Potential role of transposable elements to differentiate tuna and xoconostle *Opuntia* varieties

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ABSTRACT

Opuntia, an important horticultural crop in Mexico, is cultivated mainly for its two fruits variants: sweet fruits or prickly pears (*tunas*) and acidic fruits (*xoconostles*). The inter-retrotransposon amplified polymorphism (IRAP) technique was applied to evaluate genetic diversity of *Opuntia* varieties and to differentiate xoconostle fruits from tunas. Five IRAP primers previously described for other plant species and classified into three retrotransposon families, namely Copia, Gypsy and TRIM, were analysed in 43 *Opuntia* varieties (eight xoconostles and 35 tunas). The five individual IRAP primers generated a total of 264 fragments, where 64.8 % of them were polymorphics. The retrotransposon of the Gypsy family (60 fragments) was more represented than Copia (average of 52 fragments) or TRIM (48 fragments) families. Moreover, the percentage of polymorphic fragments was higher (61.9 %) in xoconostles than in tunas (56.5 %). A larger number of total amplified fragments (262) was found among tunas, compared to those amplified from xoconostle varieties (257 fragments). In contrast, a lower number of polymorphic bands were counted among tunas (148) than among xoconostle varieties (159). Unlike the UPGMA analysis, where three of the xoconostle-producing varieties were grouped with other tunas, the PCoA analysis allowed a better separation of all xoconostle varieties. These results suggest a potential role of the transposable elements in genetic divergence within the *Opuntia* genus.

***Keywords*:** prickly pear, tunas, transposable element, IRAP markers, xoconostles.

INTRODUCTION

Opuntia sensu stricto (“nopal” in Mexico or “prickly pear” worldwide) is the largest genus in Opuntioideae, Cactaceae family (Anderson, 2001). About 150 to 180 species are reported within the genus, of which 66-83 are found in Mexico (Stuppy, 2002; Hunt, 2006). Numerous *Opuntia* genotypes are cultivated throughout the world as fruit crops. They are also grown for forage in arid areas throughout the world, mainly in regions of Brazil and Mexico, and to a lesser extent in western Asia and Africa. In Mexico, *Opuntia* species have been cultivated for at least 14,000 years (Casas and Barbera, 2002). The Aztec and Mesoamerican civilizations used the cladodes for consumption as vegetables and/or forage, but mainly for seasonal fruit.

Opuntia species that produce xoconostles or tunas (prickly pears) are succulent and xerophyte plants. There are two types of fruits, depending mainly on the total sugar content: sweet fruits (tunas) or acid fruits (xoconostles). The *xoconostles* (from Nahuatl: xoco = acid, noxtle = tuna) have a thick inner edible wall and a thin outer wall that is not easily detached. The name *xoconostle* is used indifferently to refer to acidic fruits as well to *Opuntia* plants that produce such fruits (Reyes-Agüero *et al.,* 2005; Scheinvar *et al.,* 2009; Samah and Valadez-Moctezuma*,* 2014). In contrast, *tunas* are fruits with abundant pulp, generally sugary and thin. Their seeds are distributed almost throughout the fruit, and the funicles are sweet. During the past two decades, interest in xoconostles and tunas has increased, and they are becoming formal crops due to their nutritional and functional properties. The main uses for both fruits are in stews, as a condiment, dried, as sweetened fruits, as the basis for soft drinks, or as raw material in the production of wines, liquors, jams and jellies. They are also considered an alternative natural medicine due to their antihypoglycemic, antioxidant and cancer prevention effects (Chavez-Santoscoy *et al.,* 2009).

However, the separation between tuna and xoconostle fruit types is still contradictory. Morphologically, there is a clear separation between the two fruit types. Scheinvar *et al*. (2009) reported ten Opuntia species that produce xoconostle fruits; nine of these belong to the genus *Opuntia*, and one more species to the genus Cylindropuntia. However, studies on genetic diversity based on molecular markers revealed that genetic differences between both fruit types are narrow. Results form biochemical markers (Samah *et al.,* 2015), RAPD and ISSR (Valadez-Moctezuma *et al.,* 2015), and SSR markers (Samah *et al.,* 2016) showed a trend of separation between tunas and xoconostles. On the contrary, Espinoza Sánchez *et al.* (2014) did not find any trend of separation between these two types of fruits using AFLP markers.

Transposable elements (TEs) are DNA sequences that can insert into new chromosomal locations and often duplicate copies of these in the process. TEs are the single largest constituent of most eukaryotic genomes, although active elements comprise only a small minority of the genomic TE complement in most multicellular organisms (Oliver *et al.,* 2013; Rey-Baños *et al.,* 2017). The plant genome percentage represented by TEs in assembled genomes was found to range between 7 % of *Populus trichocarpa* to 75 % of *Zea mays* (Rey-Baños *et al.,* 2017). TEs in eukaryotes are divided into two classes, depending on whether their transposition intermediate is RNA (class 1, or retrotransposons) or DNA (class 2). Class 1 is classified into two groups depending on the presence or not of long terminal repeats (LTRs), LTR retrotransposons and non-LTR retrotransposons (Kumar and Bennetzen, 1999). LTR retrotransposons (LTR-RTs) are further classified into Ty1-copia and Ty3-gypsy families that differ from each other in their sequences similarity degree and encoded gene product order. For plants, there are two groups of this LTR-RTs, LARDs (Large Retrotransposon Derivatives) and TRIMs (Terminal-repeat Retrotranposons in Miniature) (Witte *et al.,* 2001; Kalender *et al.,* 2004; Wicker *et al.,* 2007; Yin *et al.,* 2014), which include the recently described Cassandra family (Kalendar *et al.,* 2008).

The ubiquity, abundance, dispersion, and dynamism of LTR retrotransposons in plant genomes have made them excellent sources of molecular markers (Kalendar and Schulman, 2006). The methods for their detection, generally based on PCR, consider one conserved retrotransposon site and another abundant, dispersed and conserved site whitin the genome. These second sites could be a restriction site adapter in sequence specific amplified polymorphism (SSAP; Waugh *et al.,* 1997), a microsatellite in retrotransposon-microsatellite amplified polymorphism (REMAP; Kalendar *et al.,* 1999), or another retrotransposon in inter retrotransposon amplified polymorphism (IRAP; Kalendar *et al.,* 1999; Kalendar and Schulman, 2006). All these molecular markers produce dominant markers, and their alleles do not allow distinguishing between homozygous and heterozygous loci (Vukich *et al.,* 2009).

The replicative activity of retrotransposons has made them a major force in genome diversification through insertion and recombinational loss (Kalendar *et al.,* 2000; Hawkins *et al.,* 2008; Vukich *et al.,* 2009). Given this characteristic, retrotransposon-based marker methods seem attractive to be used in Opuntia; however, these elements have not yet been identified in this genus. In this study, the IRAP technique based on three retrotransposons families (Copia, Gypsy and TRIM), was used to determine the genetic relationships between 43 varieties of Opuntia and to evaluate the ability of transposable elements to separate xoconotles from tuna fruit types.

MATERIALS AND METHODS

Plant Material and IRAP Analysis

Samples of 43 *Opuntia* varieties (35 tunas and 8 xoconostles) were obtained from the Unversity of Chapingo´s Cactus Pear Experimental Orchard (Texcoco, Mexico) germplasm bank in Mexico (Table 1). Distinction between varieties was based upon morphological characteristics and species taxonomic classification, according to Scheinvar *et al.,* 2009. Total genomic DNA was extracted using the CTAB method (Luna-Paez *et al.,* 2007). The DNA quantification was estimated by spectrophotometry (ND-1000 Thermo scientific, USA), and DNA quality was determined in 1 % agarose gels.

Five individual IRAP primers were used, one Cassandra primer (TRIM family) 5' ATTTGGATGGGTGACCTTCTGGGA 3', one Peabody primer (Gypsy family) 5' GTGTGACACCCTTGAATTGCATGT 3', both of them described in *Lens culinaris* (Rey-Baños *et al.,* 2017); and three primers from Copia family, namely Angela-like 5' ATCATGCCCCTTCGTAAGGATCAC 3' described in *Acacia*, *Delonix*, *Pisum*, *Cassia*, *Vicia*, and other species (BLAST search 2017); Glycine 5' GGCGCAATGCACTTTCTAGGTGTT 3' described in *Lens culinaris* (Rey-Baños *et al.,* 2017) and BARE-1 primer 5' TGTTTCCCATGCGACGTTCCCCAACA 3' described en *Hordeum*.

All PCRs were carried out in a final volume of 12.5 µL containing nuclease-free water, 500 mM dNTPs, 1×Taq buffer, 25 mM $MgCl_2$, 20 *p*mol primer, 1.5 U Taq DNA polymerase (Promega), and 50 ng of DNA. The thermocycling conditions (Maxy Gene Thermel Cycler, Applied Biosystem, USA) were as follows: one 2-min cycle at 94 °C, 35 cycles [94 °C for 30 s;

an annealing step for 1 min at 40 °C; 72 °C for 2 min], and one final extension cycle at 72 °C for 7 min.

Table 1. *Opuntia* varieties and their corresponding species analysed for this study.

No. [1]	Common Name	Scientific Name	No.	Common Name	Scientific Name
1	Amarilla Milpa Alta	*Opuntia ficus-indica* (L.) Mill.	23	Pico Chulo	*Opuntia megacantha* Salm-Dyck
2	Amarilla Miquihuana	*Opuntia lasiacantha* Pfeiff.	24	Rojo Azteca	*Opuntia megacantha* Salm-Dyck
3	Amarilla Montesa	*Opuntia megacantha* Salm-Dyck	25	Rojo Pelón	*Opuntia ficus-indica* (L.) Mill.
4	Amarilla Plátano	*Opuntia megacantha* Salm-Dyck	26	Sanjuanera	*Opuntia lasiacantha* Pfeiff.
5	Amarilla Zacatecas	*Opuntia megacantha* Salm-Dyck	27	Sangre de Toro	*Opuntia megacantha* Salm-Dyck
6	Bola de Masa	*Opuntia albicarpa* Scheinvar	28	Solferino	*Opuntia ficus-indica* (L.) Mill.
7	Burrona	*Opuntia albicarpa* Scheinvar	29	Tapón Aguanoso	*Opuntia robusta* H.L. Wendl.
8	Cardón Blanco	*Opuntia streptacantha* Lem.	30	Tapona de Mayo	*Opuntia robusta* H.L. Wendl.
9	Charola Tardía	*Opuntia hyptiacantha* F.A.C. Weber	31	Tobarito	*Opuntia megacantha* Salm-Dyck
10	Chicle	*Opuntia ficus-indica* (L.) Mill.	32	Torreoja	*Opuntia megacantha* Salm-Dyck
11	Color de Rosa	*Opuntia albicarpa* Scheinvar	33	Tuna Mansa	*Opuntia albicarpa* Scheinvar
12	Copena CEII	*Opuntia ficus-indica* (L.) Mill.	34	Tuna Rosa	*Opuntia albicarpa* Scheinvar
13	Copena T12	*Opuntia ficus-indica* (L.) Mill.	35	Villanueva	*Opuntia albicarpa* Scheinvar
14	Copena V1	*Opuntia ficus-indica* (L.) Mill.	36	X. Alimonado	*Opuntia joconostle* F.A.C. Weber
15	Copena Z1	*Opuntia albicarpa* Scheinvar	37	X. Blanco	*Opuntia joconostle* F.A.C. Weber
16	Cristalina	*Opuntia albicarpa* Scheinvar	38	X. Cenizo	*Opuntia joconostle* O. oligacantha
17	Gavia	*Opuntia albicarpa* Scheinvar	39	X. Cerro Blanco	*Opuntia joconostle* F.A.C. Weber
18	Larreguin	*Opuntia ficus-indica* (L.) Mill.	40	X. Chivo	*Opuntia durangensis* Britton y Rose
19	Mango	*Opuntia albicarpa* Scheinvar	41	X. Colorado	*Opuntia joconostle* F.A.C. Weber
20	Memelo	*Opuntia affinis hyptiacantha*	42	X. Borrego	*Opuntia joconostle* F.A.C. Weber
21	Milpa Alta	*Opuntia ficus-indica* (L.) Mill.	43	X. Manzano	*Opuntia joconostle* F.A.C. Weber
22	Naranjón Legítimo	*Opuntia albicarpa* Scheinvar			

[1] Numbers 1 to 35 indicate prickly pear varieties, and 36 to 43 are xoconostle types.

Three µL of the PCR products were mixed with 3 µL loading buffer containing blue and yellow dyes (5× Green GoTaq® Flexi Buffer), denatured at 95 °C for 5 min and then separated on 8 % polyacrylamide gel. The molecular weights of amplified fragments were estimated with 100 bp and 1 kb DNA ladders (Promega, USA). The electrophoresis was carried in a Dual MGV-216-33 vertical electrophoresis gel system (CBS, USA), with 220 V applied for 2 h in 1×TBE buffer (89 mM Tris-borate, 2 mM EDTA pH 8). Silver nitrate solution ($AgNO_3$) 0.2 % was used to stain DNA fragments.

Data analysis

Clear and reproducible IRAP fragments were visually identified and transformed to a binary matrix where “1” represented the presence of a band and “0” the absence of the band. For each primer, number of total bands (NTB), number of and percentage of polymorphic bands

(NPB and PPB), polymorphic information content (PIC), Marker index (MI) and resolving power (Rp), were calculated. The PIC was calculated using the formula described by Roldán-Ruiz *et al.* (2000): PIC*i* = 2f*i* (1 - f*i*), where PIC*i* is the polymorphic information content of the primer *i*, f*i* the frequency of the bands present, and (1 - f*i*) the frequency of bands absent. MI was calculated as: MI = PIC *x* polymorphic bands and Rp according to Gilbert *et al.* (1999) as Rp = ∑*I*b where Ib represents band information. It was calculated with the following formula: *I*b = 1 - (2 x |0.5 - *p*|), where *p* is the proportion of accessions containing band *I*.

Based on the basic matrix of absence/presence, the genetic similarity based on the Jaccard genetic similarity index was calculated between the 43 opuntia varieties using the SIMQUAL subprogram in NTSySpc 2.21o (Rohlf, 2002). Cluster analysis was performed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in the SAHN subprogram in NTSySpc. FigTree 1.4.2 program (Rambaut, 2014) was used to edit the dendrogram. Molecular variance analysis (AMOVA) and principal coordinate analysis (PCoA) based on the genetic similarity matrix were performed with GenAlEx 6.501 software (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION

IRAP analysis and genetic diversity

The IRAP technique allows detecting genomic loci delimited by LTR retrotransposons. In the IRAP technique, the use of a single primer allows the amplification of PCR products if two similar elements are oriented head-to-tail, or two different primers to recognize different regions head-to-head LTRs (Kalendar *et al.,* 1999; Kalendar and Schulman, 2006). In the present study, due to the absence of partial and complete sequencing of the Opuntia genome, individual IRAP primers previously published in other plant species (Kalendar *et al.,* 1999; Rey-Baños *et al.,* 2017) were used. Obtaining clear profiles and variable percentages of polymorphisms confirms the possibility of transferring this type of markers between plant species (Alavi-Kia *et al.,* 2008; Sun *et al.,* 2015; Tomás *et al.,* 2016).

Five individual IRAP primers generated a total of 264 clear and reproducible fragments in 43 Opuntia varieties, of which 171 (64.8 %) were polymorphic. These values indicate the presence of transposons (and maybe their activity). The number of fragments generated per primer ranged from 48 for the Cassandra primer to 60 for the Peabody primer, and an average of 52.8 bands/primer, revealing percentages of polymorphic bands ranging from 49.1 % (BARE) to 83.3 % (Peabody) with a general average of 64.8 %. Estimated PIC values ranged from 0.1 (Angela-like) to 0.25 (Peabody) with an average of 0.19. Estimated values of Rp ranged from 11.8 (BARE) to 20.2 (Peabody) with an overall average of 15.3. In addition, estimated values of MI varied from 3.0 (Angela-like) to 12.5 (Peabody) with a general average of 6.4. PIC and MI values are a reflection of the allelic diversity among the genotypes analysed. Caruso *et al.* (2010) reported a similar value of PIC (0.11 to 0.25) when they analysed Opuntia accessions with SSR markers. Samah *et al.* (2016) quantified higher PIC (0.25) and lower MI (2.86) values when they studied a collection of 88 Opuntia accessions

with 13 SSR markers. Considering MI values and level of polymorphism, two "Peabody" (Gypsy family) and "Glycine" (Copia family) markers could be considered more informative because they showed higher MI values than the average (6.4) and generated more than 35 polymorphic bands (Table 2).

Regarding the frequency of presence of transposable elements in Opuntia (Table 2), the Gypsy family (50 polymorphic bands of the 60 bands obtained) was more represented than Copia family (31.3 polymorphic bands of the 52 bands obtained, average of three IRAP markers). Similar results were reported in other plant species (Vukich *et al.,* 2009; Rey-Baños *et al.,* 2017). On the other hand, Cassandra's retrotransposon (TRIM family) was the less exemplified in Opuntia, considering the low number of amplified bands. This can be explained by the fact that TRIM retrotransposons tend to have a preferential distribution in the genome (Gao *et al.,* 2016), unlike other families which, in addition to being abundant and distributed randomly, move along the genomes of plants, as in the case of maize (Meyers *et al.,* 2001). In our case, the five retrotransposon primers used in 43 varieties of tuna (eight species) and xoconostle (two species) amplified numerous fragments, suggesting that the primers are not species-specific as expected, since these retrotransplants are implemented from other plant species.

Table 2. List of IRAP markers used for the analysis, numbers of bands, PIC and MI values obtained from each primer and Opuntia type.

	Opuntia types	**Tunas (35 varieties)**			**Xoconoslte s (8 varieties)**			**Total (43 varieties)**					
IRAP Primers (Family)	**Band size**	**NTB**	**NPB**	**PPB**	**NTB**	**NPB**	**PPB**	**NTB**	**NPB**	**PPB**	**Rp**	**PIC**	**MI**
Angela-like (*Copia*)	100-3000 pb	51	21	41.2	51	30	58.8	51	30	58.8	13.4	0.1	3
BARE (*Copia*)	200-2000 pb	55	24	43.6	54	22	40.7	55	27	49.1	11.8	0.15	4.1
Glycine (*Copia*)	150-1500 pb	48	33	68.8	49	36	73.5	50	37	74	16.2	0.23	8.5
Peabody (*Gypsy*)	250-1500 pb	60	47	78.3	56	46	82.1	60	50	83.3	20.2	0.25	12.5
Cassandra (TRIM)	200-1500 pb	48	23	47.9	47	25	53.2	48	27	56.3	14.7	0.2	5.4
Average		52.4	29.6	56.5	51.4	31.8	61.9	52.8	34.2	64.8	15.3	0.19	6.4
Total		262	148		257	159		264	171				

NTB number of total bands, NPB number of polymorphic bands, PPB percentage of polymorphic bands, Rp resolving power, PIC polymorphic information content, MI Marker Index

On the other hand, comparing the results of analyses from tuna and xoconostle Opuntia types, a greater number of amplified fragments (markers) were recorded: 262 from tuna varieties and 257 fragments from xoconostle varieties. On the contrary, a lower number of polymorphic bands were counted among tuna varieties (148 polymorphic bands) than among xoconostle varieties (159 polymorphic bands). In particular, the same number of markers was calculated from the Angela-like retrotransposone, between tunas and xoconostles. BARE and Cassandra retrotransposons generated one more fragment in tunas than in xoconostles, and the Peabody marker generated four more fragments in tunas. Glycine marker generated one

more fragment in xoconostles (Table 2). The Peabody marker (Gypsy family) was the most effective to differentiate varieties of each Opuntia type. The average polymorphism percentage of the five retrotransposons was higher (61.9 %, ranging from 40.7 to 82.1 %) in xoconostles than in tunas (56.5 %, ranging from 41.2 to 78.3 %). If we assume that a polymorphic band may be related to a retrotransposon insertion, this result suggests that retrotransposon activity is greater in xoconostles than in tunas. The total large number of IRAP markers observed during this study is explained because it has been known that transposable elements produce changes in genomes and can explain much about the diversification and the plants' evolution; in addition, a wide variety of changes to the expression and function of the genes, aspects that are recognized as facilitators of evolution (reviewed by Lisch, 2013 and Oliver *et al.,* 2013).

Genetic similarities, taxonomic relationships and molecular variance

Each variety of the 43 studied here presented a unique IRAP pattern, suggesting that none of them is a vegetative clone or a duplicate. Although a relatively low average PIC value was found during this study (0.19), the diversity of IRAP markers proved to be a reliable tool to discriminate all varieties. The estimated genetic similarity based on Jaccard index among the 43 varieties of *Opuntia* varied from 0.465 (between the X. Borrego and Bola de Masa varieties) to 0.894 (between the Sanjuanera and Sangre de Toro varieties). Among tuna varieties, the genetic similarity ranged between 0.635 (between Villanueva and Bola de Masa) and 0.894 (between Sanjuanera and Sangre de Toro). Meanwhile, among xoconostle varieties, the genetic similarity was greater (0.819) between X. Blanco and X. Colorado, and smaller (0.537) between X. Chivo and X. Borrego.

The relationship between the 43 *Opuntia* varieties was determined by UPGMA analysis. The grouping showed a moderate relationship to the similarity matrix as reflected by the cofenetic correlation coefficient (r = 0.89). This value was higher than that reported by Samah *et al.* (2016) (r = 0.72) when they evaluated 88 *Opuntia* accessions with 13 SSR markers. The UPGMA dendrogram showed the formation of seven groups (Figure 1). The largest group (A) formed by 30 varieties, all tuna *Opuntia* types. It was difficult to subdivide this group since very low bootstrap values were registered. Group B was formed with eight varieties; five of them were tunas (Tuna Mansa, Tuna Rosa, Tobarito, Torreoja and Villanueva) and three were xoconostle types (X. Blanco, X. Colorado and X. Manzano). The remaining five groups were formed, each of them, by an individual variety of the xoconostle type, namely: group C (X. Cerro Blanco), D (X. Chivo), E (X. Borrego), F (Cenizo), and group G (X. Alimonado). The grouping of varieties did not agree with the morphological characteristics of the fruits because both fruit types were no longer totally differentiated; these results can suggest, again, as the previous reports using molecular markers, that the genome of all varieties of *Opuntia* considerated in this study is structurally very similar, but with high variation. Perhaps this is the principal reason why *Opuntia* taxonomy classification did not agree at species level, as previously reported by Labra *et al.* (2003), Helsen *et al.* (2009), Caruso *et al.* (2010), and Valadez-Moctezuma *et al*. (2015).

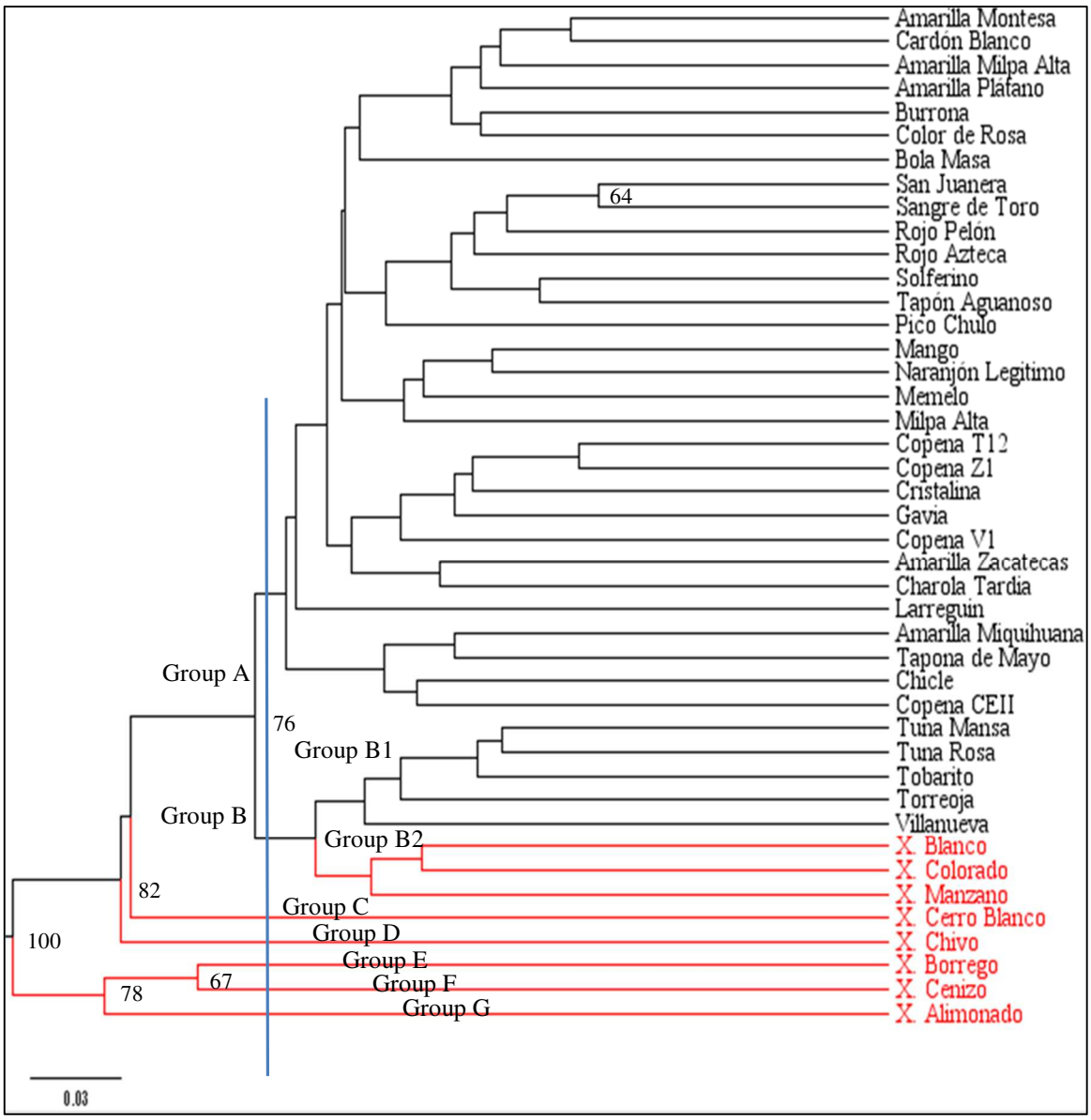

Fig. 1. UPGMA tree of 43 Opuntia varieties based on Jaccard coefficient calculated from five IRAP markers. Black and red colors indicate tuna and xoconostle varieties, respectively. Vertical line indicates the division between groups. Bootstrap values higher than 50 are indicated in the figure.

The PCoA multivariate analysis was used to confirm further this result (Figure 2). PCoA revealed that the first three coordinates explained 43.77 % of the total variability. The first two coordinates accumulated a variability percentage of 24.25 and 10.29 %, respectively. Unlike the UPGMA grouping, where three of the xoconostle type varieties were grouped in group B along with other tuna types, the two types of Opuntia (tunas and xoconostles) were separated clearly in the PCoA analysis on the first principal coordinate, where a greater dispersion was observed for xoconostle varieties than for tuna varieties (Figure 2).

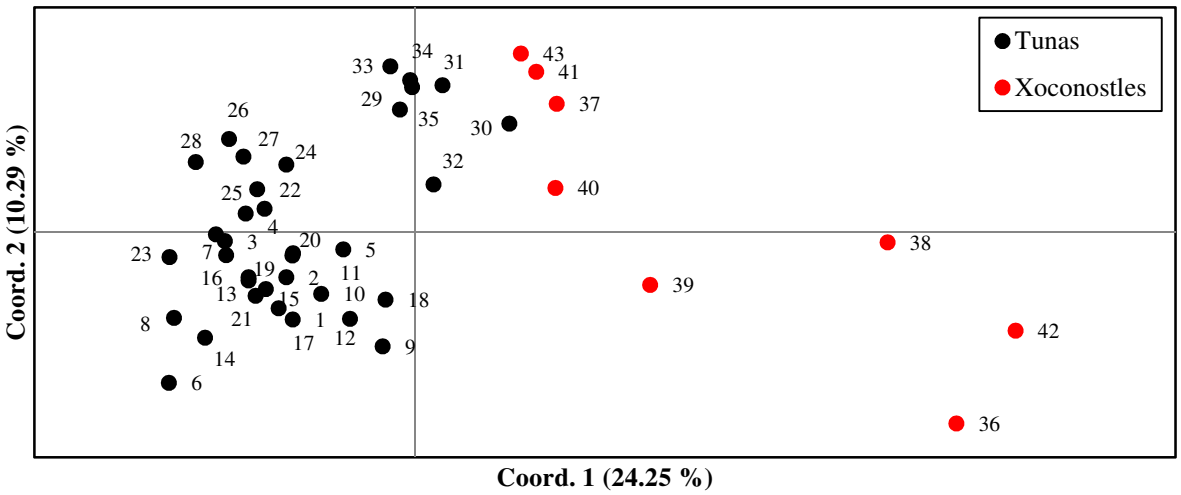

Fig. 2. Two-dimensional plot obtained from principal coordinate analysis of 43 Opuntia varieties using five IRAP markers. Numbers refer to varieties indicated in Table 1. Black and red colors of the filled circle indicate tuna and xoconostle varieties, respectively.

The genetic difference between the two main Opuntia types was estimated using the molecular analysis of variance (AMOVA). This statistical tool is an effective method to estimate the genetic distance between populations. The AMOVA analysis attributed 81 % of the variance to the genotypes (within the group) and 19 % between the groups. These values (*PhiPT* < 0.0001) suggest a different genomic constitution of the two groups, and the variation was attributed to the genotypes within groups.

The morphological differences between tunas and xoconostles have been widely studied in Mexico (Scheinvar *et al.,* 2009). Genomically, the separation of these two types of fruits has been reported as contradictory. Valadez-Moctezuma *et al.* (2015) found the presence of a slight trend of separation between tunas and xoconostles using RAPD markers and ISSR. Similar results were obtained by Samah *et al.* (2015 and 2016) applying biochemical markers, such as seed reserve proteins and SSR markers. However, AFLP and cytoplasmic markers revealed no trend of separation between these two types of fruits (Espinoza-Sánchez *et al.*, 2014; Las Casas *et al.,* 2017). In this study, the use of five IRAP retrotransposon markers allowed obtaining a better tendency to separate tuna from xoconostle types, especially when PCoA analysis was carried out. It was expected to find a not-total divergence between Opuntia fruit types, since the fruit has been the organ of interest during a domestication process. It can be assumed that the retrotransposition contributes to the differentiation of species within this genus; in this case, for the discrimination between the two types of plant fruits.

CONCLUSION

The presence of five IRAP retrotransposons in *Opuntia* varieties was determined. The number of polymorphic bands produced by the five IRAP primers suggests greater retrotransposition activity in xoconostles than in tunas under the hypothesis that a polymorphic band may be related to a new retrotransposon insertion. The PCoA analysis allowed a clear separation of the varieties producing xoconostles from those producing tunas. These results suggest a potential role of the transposable elements in the genetic divergence within the *Opuntia* genus and invite to develop specific retrotransposon markers for *Opuntia*; principally, specific markers for xoconostles and others for tunas.

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