Key words:** *Solanum tuberosum* L., random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), genetic diversity, relationships.

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most economically important crops in the world and is the fourth after rice, wheat and maize (Horton, 1987). In Mexico, potato cultivation is carried out on about 64,000 ha annually with a production of 1.7 Mt and an average

yield of 27 t/ha (FAOSTAT, 2007). Potatoes are grown in the following principal states: Chihuahua, Coahuila, Guanajuato, Jalisco, Michoacan, Nuevo Leon, Sinaloa, Sonora and Zacatecas (SAGARPA, 2010). Although Mexico is not a center of origin for potatoes, there exists

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great diversity of potato germplasm. Potato varieties in Mexico can be distinguished into three groups: genotypes from Holland, USA and varieties improved by Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP) (Ferroni, 1981). The first group represents 50% of cultivated varieties (Alpha), the second 38% and the last about 8%. The two principle centers of diversity and production of native potatoes in Mexico are Nevado de Toluca and Pico de Orizaba (Ugent, 1968). Information on the genetic diversity and relationships existing among the potato collections in Mexico is limited, which impedes their use in breeding programmes (Becerra and Paredes, 2000).

Identification of crop plants, studies in their genetic diversity and relationships is crucial for the development of breeding programmes. Historically, this has been achieved in potato through the use of morphological markers (Hijmans and Spooner, 2000). However, these markers are limited, complex and greatly influenced by the environment (Semagn, 2006). Although biochemical markers are limited and influenced by the condition of the plant, they have also been used to describe potato cultivars (Douches and Ludlam, 1991). To complement these methods and address some of the limitations, molecular markers have been developed, among which there are those based on hybridization and PCR methods (Semagn, 2006). RFLP is a molecular marker system based on hybridization and has been used extensively to construct genetic maps (Bonierbale et al., 1988), conduct phylogenetic and diversity studies in potato (Görg et al., 1992; Ritter, 2000). Nevertheless, it is time consuming, expensive and requiring high level technical support (Karp et al., 1997).

The PCR based markers developed include RAPD and ISSR (Reddy et al., 2002). These markers are simple, rapid, economic, requiring minimum level technical support and do not require prior knowledge of the genome sequence (Karp et al., 1997). RAPDs are a technique based on the amplification of discrete regions of the genome by polymerase chain reaction (PCR) with short oligonucleotide primers of arbitrary sequence (Williams et al., 1990). Although limited in reproducibility, being a dominant marker and showing problems with homology, the technique has been useful in construction of genetic maps (Perez et al., 1999), analyzing genetic diversity (Orona-Castro et al., 2006; Yasmin et al., 2006) and conducting taxonomy and phylogenetic studies in potato (Sun et al., 2003). ISSR amplification uses SSR primers (anchored or nonanchored) to amplify DNA sequences between two inverted SSRs made up of the same sequence. ISSR was first used by Zietkiewicz et al. (1994) to rapidly differentiate between closely related individuals. The technique combines most of the benefits of AFLP and microsatellite analysis with the universality of RAPD (Bornet and Branchard, 2001). ISSR markers have been successfully used in potato for fingerprinting (Bornet and Branchard, 2001) and diversity studies (Bornet et al., 2002).

There is limited use of the great potato genetic diversity existing in Mexico because of available limited genetic information concerning these genetic materials. Genetic information is paramount for the conservation, improvement and use of genetic resources. In this paper, we characterize potato genotypes in Mexico using RAPD and ISSR molecular markers with the aim of generating information on genetic diversity and relationships for better exploitation in potato breeding programs.

## MATERIALS AND METHODS

35 accessions of potato were obtained from various states in Mexico and germplasm bank at the Autonomous University of Chapingo (Table 1). Tuber sections were made from five randomly selected tubers for analysis.

### Deoxyribonucleic acid (DNA) extraction

The extraction of DNA was conducted according to a protocol by Sambrook et al. (1989) with modifications. Tuber sections weighing 0.3 g were obtained and ground in liquid nitrogen to form fine powder. The sample was then placed in a microfuge tube (Eppendorf) of 1.5 mL with 600  $\mu$ L of extraction buffer (20 mL Tris-HCl 1 M, pH 8.0; 20 mL EDTA 0.5 M, pH 8.0; 20 mL NaCl 5 M; 35  $\mu$ L  $\beta$ -mercaptoetanol; 40 mL sodium dodecyl sulfate 20%), then it was incubated at 65°C for 10 min, with occasional inversion of the tubes. After that, 200  $\mu$ L of potassium acetate 5 M was added, mixed by inversion and incubated in ice for 30 min, then it was centrifuged for 8000  $\times$  g for 10 min at room temperature, and the supernatant was transferred to another tube containing 700  $\mu$ L of cold isopropanol (-20°C). It was mixed by inversion and incubated at -20°C for 30 min and centrifuged for 5 min at 8000  $\times$  g, at room temperature. The supernatant was eliminated; the precipitate was recovered and dissolved in 200  $\mu$ L of solution (Tris-HCl 50 mM, EDTA-Na<sub>2</sub> 10 mM, pH 8.0).

To eliminate RNA, 2  $\mu$ L of RNase A (Invitrogen, U.K) were added and incubated at 37°C for 1 h. 20  $\mu$ L of sodium acetate 3 M and 200  $\mu$ L of isopropanol were added, mixed by inversion and left to precipitate at -20°C for 2 h. The mixture was centrifuged at 8000  $\times$  g for 5 min at room temperature. The supernatant was eliminated and the precipitate washed with 300  $\mu$ L of 70% ethanol. The pellet was dried and dissolved in 100  $\mu$ L of TE buffer (Tris-HCl 10 mM, EDTA-Na<sub>2</sub> 1 mM, pH 8.0) at 4°C.

The concentration of DNA was quantified using a spectrophotometer Genesys 10 uv Scanning® (Thermo Scientific) and the quality was verified by electrophoresis in agarose gel at 0.8% (w/v). The DNA was used in subsequent PCR reactions.

### Random amplified polymorphic DNA (RAPD) reaction conditions

A total of 25 RAPD primers of the series A, B and D Operon® were tested (Operon technologies Inc, Alameda, CA, USA). 19 primers that showed polymorphism and clear RAPD band patterns were selected (Table 2). The PCR reactions were realized in a thermocycler Techne®. The reaction mix was carried out in a total volume of 25  $\mu$ L, which included: 4.2  $\mu$ L of double distilled sterile water, 10  $\mu$ L of dNTPs (500  $\mu$ M), 2.5  $\mu$ L of buffer 10X Tris-HCl 750 mM, pH 8.8; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 200 mM; Tween 20 at 1% (v/v); 1.0  $\mu$ L of MgCl<sub>2</sub> (50 mM); 3.0  $\mu$ L of primer at a concentration of 10 pM; 0.3  $\mu$ L of Taq DNA polymerase enzyme (Fermentas, U.S.A)

**Table 1.** Geographic origin of genotypes used in the study.

Number	Genotype	Origin
1	CAMBRAY ROSA MORELOS	México
2	CRIOLLA EDO MEX	México
3	279 - 17	U.S.A
4	286 -31	U.S.A
5	263 -68	U.S.A
6	314 -47	U.S.A
7	ALFA GRANDE	Europe
8	ATLANTIC	U.S.A
9	ARMADA	Europe
10	FIANNA	Europe
11	LB5 -5	U.S.A
12	215 - 77	U.S.A
13	286 -60	U.S.A
14	306 -54	U.S.A
15	277 -35	U.S.A
16	263 -83	U.S.A
17	286 -35	U.S.A
18	286 -40	U.S.A
19	LB5 -92	U.S.A
20	286 -9	U.S.A
21	MONTESERRAT	Europe
22	254 -83	U.S.A
23	MUNDIAL	Europe
24	176 -62	U.S.A
25	GIGANT	Europe
26	ALFA CHICA	México
27	CAMBRAY BLANCA EDO MEX	México
28	TOLLOCAN	México
29	MOCHIS DF	México
30	CAMBRAY ROSA DF	México
31	CAMBRAY DF BLANCA	México
32	CRIOLLA BLANCA PUEBLA	México
33	CRIOLLA ROSA PUEBLA	México
34	PAPA CHICA	México
35	CAMBRAY ROSA EDO MEX	México

at a concentration of  $5\text{U } \mu\text{L}^{-1}$ ; and  $4.0\text{ } \mu\text{L}$  of genomic DNA at a concentration of  $10\text{ ng } \mu\text{L}^{-1}$ .

The reaction conditions were: one cycle at  $94^{\circ}\text{C}$ , 2 min; 38 cycles ( $94^{\circ}\text{C}$  for 30 s,  $40^{\circ}\text{C}$ , for 30 s,  $72^{\circ}\text{C}$  for 90 s); with an extension of  $72^{\circ}\text{C}$  for 2 min. The amplified fragments were separated by electrophoresis using agarose gel at 1.2% (w/v) with TAE buffer (40 mM Tris-acetate, pH 7.6; 1 mM  $\text{Na}_2\text{EDTA}$ ), for 1 h at 120 V. The gels were stained with ethidium bromide ( $0.5\text{ mg mL}^{-1}$ ) for 15 min; the excess stain was removed by rinsing in distilled water for 5 min and photographed under UV light. The amplification reactions were repeated at least twice.

#### Inter-simple sequence repeat (ISSR) reaction conditions

For the ISSR reactions, five primers were used (Table 3). The

amplification reactions were performed in a similar reaction volume and with similar reagents to that used for RAPDs except for the reaction conditions,  $94^{\circ}\text{C}$  for 5 min, 35 cycles at  $94^{\circ}\text{C}$  for 30 s, specific annealing temperature for 45 s and  $72^{\circ}\text{C}$  for 2 min and a final extension at  $72^{\circ}\text{C}$  for 10 min. The annealing temperature ranged from 45 to  $58^{\circ}\text{C}$  and the cycles were reduced to 30. The amplified fragments were separated by electrophoresis using agarose gel at 1.5% (w/v) with TAE buffer (40 mM Tris-acetate, pH 7.6; 1 mM  $\text{Na}_2\text{EDTA}$ ), for 1 h at 120 V. The gels were stained with ethidium bromide ( $0.5\text{ mg mL}^{-1}$ ) for 15 min; the excess stain was removed by rinsing in distilled water for 15 min and photographed under UV light. The amplification reactions were also repeated at least twice.

The materials were compared based on similarities and differences in band patterns. A value of 1 was assigned for the presence and 0 for the absence of bands. A data matrix was constructed in a page of calculations for obtaining a dendrogram of relations between the accessions with the coefficient of Jaccard (Jaccard, 1908) and with the Unweighted Pair Group Method using Arithmetic Averages) UPGMA. An analysis of resampling was done (Bootstrapping, 1000 repetitions) with the aim of obtaining consistent numeric data of the tree generated by the statistical programme Free Tree version 0.9.1.50 (Hampl et al., 2001). The Mantel test (Mantel, 1967) was conducted for correlation between similarity matrices, using the programme Mantel Nonparametric Test calculator for Windows Version 2.0 (1999 to 2007) (Liedloff, 1999). Nonmetric Multi-Dimensional scaling was carried out using NTSYS-pc version 2.1 (Rohlf, 2000).

## RESULTS AND DISCUSSION

### Random amplified polymorphic DNA (RAPD)

19 out of the 24 RAPD primers tested of the series RAPDs; A, B and D Operon were amplified (Table 2). The mean of the detected polymorphism by RAPD primers was 82.9%. Primers A19, D04 and D10 revealed 100% polymorphism. The mean number of bands revealed by a RAPD primer was 11.15, with oscillations between 4 and 17. A total of 222 *loci* were studied and 184 were polymorphic.

The analysis based on RAPD permitted the estimation of genomic similarities and differences. Figure 1A shows band patterns obtained by RAPD primer D10, which showed differences among the potato genotypes evaluated.

Figure 2A shows a dendrogram of relationships of similarities between different genotypes of potato using RAPD markers. The results show the formation of eight groups at a Jaccard coefficient of 0.60: Tollocan formed the first group, LB5-5 the second group, the third group included Monteserrat, the fourth group was 306-54 and the fifth group was made up of a homogeneous group with Cambray Rosa Morelos and Cambray Rosa Edo Mex, which probably represent the same genotype.

Gigant formed the sixth group and 176-62 the seventh group. The eighth group included the rest of the accessions. This big group consisted of two clusters; the first cluster was divided into 5 sub-groups: the first sub group included Mexican white fleshed varieties (Cambray Blanca, Mochis, Criolla Blanca) and Atlantic which is from

**Table 2.** List of RAPDs primers and their sequences, number of amplified products, monomorphism and polymorphism.

RAPD Primer	Sequence (5'-3')	Amplification products	Monomorphism (%)	Polymorphism (%)
A02	TGC CGA GCT G	14	21.43	78.57
A3	AGT CAG CCA C	14	14.28	85.72
A05	AGG GGT CTT G	12	25	75
A13	CAG CAC CCA C	10	40	60
A19	CAA ACG TCG G	13	0	100
A09	GGG TAA CGC C	7	28.57	71.43
D05	TGA GCG GAC A	12	16.66	83.33
D07	TTG GCA CGG G	10	30	70
D04	TCT GGT GAG G	4	0	100
D10	GGT CTA CAC C	17	0	100
D11	AGC GCC ATT G	11	27.27	72.72
D18	GAG AGC CAA C	13	23.07	76.92
D20	ACC CGG TCA C	12	16.66	83.33
D01	ACC GCG AAG G	9	11.11	88.88
D03	GTC GCC GTC A	15	6.66	93.33
D06	ACC TGA ACG G	9	11.11	88.88
D09	CTC TGG AGA C	11	9.09	90.9
D02	GGA CCC AAC C	10	20	80
B05	TGC GCC CTT C	9	22.22	77.77
	Mean	11.15	17	82.98

**Table 3.** List of ISSR primers and their sequences, annealing temperature, number of amplified products, monomorphism and polymorphism.

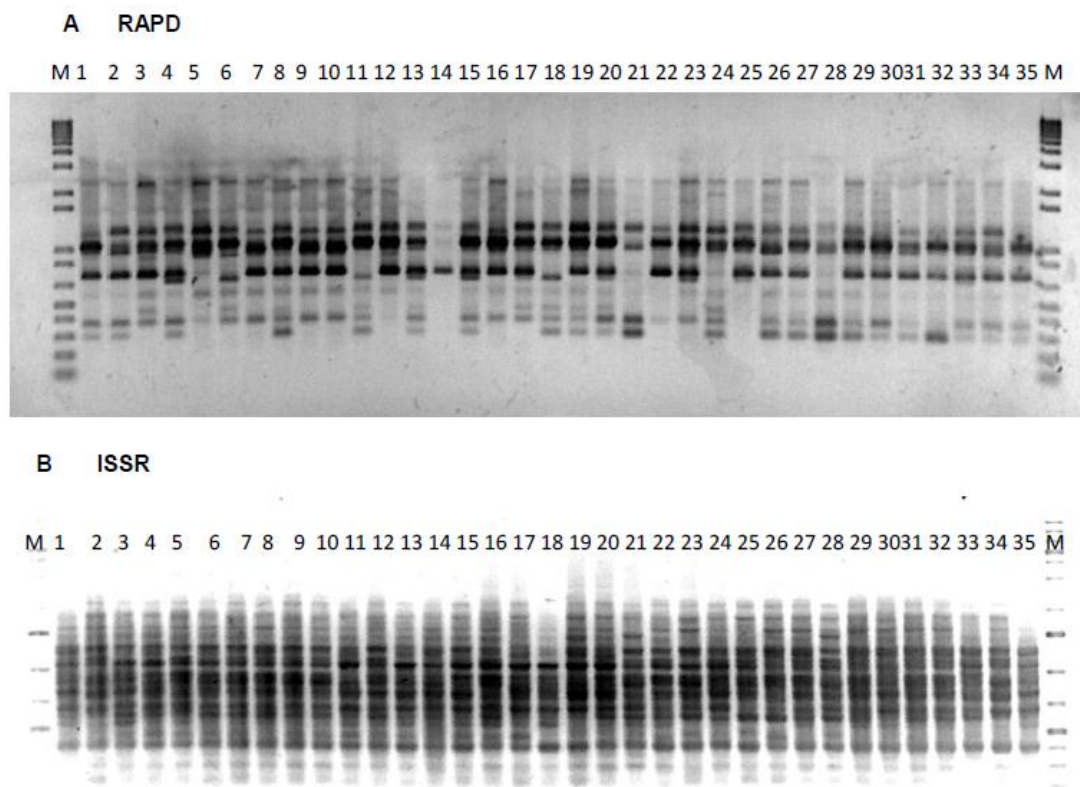
ISSR Primer	Sequence (5'-3')	TA	Amplification products	Mornomorphism (%)	Polymorphism (%)
P1	GAGCAACAACAACAACA	48	14	14.29	85.71
P2	CTGAGAGAGAGAGAGAG	58	18	22.23	77.77
P3	AGAGAGAGAGAGAGAGTG	45	21	14.29	85.71
P4	ATGATGATGATGATG	40	14	28.58	71.42
P5	AGAGAGAGAGAGAGAG	45	15	13.33	86.66
	Mean		16.4	18.54	81.45

U.S.A. The second consisted of European varieties, Alfa Grande, Fiana and Armada. The third, clones were from U.S.A (286-31, 215-77, 263-68). The fourth were, 314-47, 279-17, Criolla Edo Mex and Papa Chica. The fifth consisted of anthocyanin coloured stem Mexican varieties (Criolla Rosa Puebla, Alfa Chica, Cambray Rosa DF and 254-83). The second cluster comprised of two sub groups: Mundial, the only variety from Europe, which was also relatively more divergent at 0.65, formed the first sub group. The second sub group included clones from U.S.A. (LB5-5, 286-35, 277-35, 263-83, 286-60, 286-40 and 286-9).

Average genetic similarity between pairs of plants was  $0.61 \pm 0.02$ , with a range of 0.46-0.87. The highest similarities were obtained among European varieties. Fiana and Armada recorded 0.87, Alfa Grande and Armada, 0.83, Alfa Grande and Fiana, 0.81 and Cambray

Blanca DF and Mochis 0.81. The lowest similarities were recorded between LB5-5 and Tollocan (0.46) followed by Cambray Rosa Morelos (0.48), and Gigant and Tollocan (0.48). Tollocan was the most divergent at a similarity coefficient of (0.53) from the rest of the accessions (Figure 2A).

Multi-dimensional analysis (MDA) performed on 35 accessions of potato and 19 RAPD markers clearly divided them into two groups (Figure 3A): The first cluster was made up of clones from U.S.A (LB5-5, LB-92, 306-54, 263-83, 286-35, 286-60, 277-35, 286-31, 286-40 and 286-9). This cluster also included all of the 286 series clones. The second cluster included Mexican varieties, European varieties and the rest of the clones from U.S.A (Cambray Blanca Edo Mex, Criolla Blanca Puebla, Cambray DF Blanca, Mochis, Cambray Rosa DF, Cambray Rosa Morelos, Cambray Rosa Edo Mex, Criolla



**Figure 1.** Gel electrophoresis of amplification products obtained with RAPD primers D10 (A) and ISSR primer P2 (B) in 35 (*Solanum tuberosum* L.) accessions as listed in Table 1.

Edo Mex, Criolla Rosa Puebla, Papa Chica and Alfa Chica, Tollocan, Fiana, Armada, Alfa Grande, Mundial, Gigant, Monteserrat, Atlantic, 279-17, 263-68, 215-77, 314-47, 254-83, and 176-62).

#### Inter-simple sequence repeat (ISSR)

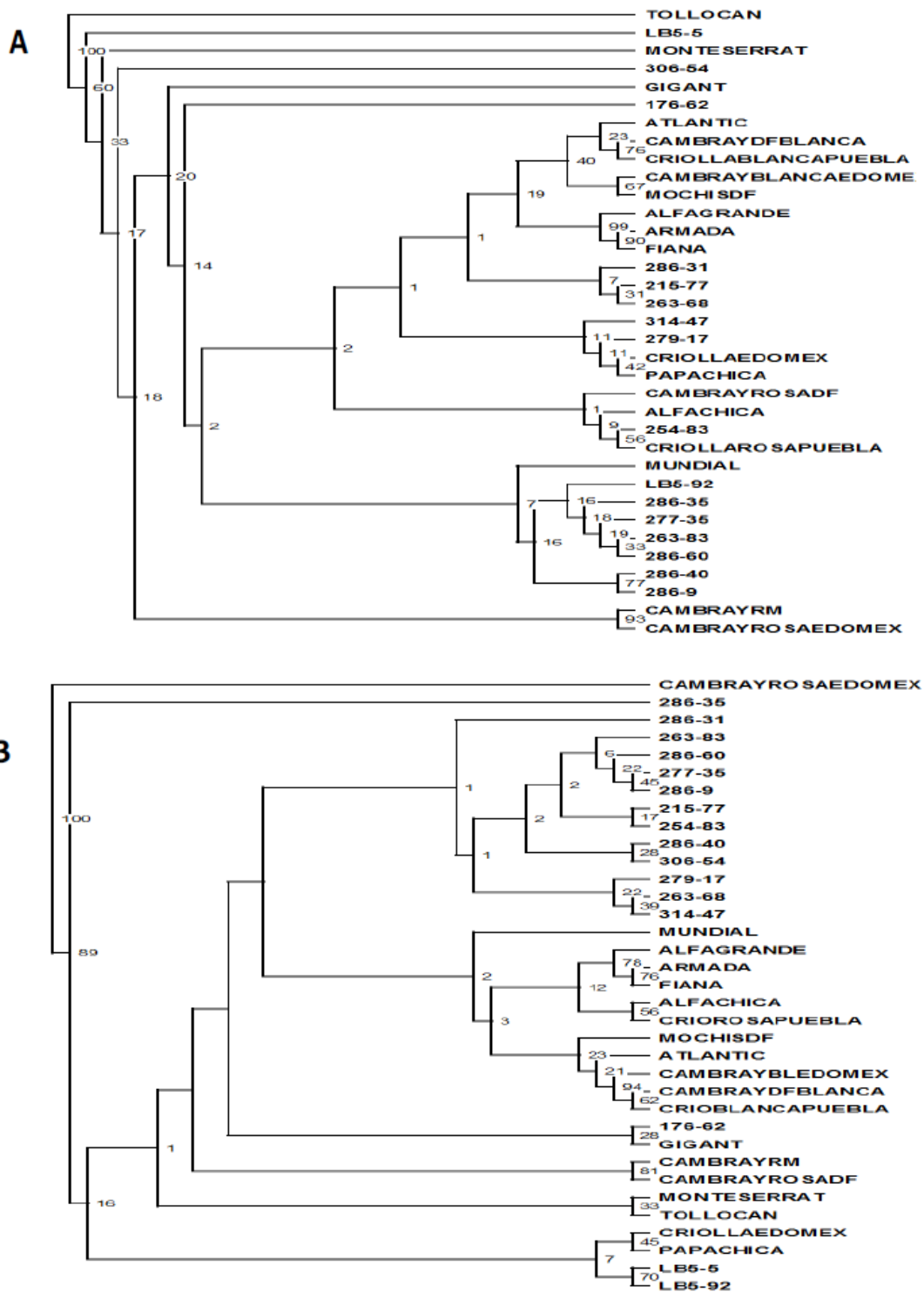
Five ISSR primers were amplified (Table 3). The mean of the detected polymorphism by ISSR primers was 81.4%, which was comparable to the mean obtained by RAPD (82.9). The mean number of bands revealed by ISSR primers was 16.4, with oscillations between 14 and 21. A total of 82 *loci* were studied and 66.7 (81.4%) were polymorphic.

The analysis based on ISSR techniques permitted the estimation of genomic similarities and differences. Figure 1B shows band patterns obtained by ISSR primer P2, which showed differences among the potato genotypes evaluated.

Figure 2B shows a dendrogram of relationships of similarities between different genotypes of potato using ISSR markers. The results show the formation of seven groups at a Jaccard coefficient of 0.70: Cambray Rosa Edo Mex formed the first group and 286-35 the second. The third group consisted of two homogenous sub

clusters: the first made of LB5-5 and LB5-92 and the second Criolla Edo mex and Papa Chica. Three homogeneous clusters of Monteserrat and Tollocan, Cambray Rosa Morelos and Cambray Rosa DF, Gigant and 176-62, formed the 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> groups, respectively. Incidentally, Cambray Rosa Edo Mex formed the main out layer from the rest of the genotypes (0.47). The seventh group consisted of the rest of the accessions. This group was divided into two clusters; the first cluster consisted of potato clones from U.S.A (286-35, 286-31, 263-83, 286-60, 277-35, 286-9, 215-77, 254-83, 286-40, 306-54, 279-17, 263-68 and 314-47). The second cluster was made up of four sub groups; the first formed by Mundial, the second sub group consisted of accessions of European varieties (Alfa Grande, Armada, Fiana). Alfa Chica and Criolla Rosa Puebla which are Mexican anthocyanin colored stem varieties formed sub group III. The fourth sub group consisted of Mexican white fleshed varieties Cambray Blanca Edo Mex, Criolla Blanca Puebla, Cambray DF Blanca, Mochis and Atlantic variety from U.S.A. The formation of the potato clone sub cluster from U.S.A was very distinct with the ISSR marker compared to RAPD.

The highest similarity was recorded between Cambray DF Blanca and Criolla Blanca Puebla (0.95), Cambray



**Figure 2.** Dendrogram generated from RAPD (A), ISSR (B) and consensus data RAPD + ISSR (C) using Jaccard similarity coefficient.

Blanca Edo Mex and Criolla Blanca Puebla (0.94), Cambray Blanca Edo Mex and Cambray DF Blanca (0.92). The European varieties also recorded high

similarity values: Fiana and Armada (0.92), Alfa Grande and Fiana (0.88) and Alfa Grande and Armada (0.86). The lowest similarity was obtained among Cambray Rosa

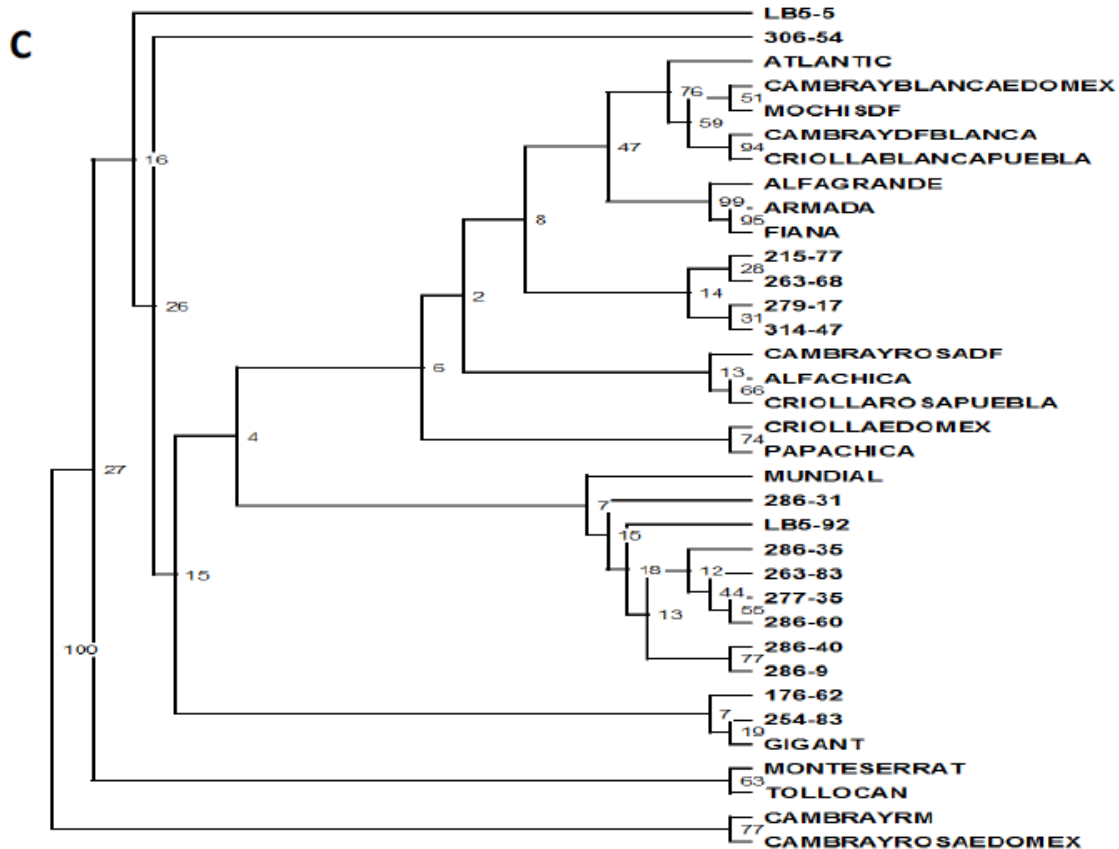


Figure 2. Contd.

Edo Mex and Monteserrat (0.40), Cambray Rosa Edo Mex and 263-68 (0.42) and Cambray Rosa Edo Mex and Armada (0.42).

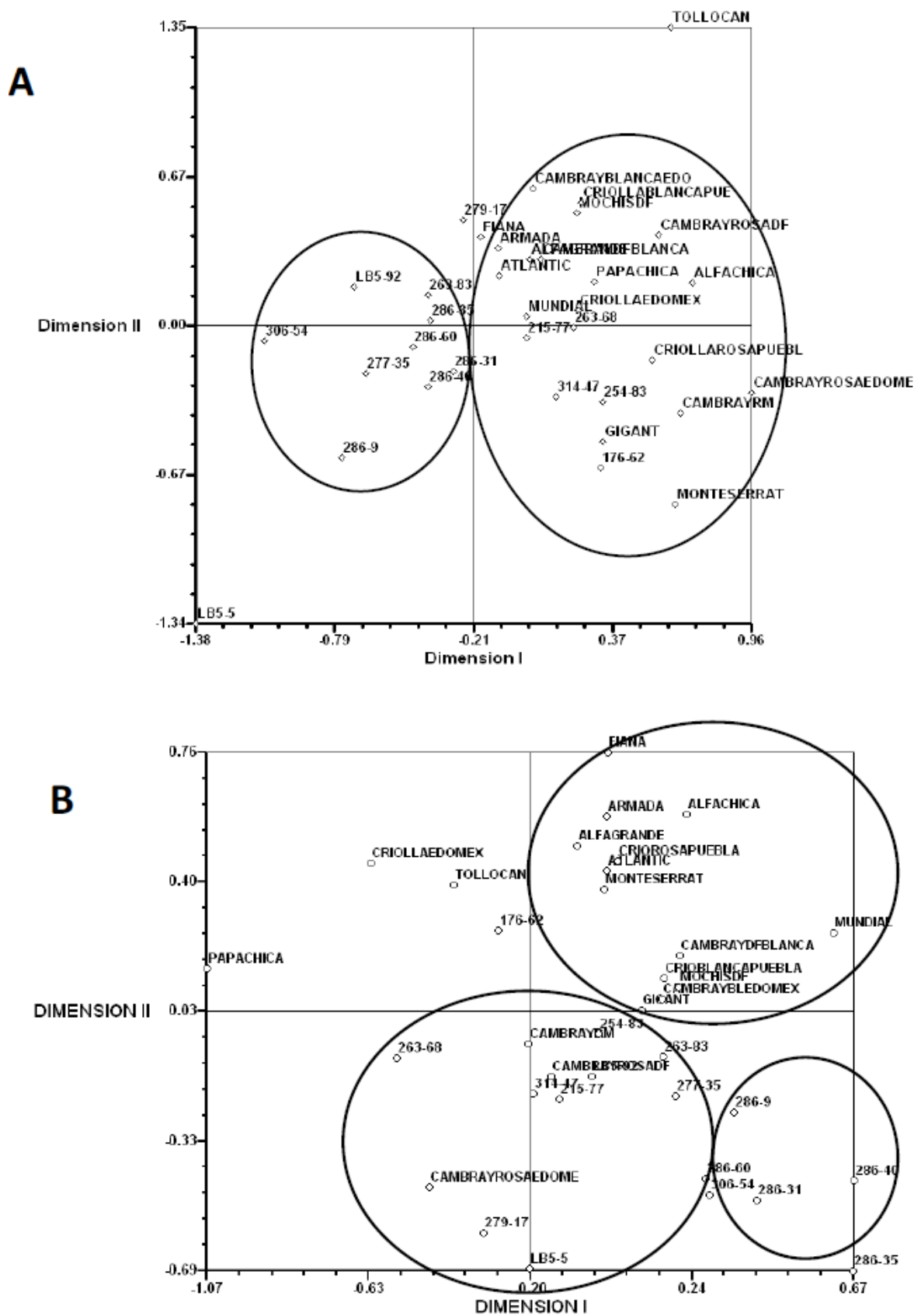
Multi-dimensional analysis (MDA) performed on 35 accessions of potato and 5 ISSR markers clearly divided them into three distinct groupings (Figure 3B): the first cluster consisted of Cambray Rosa varieties from Mexico and clones from U.S.A (LB5-5, 279-17, 263-68, 254-83, 263-83, 277-35, 215-77, 314-17, LB5-92, 254-83, and 176-62). The second cluster was made up of all the European varieties and Mexican white skinned varieties (Fiana, Armada, Alfa Grande, Alfa Chica, Monteserrat, Mundial, Cambray DF Blanca, Criolla Blanca Puebla, Mochis, Cambray Blanca Edo Mex and Gigant) apart from Criolla Rosa Puebla which is red skinned and Atlantic from U.S.A. The third cluster was mostly made up of the 286 series from U.S.A (286-9, 286-60, 286-31, 286-40, 286-35 and 306-54).

### Combined analysis

Figure 2C shows a dendrogram of relationships of similarities between different genotypes of potato using a combined analysis with RAPDs and ISSR markers. The

results show the formation of six groups. The first group consisted of a homogenous group of Cambray Rosa Morelos and Cambray Rosa Edo Mex. Monteserrat and Tollocan formed the second group. The third group was made up of LB5-5, the fourth 306-54, the fifth 176-62, 254-83 and Gigant. The sixth group consisted of the rest of the accessions. This group was divided into two clusters. The first cluster consisted of a first sub group, made up of Atlantic from U.S.A and Mexican white fleshed varieties (Cambray Blanca Edo Mex, Mochis, Cambray DF Blanca and Criolla Blanca Puebla). The second consisted of European varieties (Alpha Grande, Armada and Fiana), and the third was made up of clones from U.S.A (215-77, 263-68, 279-17 and 314-47). The fourth sub group consisted of Mexican anthocyanin pigmented stem varieties Cambray Rosa DF, Alfa Chica, and Criolla Rosa Puebla. The fifth sub group consisted of Mexican red skinned tuber varieties, Criolla Edo Mex and Papa Chica. The second cluster was made up of Mundial as the first sub group and the rest of the clones (LB5-92, 286-35, 286-83, 277-36, 286-60, 286-40 and 286-9), from U.S.A formed the second sub group. The combined analysis clearly grouped the 286 series clones from U.S.A into this second subgroup.

The highest similarity was recorded between



**Figure 3.** Scores plot of the Multi Dimensional Analysis generated from RAPD (A), ISSR (B) and consensus data RAPD + ISSR (C) of all the 35 potato genotypes.



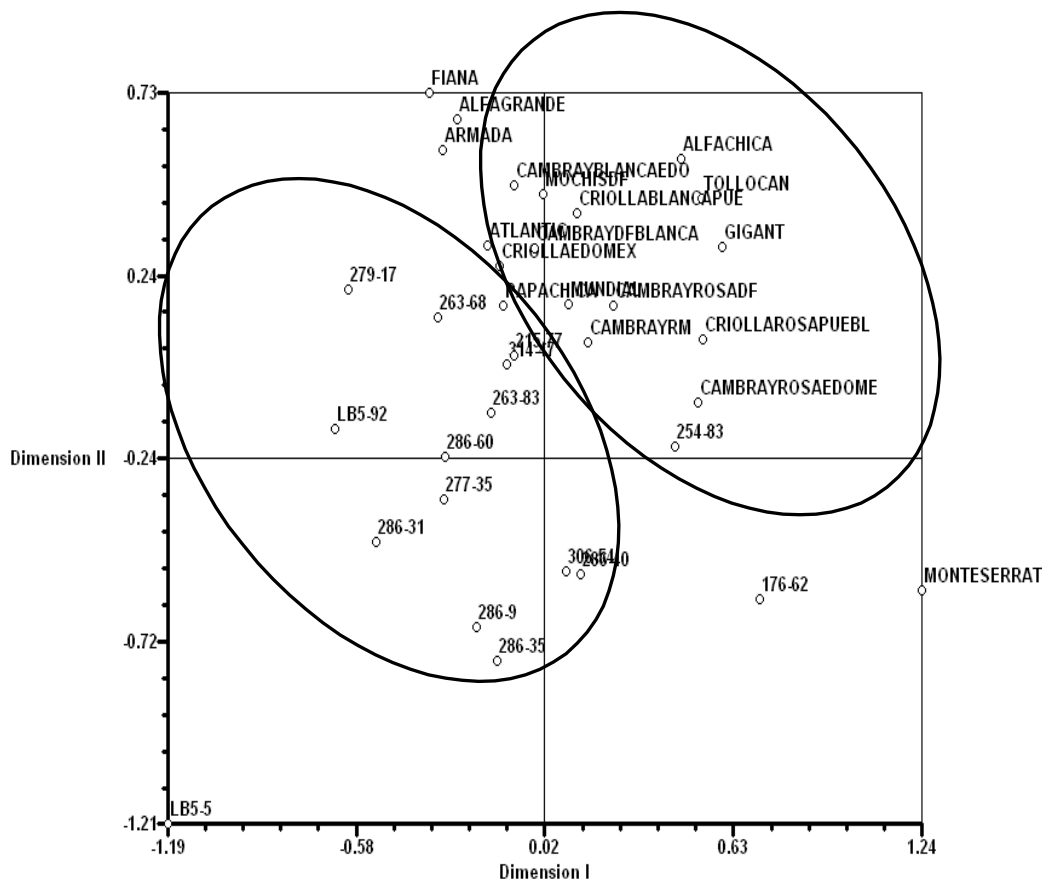


Figure 3. Contd.

European varieties: Fiana and Armada (0.88), Alfa Grande and Armada (0.84), Alfa Grande and Fiana (0.83) and Mexican white fleshed varieties: Cambray DF Blanca and Criolla Blanca Puebla (0.87), Cambray Blanca Edo Mex and Criolla Blanca Puebla (0.82) and Cambray Blanca Edo Mex and Cambray DF Blanca (0.80). The lowest similarity was obtained among Cambray Rosa Edo Mex and Tollocan (0.48), Cambray Rosa Edo Mex and LB5-5 (0.48) and Cambray Rosa Edo Mex and Mundial (0.49).

Multi-Dimensional Analysis (MDA) performed on 35 accessions of potato and 24 RAPD and ISSR markers clearly divided them into two distinct groups (Figure 3C). The first cluster was made up of potato clones from U.S.A. This cluster contained a defined subgroup of the 286 series potato clones. The second cluster consisted of all the potato varieties. Similar representation is shown in Figure 4 for three dimensional analyses.

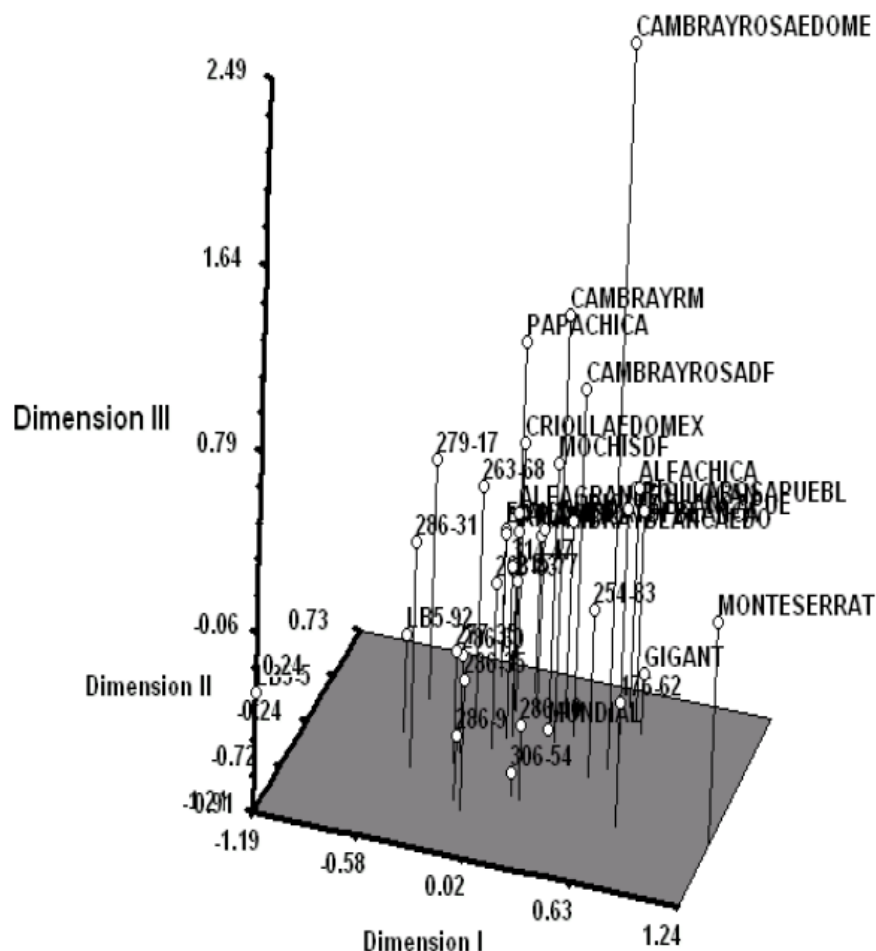
## DISCUSSION

The MD analysis provided additional information for genetic relationships among the potato accessions

studied. Cultivar identification and genetic relationships using RAPD markers in potato is well documented (Mori et al., 1993; Charchravarty et al., 2003; Orona-Castro et al., 2006; Yasmin et al., 2006 and Rocha et al., 2010).

In the present study, high polymorphism levels were detected among potato genotypes using ISSR markers. In similar studies, the ISSR makers were quite promising in the identification of potato cultivars (Prevost and Wilkinson, 1999; Borner et al., 2002).

The Mantel test (Mantel, 1967) carried out between RAPD and ISSR similarity matrices showed a positive relationship between the data but a low correlation (0.27). Previous studies of genetic diversity using PCR based molecular markers reported similar results. Rocha et al. (2010) and Ghislain et al. (2006), observed low correlation between RAPD and SSR. Similarly, Milbourne et al. (1997), obtained low correlation between RAPD, AFLP and SSR. The reason for these findings could be due to the kind of information obtained by different molecular markers since different molecular markers amplify different repetitive and non-repetitive regions of the genome (Ghislain et al., 2006). Further analysis between RAPD and RAPD + ISSR showed low correlation (0.47) and ISSR and RAPD + ISSR showed a



**Figure 4.** Tridimensional analysis generated from consensus data RAPD + ISSR of all the 35 potato genotypes.

high correlation (0.74). With only 5 primers, ISSR marker achieved comparable polymorphism (81.45%) to RAPD DNA marker (82.98%). The ISSR molecular marker has been reported to be powerful in analyzing genetic diversity in potato (Bornet et al., 2002), and in relation to RAPD marker in other plants (Rus-Kortekaas et al., 1994; Nagaoka and Ogihara, 1997; Raina et al., 2001). This may elucidate the high correlation between ISSR and RAPD + ISSR consensus data. The use of complimentary approaches, such as using both RAPD and ISSR data may provide more accurate information on genetic diversity.

Generally, the potato genotypes formed subgroups related to origin, probably due to shared parentage in breeding programs within a given region. Clustering of potato genotypes based on origin has been reported by several authors. Esfahani et al. (2009) reported clustering of potato genotypes based on origin from Europe and North America. Similarly, Bornet et al. (2002) discriminated potato genotypes based on origin from Europe and Argentina.

## Conclusion

In conclusion, RAPD and ISSR markers successfully discriminated between the 35 different potato accessions used in the study. Consensus data provided more reliable information related to the genotypes. The results from this study will be useful for assisting in the selection of parental combinations for developing progenies with maximum genetic variability for genetic mapping or further selection.

## Conflict of Interests

The authors have not declared any conflict of interests.

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## Abbreviations

**ISSR**, Inter-simple sequence repeat; **Ta**, annealing temperature; **RFLPs**, restriction fragment length polymorphism; **PCR**, polymerase chain reaction; **RAPD**, random amplified polymorphic DNA; **SSR**, simple sequence repeats; **AFLP**, amplified fragment length polymorphism.

## REFERENCES

- Becerra VV, Paredes CM (2000). Use of biochemical and molecular markers in the study of genetic diversity. *Agric. Téc. (Chile)* 60(3):270-281.
- Bonierbale M, Plaisted RL, Tanksley SD (1988). RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. *Genetics* 120:1095-1103.
- Bornet B, Branchard M (2001). Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. *Plant Mol. Biol. Rep.* 19:209-215.
- Bornet B, Goraguer F, Joly G, Branchard M (2002). Genetic diversity in European and Argentinian cultivated potatoes (*Solanum tuberosum* subsp. *tuberosum*) detected by inter-simple sequence repeats (ISSRs). *Genome* 45(3):481-484.
- Douches DS, Ludlam K (1991). Electrophoretic characterization of North American potato cultivars. *Am. Potato J.* 68:767-780.
- Esfahani ST, Shiran B, Gholamreza B (2009). AFLP markers for the assessment of genetic diversity in European and North American potato Varieties cultivated in Iran. *Crop Breed. Appl. Biotechnol.* 9:75-86.
- FAOSTAT (2007). Food and Agriculture Organization of the United Nations, FAOSTAT database. 13 Feb. 2007. <http://faostat.fao.org/site/339/default.aspx>.
- Ferroni MA (1981). The potential of potato for food and as a source of income. National Institute for Agricultural Investigation, Regional cooperative potato programe. Toluca, Mexico State, Mexico. P.30 (Special Publication No. 1.).
- Ghislain M, Andrade D, Rodríguez F, Hijmans RJ, Spooner DM (2006). Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja Group using RAPDs and nuclear SSRs. *Theor. Appl. Genet.* 113:1515-1527.
- Görg R, Schachtschabel U, Ritter E, Salamini F, Gebhardt C (1992). Discrimination among 136 tetraploid potato varieties by fingerprints using highly polymorphic DNA markers. *Crop Sci.* 32:815-819.
- Hampel V, Pavlicek A, Flegr J (2001). Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program Free Tree: application to trichomonad parasites. *Int. J. Syst. Evol. Microbiol.* 5:731-735.
- Hijmans RJ, Spooner DM (2001). Geographic distribution of wild potato species. *Am. J. Bot.* 88(11):2101-2112.
- Horton D (1987). Potatoes. West View Press, Boulder, Colorado, U.S.A. P. 164.
- Jaccard P (1908). Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaudoise Sci. Nat.* 44:223-70.
- Karp A, Kresovich S, Bhat KV, Ayada WG, Hodgkin T (1997). Molecular tools in plant genetic resources conservation: a guide to the technologies. IPGRI Tech. Bull. No. 2.
- Liedloff AC (1999). Mantel Nonparametric test Calculator. Version 2.0. School of National Resource Sciences, Queensland University of Technology, Australia.
- Mantel N (1967). The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27:209-220.
- Milbourne D, Meyer R, Bradshaw JE, Baird E, Bonar N, Provan J, Powell W, Waugh R (1997). Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. *Mol. Breed.* 3:127-136.
- Mori M, Hosaka K, Umemura Y, Kaneda C (1993). Rapid identification of Japanese potato cultivars by RAPDs. *Jpn. J. Genet.* 63:167-174.
- Nagaoka T, Ogihara Y (1997). Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theor. Appl. Genet.* 94:597-602.
- Orona-Castro F, Pecina-Quintero V, Rocha-Peña A, Cadena-Hinojosa MA, Octavio Martínez de la Vega O, Almeyda-León IH (2006). Molecular characterization of commercial and elite potato genotypes. *Agric. Téc. Méx.* 2(32):171-180.
- Perez F, Menedez A, Dehae P, Quiros CF (1999). Genomic structural differentiation in *Solanum* comparative mapping of the A- and E-genomes. *Theor. Appl. Genet.* 98:1183-1193.
- Prevost A, Wilkinson MJ (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet.* 98:107-112.
- Reddy MP, Sarla N, Siddiq EA (2002). Inter-simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128:9-12.
- Ritter A (2000). Application of biotechnology in the genetic improvement of potato. In: Vitoria-Gasteiz. POTATO, España. P. 8.
- Rocha EA, Luciano V P, Henrique de Carvalho H, Guimaraes CT (2010). Molecular characterization and genetic diversity of potato cultivars using SSR and RAPD markers. *Crop Breed. Appl. Biotechnol.* 10:204-210.
- Rohlf FJ (2000). Programme NTSYS-pc: numerical taxonomy and multivariate analysis system: version 2.1. Exeter Software, NewYork. p. 83
- Rus-Kortekaas W, Smulders MJM, Arens P, Vosman B (1994). Direct comparison of levels of genetic variation in tomato detected by a GACA-containing microsatellite probe and by random amplified polymorphic DNA. *Genome* 37:375-381.
- SAGARPA-Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (2010). Information Service Agriculture, Food and Fisheries statistics. Potato crop. Mexico, D.F. Pp. 550-563.
- Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning: A laboratory manual, 2nd ed. New York: Cold Spring Harbor Laboratory Press.
- Semagn K, Bjørnstad Å, Ndjiondjop MN (2006). An overview of molecular marker methods for plants. *Afr. J. Biotechnol.* 5(25):2540-2568.
- Sun G, Wang-Pruski G, Mayich M, De Jong H (2003). RAPD and pedigree-based genetic diversity estimates in cultivated diploid potato hybrids. *Theor. Appl. Genet.* 107:110-115.
- Ugent D (1968). The potato in Mexico: Geography and primitive culture. *Econ. Bot.* 22:108-123.
- Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531-6535.
- Yasmin S, Islam MS, Kondoker M, Nasiruddin M, Alam S (2006). Molecular characterization of potato germplasm by Random Amplified Polymorphic DNA markers. *Biotechnology* 5(1):27-31.
- Zietkiewicz E, Rafalski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20:176-183.