INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print), 2222-3045 (Online)
http://www.innspub.net
Vol. 6, No. 2, p. 175-186, 2015

RESEARCH PAPER OPEN ACCESS

DNA and morphological diversity and relationship analysis of selected cultivated, wild potatoes and some promising hybrids

Mousa Torabi-Giglou[1,4*], Panahandeh Jaber[1], Seyed Abolghasem Mohammadi[2], Fariborz Zaree Nahandi[1], Alireza Motallebi Azar[1], Jadwiga Śliwka[3]

[1]*Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, 51666-14766, Tabriz, Iran*

[2]*Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Tabriz, 51666-14766, Tabriz, Iran*

[3]*Plant Breeding and Acclimatization Institute-national, research Institute, Mł ochów research Centre, Platanowa 19, 05-831 Młochów, Poland*

[4]*Department of Plant Productions, Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran*

Key words: diversity, ISSR, Morphology, promising hybrids, *solanum*, wild relatives.

Article published on February 01, 2015

Abstract

Genetic diversity and relationships of 45 potato genotypes, consisting of three ploidy and EBN levels, assessed by ISSR markers and morphological traits. Analysis based on data of 17 morphological traits distinguished all accessions included. A total of 510 reproducible bands were amplified from 40 polymorphic fragments by means of five ISSR primers. These ISSR markers analyzed and Nei's gene diversity and genetic distance (GD) coefficient of all genotypes were calculated. The average number of scoreable bands produced per primer for genotypes using ISSRs were 8 polymorphic fragments for all genotypes. Among primers, in terms of Marker Index and PIC value, the primers UBC826, UBC820 and UBC824, gave the best results for all attributes, and thus could be used as representative ISSR primers for the genetic analysis of potatoes. Principal Coordates Analysis (PCoA) of ISSR marker separated all diploids with A and B genome from other genotypes. Cluster Analysis based on ISSR molecular data indicated that promising hybrids including *st1.k*, *A.K* and *SB.K* alongside *Agria* and *Satina* cultivars assigned in one cluster, which indicates the degree of genetic similarities, also *ES.chc* and *stbr-p* were allocated in a side branch of the same cluster and in its nearest cluster, the accessions of sto and fen were set apart in a cluster and *plt*, *hjt* and *pta* accessions were placed in a side cluster. Pairwise species matrix of Nei Genetic Distance varied from 0.058 to 0.645, and the closest species to *tbr* species, were *phu*, *plt* and *hjt* 0.058, 0.068 and 0.095 respectively. The closest genotypes to each other among all genotypes, were *sto/206×9* and *sto/206*, *Agria* and *St1.k* and *St1.k* and *AK*, with the genetic distances of 0, 1 and 3 respectively.

***Corresponding Author:** Mousa Torabi-Giglou ✉ mtorabi@uma.ac.ir

Introduction

Potato (*Solanum tuberosum* L.), is a crop of great importance and one of the four most-valuable crops worldwide, and exceeded only by wheat, rice and maize in world production for human consumption (FAO, 2012). Among the major crops, the potato probably has the largest number of wild relative species, and almost all wild species of potatoes are in the same gene pool, so there are many ways, to cross cultivated potatoes with them and exploitation of the useful properties (Bradshaw and Mackay, 1994). Potato genomes groups A and B are most important. The basic genome in cultivated autotetraploids and some wild relatives is A genome and B Genome belongs to Mexican diploids and exists also in allotetraploids and hexaploids like *S. stoloniferum, S.polytrichon, S. papita* and *S. demissum* species (Matsubayashi 1991). The taxonomy of wild tuber-bearing *Solanum* species is complicated and under continuous revision. Hawkes, 1990; Spooner and Hijmans, 2001; Spooner and Salas, 2006; Jacobs *et al.*, 2008). Considering the existence of disease-resistance genes such as late blight resistance genes in B genomes in the Mexican wild species, the analyzing the genotypes carrying resistance genes, using classical and molecular methods to generate new cultivars is essential. Markers such as microsatellites, have been used to investigate the relationships between different genomes of cultivated and wild relatives of potato and finding the genotypes with resistant to the disease (Ritter *et al.* 2009; Polzerova *et al.* 2011).

Inter-simple sequence repeats (ISSRs) involve the use of microsatellite sequences directly in the polymerase chain reaction (PCR) for DNA amplifications (Gupta *et al.* 1994; Zietkiewicz *et al.* 1994) which combines most of the benefits of AFLP and microsatellite analysis with the universality of RAPD. In potatoes, ISSRs have been successfully used to genomic fingerprinting (Prevost & Wilkinson, 1999; Gorji *et al.* 2011; McGregor *et al*, 2000), Genetic diversity and phylogenetic analysis (McGregor *et al.*, 2000; Bornet *et al.* 2002), resistance gene mapping (Marczewski 2001; Marczewski *et al.* 2002), These markers are multilocus, more reproducible, provide highly polymorphic fingerprints, are easy to develop in large numbers, have a simple assay, and are random, so that no prior sequence information is required (Bornet and Branchard 2001; Zietkiewicz *et al.* 1994). These markers have been successfully employed to estimate levels of genetic variation between and within species in potatoes (Bornet, *et al*, 2002; Aguilera *et al*, 2011). Also this technique, fingerprints with high speed and polymorphism, therefore shows genetic variations clearly between potato cultivars (Mahfouz *et al*, 2012). ISSR marker superiority over other techniques, have been identified in several studies. For example, in a study, Prevost and Wilkinson (1999) found that 5 ISSR primers were adequate to distinguish 35 varieties of potatoes. Aversano *et al.*(2009) identified thirteen ISSR primers to investigate the broad variability in cytoplasmic and nuclear DNA of *Solanum* genotypes regenerated plantlets and emphasized that ISSR markers, due to its fast, high reproducibility and low cost, according to obtained useful information, are suitable for the analysis of genetic variations in this method of proliferation (Aversano *et al*, 2009).

Considering the importance of potato and the role of morphological characteristics and molecular markers in quick and accurate breeding, this study was conducted to investigation of genetic diversity between different species of cultivated potatoes and wild relatives and some hybrids possessing genomes A and/or B, by morphological traits and ISSR markers and comparison of distinctiveness power of ISSR markers and morphological characteristics in the studied potato.

Materials and methods

Plant materials and morphological evaluation

The studied plant material, included 45 accession of potatoes, in three ploidy levels; diploid, tetraploid and hexaploid and related to cultivated and wild relatives of the Mexican potatoes possessing A and B genomes, respectively (Table 1). After complete growth of plants

in the field and in the flowering stage, data from aerial parts including leaves traits (number of lateral leaflets (number of pairs), terminal leaflet length to width ratio, petiole length and joint position and leaves length and width (cm)) and some flowers characteristics (largest and smallest diameter of corolla, style and anther length) were obtained and data of tubers characteristics (tuber shape, the dominant and secondary color of tuber skin and flesh and eyes depth, number and distribution) have got at the end of the season and after harvesting of tubers (; Chimote *et al*, 2007; Spooner *et al*, 2000). Leaf samples (the young fully expanded leaves) of plants grown in field conditions, harvested and DNA was extracted by CTAB (Saghaei *et al*, 1984). The quantity and quality of DNA samples was determined using 0.8 percent agarose gel electrophoresis and spectrophotometry. For polymerase chain reaction, DNA samples were diluted to concentration of 25 ng per µl.

Table 1. Plant materials, Abbreviations, ploidy level, EBN and accession numbers.

Species or hybrids	Ploidy level	Abbr.	EBN	Accesion No.	Species or hybrids	Ploidy level	Abbr.	EBN	Accesion No.
S. pinnatisectum	2x	pnt	1	CGN 17740, CGN 17742	*S. papita*	4x	pta	2	CGN 17830, CGN 17832
S. bulbocastanum	2x	blb	1	CGN 23010, GLK 1768, GLK 17687	*S. hjertingii*	4x	hjt	2	GLK 2405, CGN 22385
S. commersonii	2x	cmm	1	CGN 18215, CGN 22351, CGN 22359	*S. polytrichon*	4x	plt	2	CGN 22361, CGN 22362
S.cardyophyllum	2x	cph	1	CGN 22387	*S. acaule*	4x	acl	2	NIJ 934750049, CPC 1176, CPC 11767
S. chacoense	2x	chc	2	GLK 134, CGN 18294, GLK134 x IvP 35	*S. fendlerii*	4x	fen	2	GLK 437, GLK 444
S. verocosum	2x	ver	2	CGN 20571, CGN 17675	*S. stoloniferum*	4x	sto	2	NIJ 80/206, CGN 18333, NIJ 470206, NIJ80470206 x CPC 9
S. tuberosum(dH)	2x	tbr	2	IGC-02/203, IGC-02/204, IGC-dhNortena(op)	*S. tbr x S. sto*	4x	stbr	4	stbr-w, stbr-p
S. phureja	2x	phu	2	IvP 35(op), GLK 1497	*(S.tbr x S.sto) x tbr cv. Kizer*	4x	stbr	4	Stbr1.K
S. brevidense	2x	brd	1	NIJ 924750177	*(S.tbr x S.sto) x tbr cv. Kizer*	4x	stbr	4	SB.K
S. demmisum	6x	dms	4	CPC-dms4, CPC-dms21	*S. tuberosum ssp. andigena*	4x	tbr	4	*GLK,CGN Adg
					S. tuberosum	4x	tbr	4	cv. Agria, cv. Satina, A.K

*Clones selected from the mass selection program for creating adapted clones to long-day conditions, original seeds were coming from CGN and GLK..

In this study, 15 ISSR primers (UBC set 9, from the Biotechnology Laboratory, The University of British Columbia Canada) were used for initial screening in four representative samples. Out of the 15 primers, 5 of them that gave the most informative patterns (in terms of repeatability, score-ability and the ability to distinguish between accessions) were selected for further analyses (Table-3). ISSR amplification was performed in a 20 ml volume containing 20 ng genomic DNA, 1 µl Taq buffer, 2.0 mM MgCl2, 0.2 mM dNTPs, 0.75 mM primer, 0.5 units of Taq DNA polymerase. The amplification reaction consisted of an initial denaturation step at 94 °C for 3 min, followed by 45 cycles of 60 s at 94°C, annealing at 42°C

for 60 s, extension at 72°C for 90 s, and ended with extension at 72°C for 7 min. The amplified products were loaded electrophoretically on 1.4% agarose gels run at 100 V in 1X TBE buffer, visualized by staining with ethidium bromide (0.5 mg/ml), and photographed under ultraviolet light by GelDuc. The amplifications were repeated twice and only clear repetitive bands were used in scoring and data analysis, and molecular weights were estimated using a 100 bp DNA ladder (Thermo Scientific Co., Ltd).

Data analysis

Statistical analysis of morphological traits data

Analysis of variances in the morphological data was performed using SPSS statistical software, based on the experimental design. According to data obtained from morphological characteristics, genotypes were classified. Tuber morphology analysis was performed using Xlstat and GenALex software to construct a dendrogram.

Determination of ISSR marker polymorphism

The amplified fragments were scored for band presence (1) or absence (0) with all the accessions studied to form a binary matrix. Obscure bands were discarded and distinct and unequivocal bands were scored only. Jaccard similarity coefficients were performed by using the POPGENE version 1.32 computer program package, Xlstat 2014.5 and GenALex 6.4 (Peakall and Smouse, 2010) to construct a dendrogram using the different methods. The observed number of bands, the Percentage of Polymorphic Bands (**PPB**), Nei's (1973) gene diversity or Expected Heterozygosity (**He**) and number of effective alleles (**Ne**) were calculated, and genetic diversity within accessions was measured by PPB.

To determination of ISSR markers discriminatory power, scored as a dominant marker, Nei gene diversity index (the expected heterozygosity) and polymorphism information content (PIC) was the same, and was calculated using the following formula in which P_i is the i^{th} allele presence frequency and q_i is the i^{th} allele absence frequency(Raina *et al.* 2001) . In addition, genetic relationships were compared by visual examination of dendrograms.

$PIC=1-p_i^2-q_i^2$

Analysis of molecular variance (AMOVA)

For separation of the total molecular variances to genetic variance between and within groups, analysis of molecular variance (AMOVA) was used. Genotypes classification using cluster analysis based on ISSR markers data as well as the data was obtained using the morphological characteristics of leaves, flowers and tubers. Software GenALex 6.4 (Peakall and Smouse 2010) were used.

Results and discussion

Morphological Characteristics analysis

Similarity analysis based on data of 17 morphological traits distinguished all the accessions included in the present study. Dendrogram tree showed clear broad grouping with hybrids and cultivated species as the most distinct clade and maximum divergence for the genotypes originated from Mexico. Some genotypes like *pnt/40* and *plt62* were placed on the right side of first component of the PCoA apart from the rest of similar genotypes as well as *ES.chc* of second component (Fig. 1). Cluster analysis based on tuber morphology data divided genotypes into four main groups. Accordingly, hybrids, dihaploid and diploid cultivated species were in Group 1. In the second group, one genotype of each species pta, fen and hjt, was placed. Other genotypes were classified into two groups in distinct clusters. Hybrids stbr-w and stbr-p were placed in clusters 1-4 and approximately were isolated morphologically from other genotypes (Fig. 1).

According to the results of leaves and flowers morphology data, genotypes were divided into three groups; Cluster of 4EBN genotypes and hybrid and cluster of 2EBN dihaploid, cultivated diploid and close tetraploid relatives, were placed respectively in two adjacent clusters, and the rest of more distant wild relatives by different EBN No. were distinguished in 3rd cluster. Promising hybrids together with Agria, Satina and *Andigena* were placed

in a separate cluster adjacent of the cluster including 2EBN *dihaploids*, cultivated diploids and close tetraploid relatives (Fig. 3).

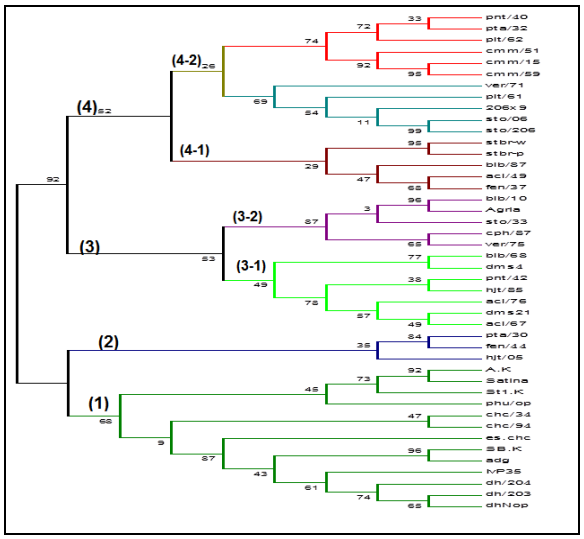

Fig. 1. Cluster analysis of genotypes based on minimum evolution method using tuber morphology data.

Based on results obtained from leaves and flowers morphology data, genotypes divided into three groups; 4EBN hybrid and genotypes cluster, 2EBN cultivated diploids, dihaploids, and close tetraploid relatives cluster, were placed into two adjacent clusters, and were distinguished from the rest of more distant wild relatives cluster with different EBN numbers. Promising hybrids associated with Agria, Satina and Andigena were placed in a separate cluster adjacent to the cluster of 2EBN cultivated diploids, dihaploids, and close tetraploid relatives (Fig. 3).

ISSR analysis

A total of 40 scoreable amplified DNA fragments with totally 908 amplified band ranging in size from 100 to 1300 bp were observed using the seven primers, whereas all fragments were polymorphic (Fig-2 and Table-3). Analysis of molecular variance based on ISSR markers are provided in Table 2. Variances between and within groups were significantly different at the 0.01 level and variance between species (61%) were estimated to be about twice the variance within species (39%). This showed a high genetic diversity between species, with a good diversity within species, which can be used in breeding programs (Table 2). In this study the polymorphism was 100%, the index of polymorphism information content (PIC) estimated 0.15 to 0.34, MI index ranged from 1.2 to 3.06 and the Nei genetic diversity of studied species was estimated to be 0.058- 0.638. Average number of amplified fragments for each primer was 8 fragments, the highest number and the minimum number of amplified fragment corresponded to the primer UBC 826 with 9 fragments and UBC 844 with 7 had been respectively. Among used primers, the index of polymorphism information content (PIC) for UBC818, UBC820and UBC813 were 0.78, 0.7 and 0.67 respectively, Also in terms of MI, the primers UBC818, UBC813 and UBC824, showed the best results, 6.24, 5.36 and 5.2 respectively (Table 3).

In order to determining the genetic relationships among the genotypes, Evolution Minimum algorithm and evolutionary distance coefficients including Jukes-Cantor, Kimura 2-Parameter, P-distance, Number of Differences and Maximum Composite Likelihood-based data ISSR markers were used. To determine the genetic relationships between species and genotypes, Nei genetic distance coefficient was used. According to the analysis conducted on the basis of the Nei genetic distances matrix, the closest species to tbr, were phu, plt and hjt respectively at 0.058, 0.068 and 0.095, and the most distant species to tbr, were pnt, cph and blb, with distances 0.302, 0.279 and 0.275, respectively. As well as genetic distance of pta with hjt and plt were 0.068 and hjt with plt and fen were 0.092, 0.094, respectively (Table 4).

Table 2. Summary of ISSR AMOVA Table.

Source	df	SS	MS	Est. Var.	%
Among Pops	15	185.267	12.351	3.568	61%
Within Pops	31	69.712	2.249	2.249	39%
Total	46	254.979		5.817	100%

Table 3. ISSR primers used in this study and summary of ISSR markers results.

Primer name	Primer sequence	Annealing temperature (°C)	scored Fragment size range	PIC	np1	ß2	PPB3 (%)	EMR4	MI5
UB C813	(CT)8T	42	200-1000	0.22	8	1	100	8	1.76
UBC 818	(CA)8G	42	200-800	0.15	8	1	100	8	1.20
UBC 820	(GT)8T	42	200-900	0.30	7	1	100	7	2.1
UBC824	(TC)8G	42 or 44	200-1300	0.23	8	1	100	8	1.84
UBC 826	(AC)8C	42	200-900	0.34	9	1	100	9	3.06

[1] n_p (No. of polymorphic bands)

[2] ß = n_p/ (total band No.)

[3] Percentage of Polymorphic Bands

[4] EMR(Effective Multiplex Ratio)= np× β

[5] Marker Index(MI=(PIC*EMR)).

Table 4. Pairwise species matrix of Nei Genetic Distance.

pnt	blb	cmm	cph	chc	phu	ver	brd	tbr	pta	hjt	plt	acl	fen	sto	dms	
0.000																pnt
0.241	0.000															blb
0.375	0.332	0.000														cmm
0.311	0.245	0.391	0.000													cph
0.224	0.312	0.162	0.328	0.000												chc
0.308	0.276	0.196	0.256	0.108	0.000											phu
0.367	0.341	0.300	0.393	0.199	0.242	0.000										ver
0.483	0.636	0.478	0.693	0.406	0.445	0.553	0.000									brd
0.214	0.195	0.173	0.282	0.080	0.054	0.195	0.476	0.000								tbr
0.341	0.353	0.265	0.466	0.174	0.250	0.243	0.490	0.166	0.000							pta
0.226	0.270	0.203	0.462	0.143	0.210	0.210	0.511	0.120	0.077	0.000						hjt
0.225	0.273	0.179	0.337	0.131	0.166	0.282	0.490	0.080	0.143	0.094	0.000					plt
0.415	0.303	0.288	0.326	0.325	0.312	0.233	0.534	0.298	0.399	0.422	0.345	0.000				acl
0.304	0.317	0.246	0.490	0.143	0.183	0.342	0.604	0.126	0.182	0.127	0.147	0.431	0.000			fen
0.258	0.351	0.261	0.412	0.169	0.171	0.347	0.516	0.132	0.294	0.190	0.141	0.457	0.116	0.000		sto
0.307	0.276	0.279	0.483	0.205	0.329	0.277	0.704	0.189	0.252	0.181	0.233	0.388	0.179	0.289	0.000	dms

Cluster Analysis based on ISSR molecular data indicated that promising hybrids including st1.k, A.K and SB.K alongside Agria and Satina cultivars assigned in one cluster, which indicates the degree of genetic similarities, also *ES.chc* and *stbr-p* were allocated in a side branch of the same cluster and in its nearest cluster, the accessions of *sto* and *fen* were set apart in a cluster and *plt*, *hjt* and *pta* accessions were placed in a side cluster. These results have been consistent with the Spooner *et al.* (2004 and 2006) theory of species reduction and the results of Jacobs *et al.* (2008) and in accordance with the information reported by Hardigan *et al.* (2014). As spooner *et al.* (2004 and 2006) placed accessions of the studied species in a clade, and were approved by Jacobs *et al.* (2008) with a little proof, used accessions of pnt, *blb* and *cph* species in our study were set apart in one cluster. Spooner *et al.* (2004) placed accessions of *pnt* species in the clade of *Pinnatisecta* and accessions of *blb* and *cph* species in *Bulbocastana* clade.

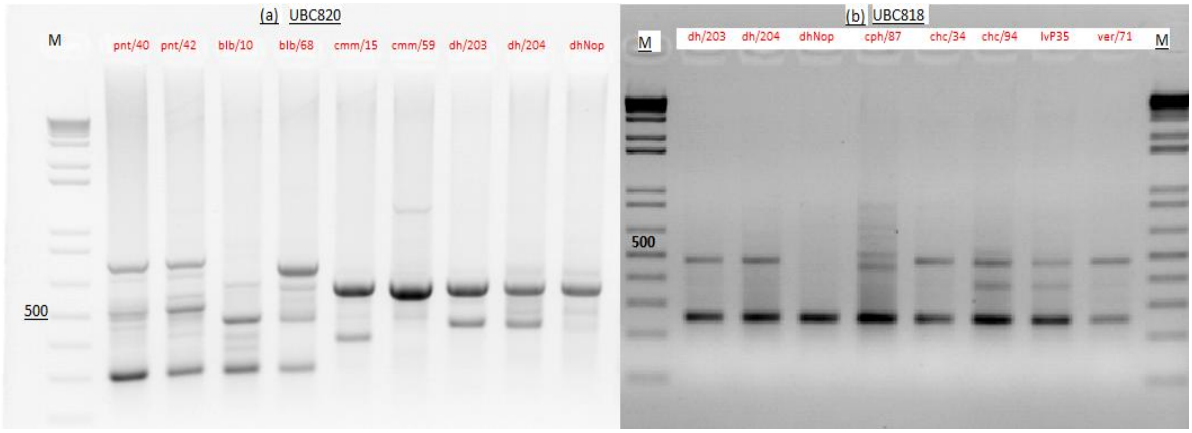

Fig. 2. ISSR profiles of 16 potato accessions obtained by using UBC820 (a) and UBC818 (b**)** primers, M: 100 bp molecular size marker (Thermo Scientific Co., Ltd).

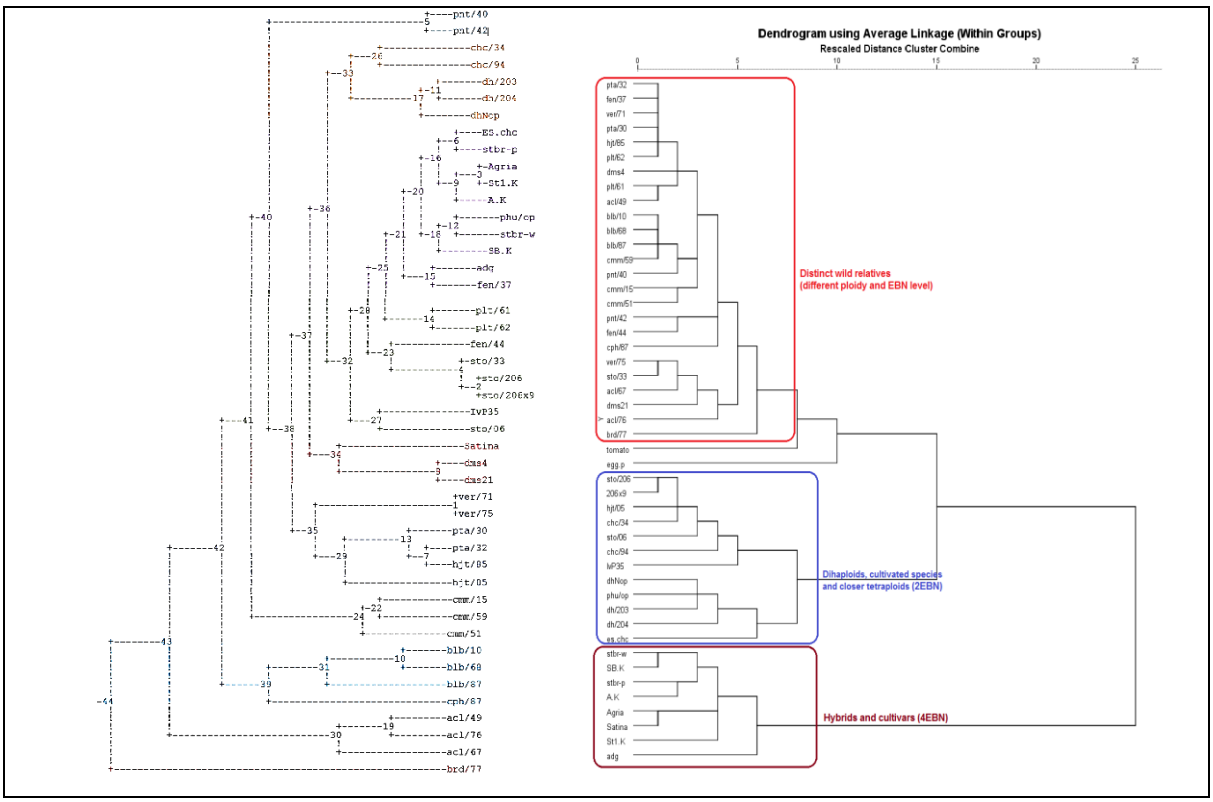

Fig. 3. Dendrogram Based Nei's (1972) Genetic distance using UPGMA Method Modified from NEIGHBOR procedure of PHYLIP Version 3.5 (left) and dendrogram using genotypes leaves and flowers morphology data using linkage method (within groups) with Euclidean distance.

Wild genotypes possessing A genome which have been separated from the group of the zone 2, were placed into two districts: IvP35 and cmm59 were placed in zone 1 and *ES.chc* and *phu-op* in zone 3, which represents their further genetic and morphological proximity. In zone 4, placed accessions with B genome which were quite distinct from the other genotypes, as well as *brd/77* possessing E genome was placed in the zone 4 far from genotypes containing B genome representing it's genetically distance from other studied genotypes (Fig. 4). Principal Coordates Analysis, performed as a complementary method for ISSR cluster analysis results, showed that, the first two components cover a

significant portion of percentage variations. According to Fig. 4, the commercial cultivars, dihaploids and allotetraploids, along with the promising hybrids were placed in region left side of the first component, which confirmed the results of morphological data and clustering results based on ISSR markers. Also in right side of the first component on the upper part of the second component genotypes were located which possess "A" genome. Mexican diploids with 1EBN level separated from the other potatoes and located in the left side of the first coordinate and the down side of second coordinate.

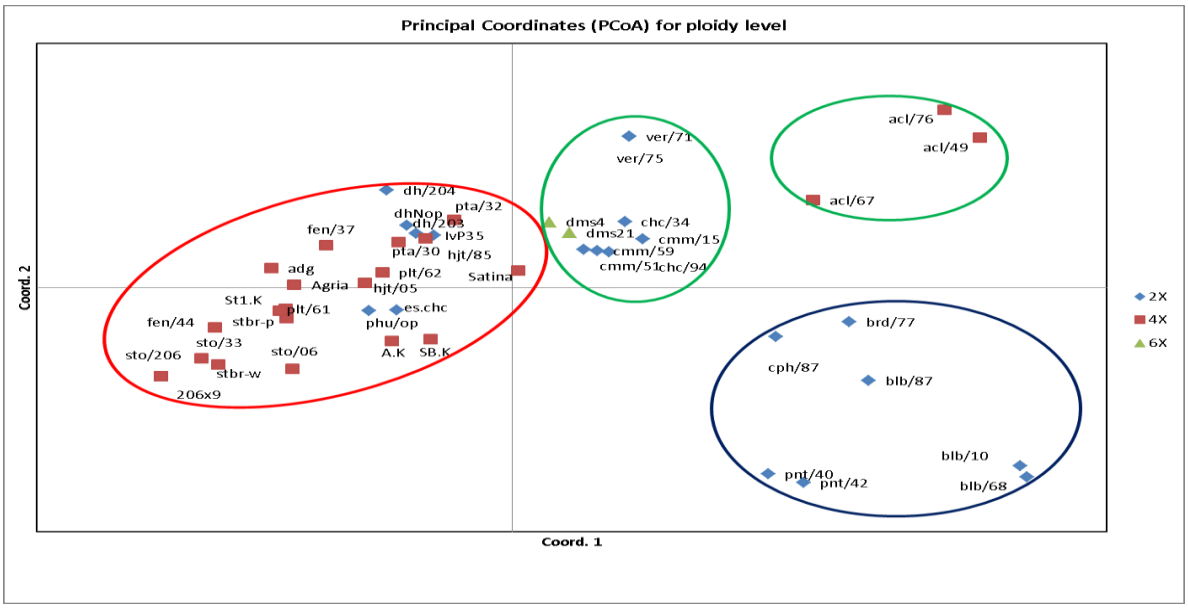

Fig. 4. Principal Coordates Analysis (PCoA) of ISSR marker results based on ploidylevel.

In order to determine the genetic relationship between species and genotypes, Nei genetic distance coefficient was used. According to conducted analysis on the basis of the matrix of Nei genetic distances, the closest species to *tbr* were *phu, plt* and *chc,* and *fen* with distances of 0.054, 0.080 and 0.126 respectively, and the most distant species to *tbr*, were *brd, acl* and *cph*, with distances of 0.476, 0.298 and 0.282, respectively. The genetic distance of *pta* with *hjt*, *plt*, *fen* and *sto* was 0.77, 0.143, 0.182 and 0.294 respectively, and *hjt* distance with *plt, fen* and *sto* were 0.094, 0.127 and 0.190, respectively, so that pta, hjt, fen and plt are closer to each other rather than sto (Table 4). Pairwise matrix of genetic distances showed that the closest genotypes to hybrid stbr-w (promising studied hybrid), were *sto/06* and *phu/op* with Nei genetic distance 3, and *adg* at a distance of 4. The closest genotypes to *Agria* (as a standard commercial cultivar), were *ES.chc, adg* and "*plat61* and *phu/op*" with distance 3, 4 and 5 respectively and the most distant genotypes, were brd/77, cph/87 and acl/67 having Nei genetic distances of 19, 15 and 15 respectively (Data table not shown).

ISSR markers and morphological traits successfully distinguished the 45 potato accessions. 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). In the present study, the average number of scoreable bands produced per primer for genotypes using ISSRs were about 8 bands, those produced polymorphic fragments for all 45 genotypes.

The morphological results in our work, were very similar to those of Huaman and Spooner (2002) and

Gavrilenko *et al.* (2010). Huaman & Spooner (2002) examined the morphological support for the classification of landrace populations of cultivated potatoes, using representatives of all seven species and most subspecies as outlined in the taxonomic treatment of Hawkes (1990). Most morphological support was based on a suite of characters, all of which were shared with other taxa. These results, combined with their probable hybrid origins, multiple origins and evolutionary dynamics of continuing hybridization, led Huaman & Spooner (2002) to recognize all landrace populations of cultivated potatoes as a single botanical species, *S. tuberosum*, with the eight cultivar groups. Gavrilenko *et al.* (2010) studied the morphological and microsatellite markers support for the Russian National Cultivated Potato Collections at the N. I. Vavilov Institute for Plant Industry. The morphological taxonomic results were similar to those of Huaman and Spooner (2002) in recognizing entities corresponding to most of examined species, despite using a different germplasm base, a different evaluation environment and different scoring methods for some of the traits.

Molecular data have been used to establish the phylogeny of groups of tuber-bearing *Solanum spp*., to evaluate hybridization hypotheses, intraspecific classifications, to establish the ancestry of the cultivated potato, to trace introgression from wild species and to assess genetic diversity within species and cultivated material. Furthermore, molecular data allow checking for misidentifications and can be utilized in risk-assessment studies. In addition, molecular data have been used to address three main issues about the cultivated potato:(1) the mode of origin of the crop and the relationships with its wild relatives; (2) the relationship between the Andigena and Tuberosum groups and the introduction of the cultivated potato from South America to Europe and the rest of the world; (3) the genetic diversity of the crop.

ISSR primers produced different numbers of DNA fragments, depending upon their simple sequence repeat motifs. Among the initially tested primers, seven of the 15 primers generated useful fingerprints based on the very high number of bands and the level of detectable polymorphism (Table 3; Fig. 2). These primers corresponded to dinucleotide motifs with an anchoring end. The nonanchored dinucleotide primers tested in previous studies by Gupta and Varshney (2000), only smears were obtained. These results show the great interest of our strategy: the use of only a few anchored primers, but with specific and optimal PCR conditions for each one. Bornet *et al.* (2002) identified 28 cultivars using 15 ISSR primers in an optimal condition and their amplifications led to a high level of polymorphism, indicating that ISSR markers were very appropriate in evaluating the genetic diversity of the potato population. The low level of genetic diversity of European cultivated potatoes was confirmed in an analysis using ISSRs by Bornet *et al.* (2002). Their results showed that European potatoes are completely homogenous, and the genetic diversity was very low compared with Argentinian cultivars. The conclusions about these matters are not conclusive. Eventually, the completely classification of the modern cultivars of potato in subgroups has not really been associated yet with molecular markers.

According to Gupta and Varshney (2000) results, dinucleotide and trinucleotide motifs represented the most abundant microsatellites in plants. Such a result was in agreement with our analysis results. The results revealed that ISSR is more advantageous in showing intra- and interspecies differences rather than differences depending on morphological appearance. We think that it can contribute to the systematic and help people dealing with plant breeding for selecting the genotypes they want in an easier and safer way. With the present study, the ISSR fingerprinting technique used was confirmed to be a reproducible and sensitive tool for the identification of potato accessions. Our results have demonstrated that selection of distinct genotypes within a species increases the chance of a successful breeding program.

Ploidy level has been important in the classification of cultivated potatoes, but our results show so many exceptions that it is a poor character to explain gene pools. Spooner *et al.*(2007) provided one of the largest molecular marker studies of potato, to include an extensive study of 742 landraces of all cultivated species (or Cultivar Groups) and 8 closely related wild species progenitors, their results highlighted a tendency to separate by ploidy level, However they mentioned, there are many exceptions to grouping by ploidy. Our results emphasized the results of Spooner *et al.* (2007) as they find many accessions of S. phureja to cluster with the polyploids, because such manner also were observed in our materials (fig. 3 and 4). Mexican species represent such a great, largely untapped pool for breeding that promoted Toxopeus (Toxopeus, 1964) to describe his work with some of these species as "treasure digging"(Spooner, *et al.*1991). Differences between species can be caused and justified by several factors such as genetic drift and genetic separation (Hagbin and Peakall, 1999). Also, when populations are small and isolated, genetic drift has a greater impact on the genetic structure of populations, ands increases the differences between species (Ellstrand and Elam, 1993).

In conclusion, ISSR markers was successfully used for the quick identification of genotypes, to investigate diversity between different genotypes of potatoes, in order to identifying differences and closeness of distinguished plants cultivated from different species, and to show the potential possibility of ISSR markers for identification of plant material for cross programming between different potatoes. Some cultivated species and Mexican potatoes consisted of closely related morphogical characteristics to commercial cultivars, which were distinguished from each other using the ISSR markers. Our results showed that some Mexican potatoes (possessing B genome and resources of many useful resistances) are clearly close to *S.tuberosum* and as a promising gene pool can be utilized in the development of modern cultivars by manipulation the ploidy level and using bridge species for overcoming crossing barriers to EBN difference. Detailed knowledge of the genetic of EBN would facilitate a more efficient utilization of this germplasm in potato breeding programs.

References

Aguilera JG, Pessoni LA, Rodrigues GB, Elsayed AY, Silva DJH, de Barros EG. 2011. Genetic variability by ISSR markers in tomato (Solanum lycopersicon Mill.). Revista Brasileira de Ciências Agrarias **6(2),** 243-252.

Allainguillaume J, Wilkinson M, Clulow SA, Barr SNR. 1997. Evidence that genes from the male parent may influence the morphology of potato dihaploids. Theoretical and Applied Genetics **94,** 241-248.

Aversano R, Savarese S, Maria J, Nova D, Frusciante L, Punzo M, Carputo D. 2009. Genetic stability at nuclear and plastid DNA level in regenerated plants of Solanum species and hybrids. Euphytica **165,** 353–361.

Bedoni MC, Camadro EL. 2009. Morphological and molecular evidence of natural interspecific hybridization in the diploid potato Solanum kurtzianum from Argentina. Botany **87(1),** 78-87.

Bornet B, Branchard M. 2001. Nonanchored Inter Simple Sequence Repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Molecular Biology Reporter **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,** 480-484.

Bradshaw JE, Mackay GR. 1994. Breeding strategies for clonally propagated potatoes. In: Bradshaw JE,Mackay GR (eds.) Potato genetics. CAB International, Wallingford 467–497.

Bussell JD, Waycott M, Chappill JA. 2005. Arbitrarily amplified DNA markers as characters for phylogenetic inference. Perspectives in Plant Ecology, Evolution and Systematics **7,** 3–26.

Chimote VP, Pattanayak D, Naik PS. 2007. Molecular and morphological divergence studies in Indian potato varieties. Indian Journal of Biotechnology **6(2),** 216-223.

Ellstrand NC Elam DR. 1993. Population genetics consequences of small population size: Implications for plant conservation. Annual Review of Ecology, Evolution, and Systematics **24,** 217-243.

FAO. 2012. http://faostat.fao.org.

Gavrilenko T, Antonova O, Ovchinnikova A, Novikova L, Krylova E, Mironenko N, Pendinen G, Islamshina A, Shvachko N, Kiru S, Kostina L, Afanasenko O, Spooner D. 2010. A microsatellite and morphological assessment of the Russian National cultivated potato collection. Genetic Resources and Crop Evolution **57,** 1151–1164.

Gorji AM, Poczai P, Polgar Z, Taller J. 2011. Efficiency of Arbitrarily Amplified Dominant Markers (SCOT, ISSR and RAPD) for Diagnostic Fingerprinting in Tetraploid Potato. American Journal of Potato Research **88,** 226–237.

Gupta M, Chyi YS, Romero-Severson J, Owen JL. 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theoretical and Applied Genetics **89,** 998–1006.

Gupta PK, Varshney RK. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica **113(3),** 163-185.

Hagbin PM, Peakall R. 1999. Evaluation of the conservation of genetic research to the management of endangered plant *Zieria prostrate*. Conservation Biology **13,** 514-522.

Hardigan MA, Bamberg J, Buell CR, Douches DS. 2014. Taxonomy and genetic differentiation among wild and cultivated germplasm of *Solanum* sect. *Petota*. The Plant Genome, in press.

Hawkes JG. 1990. The Potato: Evolution, Biodiversity and Genetic Resources. Belhaven Press, London.

Huaman Z, Spooner DM. 2002. Reclassification of landrace populations of cultivated potatoes (Solanum sect. Petota). American Journal of Botany **89,** 947-965.

Jacobs MM, van den Berg RG, Vleeshouwers VG, Visser M, Mank R, Sengers M, Hoekstra R, Vosman B. 2008. AFLP analysis reveals a lack of phylogenetic structure within Solanum section Petota. BMC Evolutionary Biology **8:145,** 1-12.

Mahfouze SA, El-Dougdoug, KAF, El-Sayed, OE, Gomaa MA Allam EK. 2012. Genetic diversity of cultivated and wild-type potatoes under potato spindle tuber viroid infection. New York Science Journal **5(7),** 9-18.

Marczewski W. 2001. Inter-simple sequence repeat (ISSR) markers for the Ns resistance gene in potato (Solanum tuberosum L.). Journal of Applied Genetics **42(2),** 139–144.

Marczewski W, Hennig J, Gebhardt C. 2002. The Potato Virus S resistance gene Ns maps to potato chromosome VIII. Theoretical and Applied Genetics **105,** 564–567.

Matsubayashi M. 1991. Phylogenetic relationships in the potato and its related species. In: Tsuchiya T. and Gupta P.K. (eds.) Chromosome engineering in plants: genetics, breeding and evolution, part B. Elsevier, Amsterdam, 93–118.

McGregor, C.E., Lambert, C.A., Greyling, M.M., Louw, J.H. and Warnich, L. (2000). A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (Solanum tuberosum L) germplasm. Euphytica **113,** 135–144.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences **70,** 3321-3323.

Prevost A, Wilkinson MJ. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and Applied Genetics **98,** 107–112.

Peakall R, Smouse PE. 2010. GenALex 6.4: Genetic analysis in Excel: Population genetic software for teaching and research. Molecular Ecology Notes **6,** 288-295.

Raimondi JP, Peralta IE, Masuelli RW, Feingold S, Camadro EL. 2005. Examination of hybrid origin of wild potato Solanum ruiz-lealii Brücher. Plant Systematics and Evolution **253,** 33-51.

Raina SN, Rani V, Kojima T, Ogihara Y, Singh KP, Devarumath RM. 2001. RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification and phylogenetic relationships in peanut (Arachis hypogaea) cultivars and wild specious. Genome **44,** 763-772.

Ritter E, de Galarreta JIR, Hernandez M, Plata G, Barandalla L, Lopez R, Sanchez I, Gabriel J. 2009. Utilization of SSR and cDNA markers for screening known QTLs for late blight (Phytophthora infestans) resistance in potato. Euphytica **170,** 77–86.

Saghai-Maroof MA, Solaiman K, Tprgensen RA, Allard RW. 1984. Ribosomal DNA spacer-lenth polymorphism in barely: Mendelian inheritance, chromosomal location and population dynamics. Proceedings of the National Academy of Sciences **81,** 8014-8018.

American Journal of Botany, Hjerting BJ, Gomez J. 1991. Mexico, 1988. potato germplasm collecting expedition and utility of the Mexican potato species. American Journal of Potato Research **68,** 29-43.

Spooner DM, Hijmans RJ. 2001. Potato Systematics and Germplasm Collecting, 1989-2000. American Journal of Potato Research **78,** 237-268.

Spooner DM. Salas A. 2006. Structure, biosystematics, and genetic resources. In: J. Gopal and S.M. Paul Khurana (eds.) Handbook of potato production, improvement, and post-harvest management. Haworth's Press, Inc., Binghampton, New York, 1-39.

Spooner DM, van den Berg RG, Bryan G, Del Rio A. 2004. Species concepts and reduction of species in wild and cultivated potatoes (Solanum section Petota). Acta Horticulturae **619,** 63-75.

Spooner DM, Nunez J, Trujillo G, Del Rosario Herrera M, Guzman F, Ghislain M. 2007. Extensive simple sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. Proceedings of the National Academy of Sciences **104,** 19398–19403.

Spooner DM, van den Berg RG, Miller JT. 2000. Species and series boundaries of Solanum series Longipedicellata (Solanaceae) and phenetically similar species in series Demissa and series Tube,vsa: Implications for a practical taxonomy of Section Petota. American Journal of Botany **87(12),** 113-1300.

Toxopeus HJ, 1964. Treasure-digging for blight resistance in potatoes. Euphytica **13,** 206-222.

Zietkiewicz E, Rafalski A, Labuda D. 1994. Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics **20**, 176–183.