

Full Length Research Paper

Molecular based assessment of genetic diversity of xoconostle accessions (*Opuntia* spp.)

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Accepted 2 January, 2014

Xoconostle or acidic prickly pear is an important fruit in Mexico; it is produced by a group of *Opuntia* plants known for their nutritional qualities and adapted to harsh environmental conditions. In this study, we report for the first time the estimation of genetic diversity within a set of 24 xoconostle accessions using inter simple sequence repeats (ISSR) markers. Five ISSR primers were selected to detect DNA polymorphisms in these plants. Broad genetic diversity was detected (0.41-0.82, Dice coefficient) and the unweighted pair group method with the arithmetic averaging (UPGMA) analysis allowed discriminating all genotypes and enabled their clustering into six groups; the 'Matizado' accession was significantly divergent from all tested genotypes. In addition, as shown by the clustering analysis and principal component analysis (PCA), the tested genotypes did not group according to their origin, to culture conditions, or to the current species classification. This work, on one hand, demonstrates the high genetic variation among genotypes of xoconostles, and on the other hand, it suggests a taxonomic revision and raises doubts about the number of species reported for these plants.

Key words: *Opuntia* spp., acidic prickly pear, inter simple sequence repeats (ISSR) markers, taxonomy.

INTRODUCTION

Xoconostles are fruits produced by a group of plants included in *Opuntia* genus (from Nahuatl: xoco = acid, noxtle = tuna). Xoconostles have a wide inner edible wall, a thin outer wall that is not easily removed, and seeds arranged in the center of the fruit with dry funiculus (Scheinvar et al., 2009; Gallegos-Vázquez et al., 2011). *Opuntia* species that produce xoconostles, as well as sweet prickly pear, are succulent and xerophytes. They are found naturally in arid and semi-arid areas of the central part of Mexico (Scheinvar et al., 2009). Wild plants represent natural reservoirs for farmers to collect fruit and/or cladodes.

Ten species that produce xoconostle fruits had been reported by Scheinvar et al. (2009), nine of these belong to the genus *Opuntia* (*O. heliabravoana* Scheinvar, *O. elizondoana* E. Sánchez and Villaseñor, *O. joconostle* F.A.C. Weber, *O. matudae* Scheinvar, *O. spinulifera* Salm-Dyck, *O. leucotricha* DC, *O. zamudioi* Scheinvar, *O. durangensis* Britton and Rose, *O. oligacantha* C.F. Förster), and one more specie to the genus *Cylindropuntia* (*Cylindropuntia imbricata* DC). Gallegos-Vázquez et al. (2011) described others more.

The name xoconostle is used indifferently to refer to acidic fruits as well to *Opuntia* plants that produce such

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Abbreviations: ISSR, Inter simple sequence repeats; UPGMA, unweighted pair group method with the arithmetic averaging; PCA, principal component analysis; PIC, polymorphic information content; MI, marker index; Rp, resolving power; PCR, polymerase chain reaction.

fruits. During the past two decades the interest in xocostles has increased, and they are becoming formal crops due to their nutritional and functional properties. Xocostles have agronomic and economic potential because the fruits remain on the cladodes for a whole year, and even longer (Venegas and Delgado, 1996; Ávalos-Andrade et al., 2006). Xocostles are consumed in different ways: in stews, as a condiment; dried, as sweetened fruits, as the basis for soft drinks, or as a raw material in the production of wines, liquors, jams and jellies, among others, which are favored by its special acidic taste (Gallegos-Vázquez et al., 2012). They are also considered an alternative natural medicine due to their antihypoglycemic, oxidative stress and cancer prevention effects (Chavez-Santoscoy et al., 2009). They also have antihyperlipidemic, antiinflammatory, antidiuretic, hypocholesterolemic, immunostimulatory and antiulcerogenic activity, including weight loss effects (Osorio-Esquivel et al., 2011, 2012; Morales et al., 2012; Patel, 2013). The cladodes of these plants contain abundant digestible fiber, high mucilage and mineral contents, and they are a good fodder (Scheinvar et al., 2009).

Generally, nopal and xocostle are known for their confusing taxonomy due to few morphological characters taken into account to classify them into the genus (Rebman and Pinkava, 2001); also, the different ploidy levels and inadequate descriptors have led to misclassification (Caruso et al., 2010; Majure et al., 2012). All these attributes make their classification difficult and limit formal proposals for their rescue, conservation and sustainable use (Scheinvar et al., 2009).

At present, molecular markers have proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. Polymerase chain reaction (PCR) technology has led to the development of different techniques, some of which have been used successfully in the *Opuntia* genus for genetic analysis (Arnholdt-Schmitt et al., 2001; Labra et al., 2003). In particular, inter simple sequence repeats (ISSR) markers generate many highly polymorphic multilocus with dominant inheritance, distributed throughout the genome (Zietkiewicz et al., 1994).

This technique has proven to be consistent and robust to identify cultivars and closely related species (Godwin et al., 1997; Bernet and Branchard, 2001), as well as to estimate the genetic diversity, population structure, genome map and evolutionary processes of plants (Wolfe et al., 1998a; Reddy et al., 2002; Vargas-Ponce et al., 2009). The method has been successfully used in studies on the genetic diversity of complex species, and also in cereals and vegetables (Salhi-Hannachi et al., 2004; Xiao et al., 2004; Taylor and Barker, 2012).

Due to the interest in knowing about the genetic variation of several xocostle accessions recognized in Mexico as genetic resources, ISSR DNA markers were used to investigate DNA polymorphism and to determine

the degree of relationship between wild and semidomesticated xocostle accessions. The results from this work will help to make decisions regarding the establishment of a reference collection by germplasm banks; indeed, although this crop is widely desired in the country, people can have crop options with respect to the possible uses based on genetic/genomic xocostles similarities.

MATERIALS AND METHODS

Plant material

Twenty four (24) accession samples producing xocostles obtained from CRUCEN-UACH Germplasm Bank and from Biology Institute, UNAM, were considered in this study. These accessions belonged to nine species and three more reported as "hybrids". Also, we have included a sample of sweet prickly pear (*Opuntia albicarpa* Scheinvar) as outgroup. The common name, the species name, the locality of sampling and the culture condition are summarized in Table 1.

DNA extraction

In *Opuntia*, the isolation of DNA from cladodes is difficult due to the presence of high amounts of mucilage that interferes with DNA quality. In this study, seeds were used for DNA extraction because these structures help to overcome the difficulties caused by the mucilage. Seed mixtures of two or three fruits of independent plants of the same accession were used.

The seeds were cleaned, dried at room temperature and then pulverized. DNA was obtained according to Luna-Paez et al. (2007), and then the DNA was treated with phenol to remove proteins. DNA was quantified by spectrophotometry and the quality was determined in 1% agarose electrophoresis gel with 1X TAE buffer (40 mM Tris-acetate, 1 mM EDTA pH 8.0); the gel was stained with 0.5 $\mu\text{g}\cdot\text{mL}^{-1}$ ethidium bromide, documented and analyzed with the Kodak Digital Science 1D software V.2.0.

ISSR amplification and electrophoresis

ISSR analysis were performed using the same informative anchored primers synthesized in SIGMA Co., and previously used by Luna-Paez et al. (2007) (Table 2). PCR reactions were performed in a 25 μL reaction mixture containing: 50 ng of template DNA, 500 μM dNTPs, 1X Taq buffer, 25 mM MgCl_2 , 10 pmol of primer and 1.5 U of DNA Taq-polymerase (Promega); PCR was performed in the MaxyGene Thermal Cycler (Applied Biosystem, USA).

The thermocycling conditions were: 1 cycle at 94°C 1 min, 35 cycles [94°C 30 s, 48°C 30 s for the $(\text{CA})_8\text{RT}$ primer alignment and 56°C for the remaining primers, 72°C 1.5 min] and one final extension cycle of 72°C for 5 min. Finally, the PCR products were separated in polyacrylamide gels (29:1) 8%. The molecular marker GeneRuler 1 kb DNA ladder (MBI Fermentas) was used as reference. The gels were run at 280 v for 2.5 h in 1X TBE (89 mM Tris-borate, 2 mM EDTA pH 8.0), pH buffer and stained with silver nitrate (AgNO_3) 0.2%. Photographs were taken with a Kodak Digital Camera.

Data analysis

Only consistent and reproducible ISSR amplified bands in the three

Table 1. List of *Opuntia* accessions (xoconostles and sweet prickly pear) studied by ISSR markers.

Label	Accession	<i>Opuntia</i> Species	Locality	Condition
1	Alimonado	<i>O. joconostle</i> F.A.C Weber	Hidalgo	Homegardens
2	Apan	<i>O. joconostle</i> F.A.C Weber x <i>O. matudae</i> Scheinvar	Hidalgo	Homegardens
3	Blanco arroyo hondo	<i>O. matudae</i> Scheinvar	Zacatecas	Wild Stock
4	Café	<i>O. leucotricha</i> Salm-Dyck x <i>O. joconostle</i> F.A.C Weber	Zacatecas	Wild Stock
5	Cambray	<i>O. duranguensis</i> Britton and Rose	Zacatecas	Wild Stock
6	Chapeadito	<i>O. matudae</i> Scheinvar	Zacatecas	Wild Stock
7	Chaveñito	<i>O. sainaltense</i> Scheinvar	Zacatecas	Wild Stock
8	Cenizo	<i>O. oligacantha</i> Förster	Zacatecas	Wild Stock
9	Colorado	<i>O. matudae</i> Scheinvar	Zacatecas	Homegardens
10	Cuaresmeño 1	<i>O. matudae</i> Scheinvar	Zacatecas	Wild Stock
11	Cuaresmeño 2	<i>O. matudae</i> Scheinvar	Zacatecas	Wild Stock
12	Cuaresmero	<i>O. matudae</i> Scheinvar	Zacatecas	Wild Stock
13	Cuerón	<i>O. matudae</i> Scheinvar	Zacatecas	Wild Stock
14	Cultivar V	<i>O. reflexispina</i> sp. nov.	Zacatecas	Wild Stock
15	De Borrego	<i>O. oligacantha</i> Förster	Hidalgo	Homegardens
16	De Cerro	<i>O. megacantha</i> Salm-Dyck x <i>O. matudae</i> Scheinvar	Hidalgo	Wild Stock
17	De Invierno	<i>O. tezontepecana</i> Scheinvar and Gallegos	Hidalgo	Homegardens
18	Del Ranchito	<i>O. joconostle</i> F.A.C Weber	Zacatecas	Wild Stock
19	Güerito	<i>O. oligacantha</i> Förster	Zacatecas	Wild Stock
20	Manso	<i>O. joconostle</i> spp. Rubra	Hidalgo	Homegardens
21	Matizado	<i>O. leiascheinveriana</i> Martínez and Gallegos	Hidalgo	Homegardens
22	Rojo Sainero	<i>O. matudae</i> Scheinvar	Zacatecas	Wild Stock
23	Sardo	<i>O. galleguiana</i> Scheinvar and Olalde	Zacatecas	Wild Stock
24	Tempranillo	<i>O. joconostle</i> F.A.C Weber	Zacatecas	Wild Stock
25	Sweet prickly pear	<i>O. albicarpa</i> Scheinvar	Zacatecas	Cultivated

Table 2. Selected ISSR primers for the study of genetic diversity in 24 accessions of xoconostles and one prickly pear (outgroup).

Primer ¹	Sequence (5'-3')	Total bands	Polymorphic bands	PPB	Rp	PIC	MI
IS-01	AC(GACA) ₄	19	15	78.9	6.64	0.32	4.80
IS-02	(AC) ₈ YG	12	11	91.7	8.00	0.43	4.73
IS-03	(AGAC) ₄ GC	10	06	60.0	4.00	0.42	2.52
IS-05	(CA) ₈ RT	05	02	40.0	1.28	0.42	0.84
IS-06	(GA) ₈ YC	26	24	92.3	12.6	0.36	8.64
Total		72.0	58.0		32.6	1.95	21.5
Average		14.4	11.6	72.6	6.51	0.39	4.31

PPB, Percentage of polymorphic bands; Rp, resolving power; PIC, polymorphic information content; MI, Marker Index. ¹SIGMA Co products. Y substitutes C (cytosine) residue and T (thymine), R substitutes G (guanine) and A (adenine).

repetitions were considered for the corresponding analysis in order to ensure the absence of gel artefacts. Polymorphic DNA bands were scored as discrete variables "1" for the presence of a band and "0" for its absence and were transformed into a binary character matrix. Then, data were processed by FreeTree software (Version 0.9.1.5) to produce a genetic distance matrix using Dice coefficient, also known as the similarity coefficient of Nei and Li (1979). The resulting matrix was computed with the unweighed pair group method with arithmetic averaging algorithm (UPGMA) in

order to construct the dendrogram with 1000 bootstrap replicates. The Tree View 1.6.6 software was used to display the tree obtained.

The ability of the most informative primers to differentiate between accessions was assessed by the estimation of their polymorphic information content (PIC), marker index (MI) and resolving power (Rp). The PIC was calculated using the formula described by Roldán-Ruiz et al. (2000): $PIC_i = 2fi(1-fi)$, where PIC_i is the polymorphic information content of the primer i , fi the frequen-

cy of the present bands, and $(1 - fi)$ represent the frequency of the absent bands. The maximum value of PIC for dominant markers is 0.5 (De Riek et al., 2001). MI was calculated as $MI = PIC \times \text{number of polymorphic bands}$ and R_p (Prevost and Wilkinson, 1999) according to the following Gilbert et al. (1999) formula: $R_p = \sum I_b$, where I_b represents the information band and was calculated with the formula: $I_b = 1 - (2 \times |0.5 - p|)$, where p is the proportion of accessions containing B and I; as well as the percentage of polymorphic bands (PPB). The origin and the culture condition of the accessions under study were considered in order to examine their potential effects on genetic clustering.

RESULTS

DNA extraction

DNA isolated from the studied samples was appropriate for ISSR analysis because it presented high quantity and quality. The photometric measurement quotient (260/280) of DNA varied between 1.6 and 1.8, indicating high quality of genomic DNA. Moreover, the yield of DNA was about 50 ng/μL out of 300 mg of seed flour used.

ISSR analysis and genetic diversity

The five ISSR primers produced several polymorphic amplicons. A total of 72 bands were amplified and 58 revealed polymorphism (80.6%). The number of polymorphic bands varied from 2 (IS-05) to 24 (IS-06) with an average of 11.6 polymorphic bands per primer (Table 2). The size of the PCR products considered in the analysis ranged between 350 and 2500 bp. Polymorphism percentage of primers ranged from 40% (IS-05) to 92.3% (IS-06), with an average of 72.6%.

Estimating R_p values exhibited a total rate of 32.6 and ranged from 1.28 (IS-05) to 12.6 (IS-06) with an average of 6.51 (Table 2). Also, the primer IS-06 seems to be the most efficient for assessing genetic diversity as presented with a high rate of R_p . The PIC ranged from 0.32 (IS-01) to 0.43 (IS-02) with an average of 0.39 and the MI varied from 0.84 (IS-05) to 8.64 (IS-06) with an average of 4.31. Among the ISSR primers used, IS-06 was the most informative.

Genetic similarities and accessions relationships

Estimates of genetic relationship between accessions were obtained from marker data using the Dice similarity coefficient. Among the accessions analyzed, the genetic distance matrix displays an average distance range from 0.41 to 0.82 (Table 3). Therefore, the genotypes analyzed in this study are highly divergent at the DNA level. The smallest distance value (0.41) was observed between the 'Matizado' and 'Café' accessions (*O. leiascheinveriana* Martínez and Gallegos and *O. leucotricha* Salm-Dyck × *O. joconostle* F.A.C Weber).

The maximum distance value of 0.82, suggests great similarities, it was observed between several pairs of accessions: 'Sardo' (*O. galleguiana* Scheinvar and Olalde) and 'Cuaresmeño 1' (*O. matudae* Scheinvar); 'Blanco arroyo hondo' and 'Apan' (classified as *O. matudae* Scheinvar and *O. joconostle* F.A.C Weber × *O. matudae* Scheinvar, respectively); 'Rojo Sainero' and 'Colorado' (both classified as *O. matudae* Scheinvar); 'Blanco arroyo hondo' (*O. matudae* Scheinvar) and 'De cerro' (*O. megacantha* Salm-Dyck × *O. matudae* Scheinvar). All the remaining accessions showed different intermediate levels of similarity.

The UPGMA analysis (Figure 1) confirmed the genetic divergence described above (Table 3). In fact, the dendrogram exhibited six main groups (a, b, c, d, e and f). Furthermore, all genotypes were distinguished from each other suggesting the high level of genetic variation among xoconostle accessions studied here. The group "a" was composed of a single accession ('Matizado'); this accession was significantly divergent from all genotypes because it was separated into an independent group, suggesting that it was the more distinct accession at the genomic level (Figure 1 and Table 3). In addition, a high level of variation is obtained in the case of the "b", "c" and "f" groups, since they contain more different accessions (8, 5 and 7 accessions, respectively). The opposite situation is observed in the case of the "d" and "e" groups, with only 2 accessions. The groups do not contain accessions of the same specie; moreover, the dendrogram illustrates that grouping the accessions was also independent of the geographic region as genotypes housed in all groups did not differ significantly (Table 1), nor did those grouped according to the culture conditions, as they can include cultivated genotypes from homegardens and wild conditions in the same group (Table 1).

The PCA results showed that the first three PCA axes account for only 32.36% of the total variability. In fact, the first two principal components accounted for 23.36% of the variability, whereas the first component gave 12.95%. In general, the PCA analysis grouped the accessions into five groups: first [2, 3, 8, 10, 11, 13, 15, 16 and 23], second [1, 4, 6, 7, 9, 14, 18, 22 and 24], third [5, 12, 17, 19 and 20], and accession 21 (Matizado) and accession 25 (sweet prickly pear) independently.

DISCUSSION

Knowledge of genetic variability of the genus *Opuntia*, which also includes xoconostles, is of great importance to determine their diversification, to aid in germplasm installation and to determine their genetic potential in breeding programs (Zoghalmi et al., 2007; Caruso et al., 2010; Bendhifi et al., 2013). The DNA obtained from *Opuntia* seeds for genotyping purposes is a good alternative as they do not have mucilage (Labra et al., 2003; Montiel et al., 2012) and also because this tissue is

Table 3. Genetic distances matrix between 24 accessions of xoconostles and one prickly pear based on ISSR data and computed using dice coefficient.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1																								
2	0.61	1																							
3	0.63	0.82	1																						
4	0.63	0.49	0.62	1																					
5	0.54	0.70	0.79	0.62	1																				
6	0.63	0.63	0.59	0.68	0.62	1																			
7	0.65	0.68	0.70	0.78	0.74	0.74	1																		
8	0.54	0.72	0.79	0.59	0.78	0.66	0.65	1																	
9	0.68	0.75	0.79	0.62	0.70	0.69	0.74	0.70	1																
10	0.56	0.74	0.76	0.61	0.69	0.61	0.73	0.60	0.75	1															
11	0.55	0.69	0.79	0.49	0.59	0.43	0.49	0.69	0.69	0.65	1														
12	0.58	0.68	0.75	0.60	0.80	0.57	0.62	0.71	0.65	0.67	0.60	1													
13	0.44	0.63	0.65	0.50	0.66	0.58	0.60	0.66	0.66	0.71	0.61	0.63	1												
14	0.64	0.61	0.69	0.62	0.70	0.69	0.64	0.70	0.63	0.59	0.66	0.58	0.62	1											
15	0.53	0.74	0.73	0.51	0.69	0.68	0.63	0.71	0.75	0.65	0.61	0.64	0.80	0.66	1										
16	0.71	0.72	0.82	0.52	0.67	0.52	0.61	0.72	0.67	0.63	0.69	0.68	0.59	0.70	0.63	1									
17	0.56	0.54	0.62	0.46	0.69	0.54	0.57	0.60	0.63	0.62	0.54	0.57	0.58	0.69	0.58	0.63	1								
18	0.67	0.69	0.74	0.64	0.76	0.57	0.74	0.62	0.62	0.71	0.64	0.63	0.57	0.69	0.58	0.66	0.64	1							
19	0.72	0.76	0.74	0.51	0.70	0.59	0.64	0.67	0.67	0.63	0.66	0.74	0.59	0.67	0.66	0.73	0.59	0.76	1						
20	0.60	0.69	0.70	0.54	0.78	0.61	0.67	0.63	0.63	0.68	0.48	0.73	0.65	0.69	0.68	0.60	0.68	0.68	0.75	1					
21	0.55	0.50	0.56	0.41	0.67	0.50	0.49	0.60	0.56	0.48	0.42	0.71	0.55	0.48	0.55	0.56	0.62	0.49	0.59	0.69	1				
22	0.61	0.61	0.71	0.76	0.70	0.72	0.74	0.70	0.82	0.69	0.56	0.62	0.63	0.67	0.69	0.55	0.56	0.66	0.57	0.60	0.53	1			
23	0.61	0.80	0.78	0.59	0.72	0.75	0.74	0.67	0.78	0.82	0.66	0.76	0.69	0.67	0.77	0.67	0.63	0.63	0.75	0.71	0.60	0.69	1		
24	0.52	0.60	0.63	0.62	0.67	0.65	0.71	0.57	0.67	0.62	0.51	0.71	0.55	0.56	0.56	0.57	0.59	0.54	0.56	0.62	0.63	0.60	0.73	1	
25	0.50	0.71	0.75	0.42	0.63	0.49	0.55	0.63	0.60	0.70	0.68	0.61	0.59	0.54	0.65	0.69	0.65	0.55	0.60	0.59	0.52	0.49	0.71	0.56	1

Labels 1 to 25 correspond to the accessions mentioned in Table 1.

genetically similar to the mother plant. This is due to the main type of reproduction that occurs in this group of plants known as apomixis (Mondragón-Jacobo, 2003). The yield and quality of the DNA obtained in this study was appropriate for molecular analysis using PCR as reported by Luna-Paez et al. (2007) who have worked with different *Opuntia* varieties.

ISSR profiles obtained were informative and clear, so this technique proved to be very useful for estimating genetic variation in xoconostle accessions, and this technique has also been reported in many other studies at species and variety levels (Behera et al., 2008; Badfar-Chaleshtori et al., 2012). In our study, each ISSR primer fingerprinted each accession. The five

ISSR markers used provided a high total Rp (32.6), MI (4.31) and high average PPB (80.6%), which indicates a wide genetic variation; this assumption strongly supports the genetic distances obtained between the xoconostle accessions (0.41-0.82). The accessions studied here are less domesticated or wild types, and still retain their high level of genetic variability as a

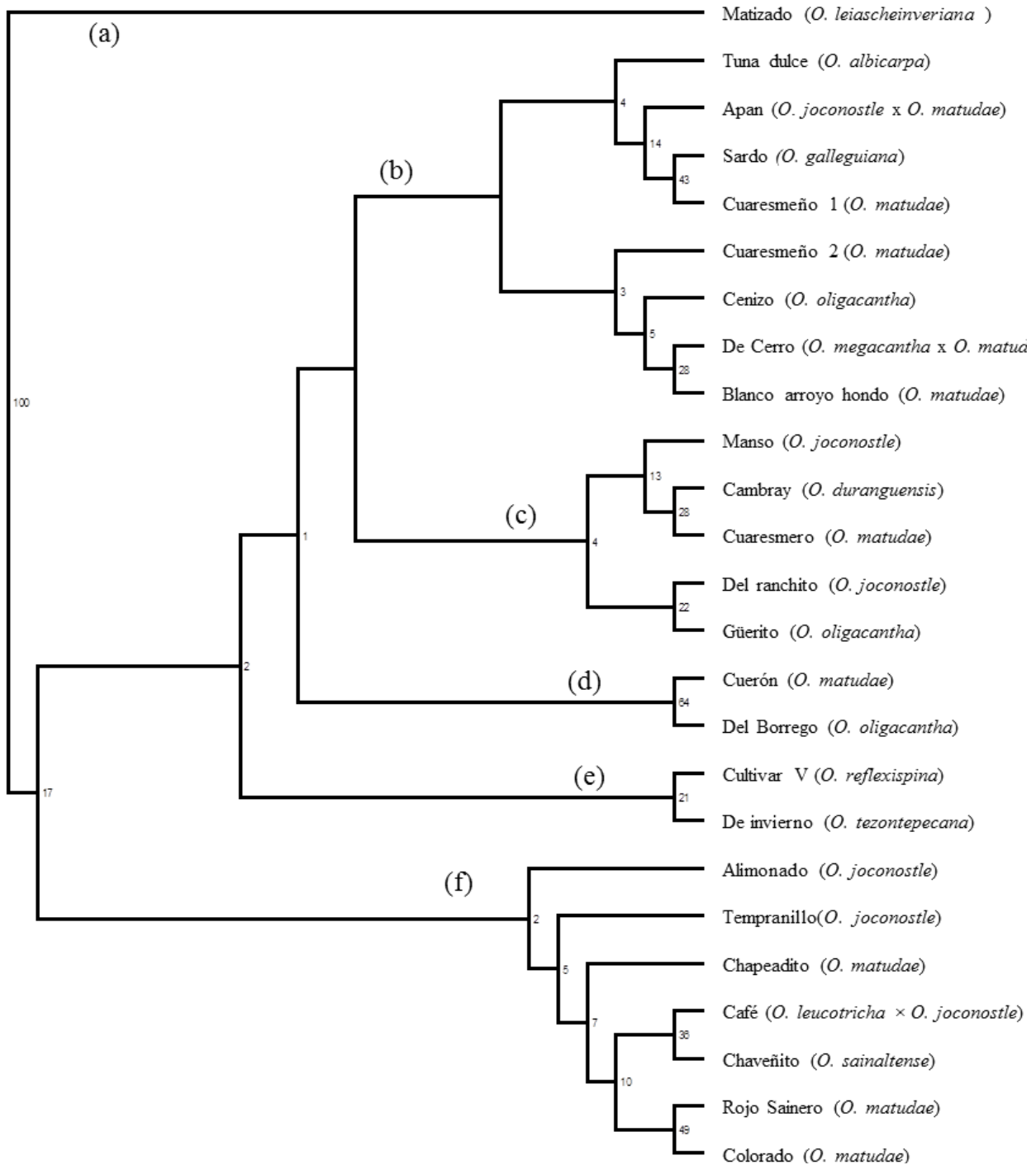


Figure 1. Dendrogram derived from UPGMA cluster analysis using Dice coefficient of ISSR markers in 24 accessions of xoconostle and one of sweet prickly pear. The numbers of the nodes indicate the percentage obtained with 1000 bootstrapping.

result of not having been subject to breeding programs.

Genomic characterizations and the corresponding analysis reported here demonstrate their usefulness in estimating genetic variation in less time, and with more efficiency, compared to morphological descriptors.

Gallegos-Vázquez et al. (2011) characterized the majority of these same xoconostle accessions with morphological descriptors and were unsuccessful in grouping them by similarity, by taxonomical groups (species) or by origin. In this sense, it is possible that the high genomic variation

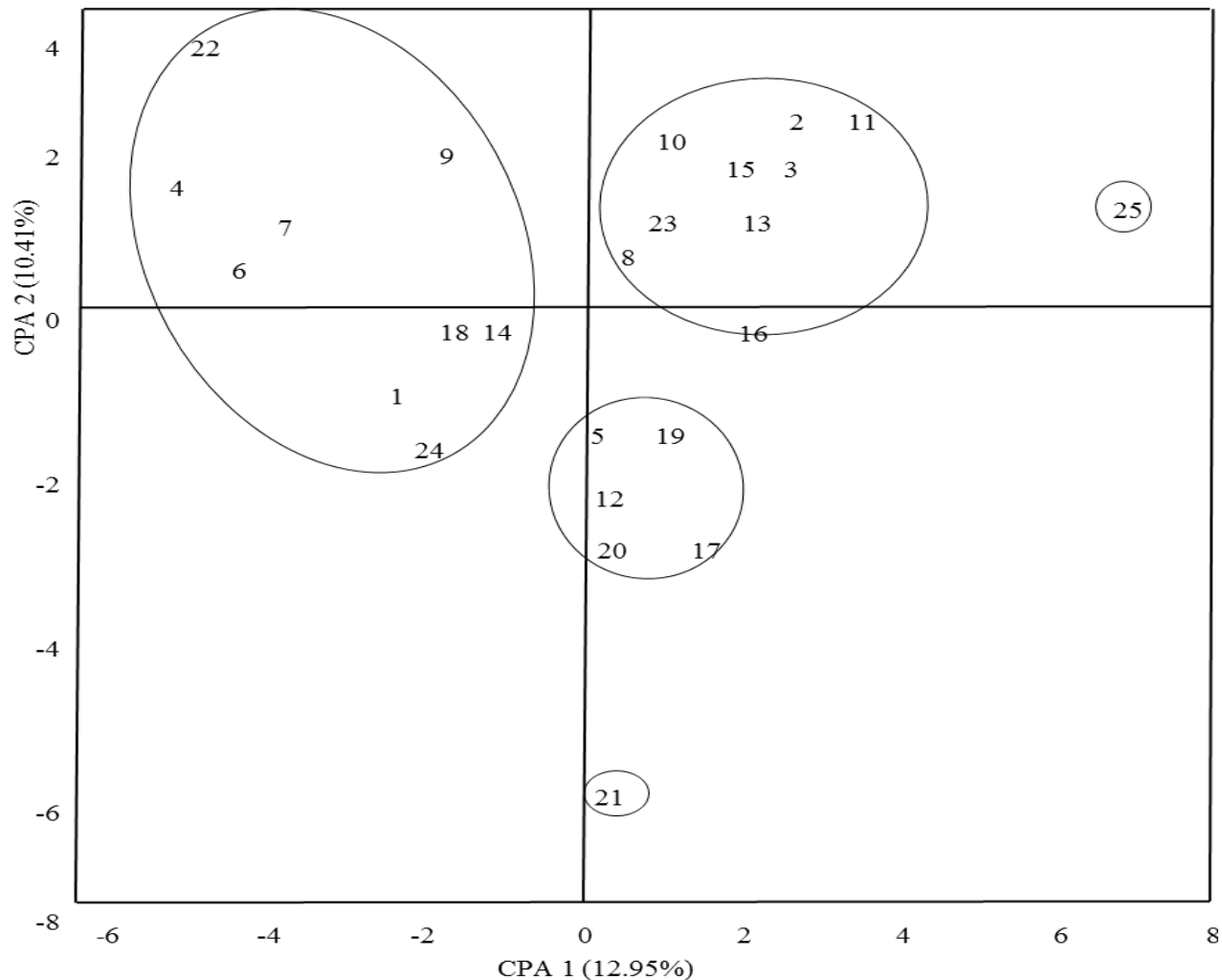


Figure 2. Results of PCA plotted along the first two-discriminant axes for the 24 accessions of xocnostles and one prickly pear produced by five ISSR markers.

detected in these plants with molecular genetic markers, may be due to frequent mutations, and that they are also favored by exchange and dispersion of genotypes among different geographic areas, which is the principal source of variation in this type of plants with asexual reproduction. However, other aspects such as the high level of phenotypic plasticity and polyploidy level should also be considered (Scheinvar, 1999; Wallace and Gibson, 2002; Caruso et al., 2010).

The UPGMA analysis (Figure 1) evidenced the degree of genetic divergence and separated the accessions into six major groups; however, the outgroup (prickly pear) did not separate as expected, since these two types of *Opuntias*, ranked as different species, share similar amplified DNA fragments with some similar primers (data not shown). Similar behavior was found by Luna-Paez et al. (2007), where the xocnostle accessions were housed in sister groups of prickly pear without showing greater genetic distinction to characterize with RAPDs and

ISSRs. Also, clusters formed (Figure 1) were independent of the geographical origin, and showed no significant divergence in this regard (Table 1). Likewise, in other studies with prickly pear, the same results were found (Wang et al., 1998; Caruso et al., 2010). None were grouped by culture condition, probably because the xocnostles studied were not the product of a breeding program, but only a product of collection and selection. In fact, farmers are responsible for collecting cladodes of genotypes that they like, because of their taste, and for cultivating them in homegardens or intensive crops depending on their financial resources.

In the PCA analysis (Figure 2), the prickly pear and "Matizado" were separated from the other accessions; this was mainly because this analysis requires few highly informative characters to establish separation. Particularly, 'Matizado' accession proved to be the material with most genetic variation (-6.94 in Eigenvectors axis 2, Figure 2) compared to remain accessions, and thus re-

presents a good example for further studies.

It is noteworthy that our results did not allow checking the hybrid nature of the accessions referred to as "hybrids" in the present study. For this, we contrasted the genetic profiles of the "hybrids" with profiles of the putative parentals represented in the same group of accessions (data not shown). There was no profile to indicate the type of inheritance of the markers from the putative parents in either intra- or interspecific hybrids. In this sense, there is not a scientific report yet where hybrids of xocconostles are reported. Nevertheless, the molecular markers, like ISSR, have been used for this purpose in plant studies (Wolfe et al., 1998b; Košnar et al., 2010).

In another study, Ruas et al. (2003) revealed the efficiency of markers in the genetic differentiation between *Coffea* species and the parents identification of interspecific hybrids; and even, for *Opuntia*, Helsen et al. (2009) tried to demonstrate the occurrence of hybrids between two varieties of *O. echios* (*echios* and *gigantea*) in the Galapagos Islands, that showed intermediate morphological characters, using microsatellites, without success. Obviously, to test the hybrid nature of any organism, further analysis are necessary, such as comparative morphological analysis of the parentals and hybrids, reproductive analysis, cytogenetic and DNA markers (Lau et al., 2005).

Finally, *Opuntia* species, including those producing xocconostle, are known for their taxonomic difficulty. Different studies based on molecular markers have suggested that the current taxonomy of many species of *Opuntia* is erroneous (Luna-Paez et al., 2007; Zoghlami et al., 2007; Helsen et al., 2009; Souto-Alves et al., 2009; Caruso et al., 2010; Majure et al., 2012). However, the question remains whether the phenotypic differences observed in this group of plants are caused by cross-pollination, adaptive genetic response to environmental differences, phenotypic plasticity, epigenetic bases, or by somatic mutations occurring in time and space resulting of the wide genomic variation observed. All these questions have caused confusion among taxonomists; but in this study our results did not confirm that the xocconostle accessions represent several species or hybrids; but rather, maybe only few (or maybe only one) species with high genetic variability.

Conclusion

In this study, we used molecular tools to investigate for the first time the genetic background of xocconostle accessions belonging to *Opuntia*, since the importance of these fruits are increasing in Mexico. The ISSR technique is useful in genomic characterization of this type of plants and certainly shows its effectiveness for the discrimination of elite genotypes. In fact, the most studied accessions showed divergence at the DNA level and represent good candidates to be preserved in germplasm banks.

Firstly, to set up a definitive taxonomic sorting, which could be of great interest for breeders; and second, to select interesting genotypes that could to be included in human diets. Similarly, ISSR markers are helpful to better support the taxonomic position of the accessions, in addition to identifying hybrids. A clear conclusion is that the xocconostles studied here were not grouped according to their current taxonomic classification, and therefore it is necessary to reconsider the biological specie concept. None of the xocconostle accessions were grouped based on the place of origin or on the crop condition, but rather they were grouped based on genomic similarities and differences accumulated over time.

ACKNOWLEDGEMENTS

This work was supported by UACH and SNICS–UACH, México. We are grateful to Dr. Clemente Gallegos-Vázquez through the "Red Nacional de Nopal" and Dr. Leia Sheinvar (Jardín Botánico Exterior, UNAM) also, for providing the study materials. The authors specially acknowledge the anonymous referees for their comments on this paper.

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