Genetic diversity and phylogenetic relationship among spineless cactus species accessions *(opuntia ficus-indica)* as revealed by ISSR markers

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ABSTRACT

The present study, *"*Genetic diversity and phylogenetic relationship among spineless cactus species accession *(Opuntia ficus-indica)* as revealed by ISSR markers*"*, characterized genetic variability and relatedness among diverse spineless cactus genotypes from Italy, Brazil, Mexico, the USA and Chile. Genomic DNA from cladode tissue was amplified using ISSR primers, producing clear and highly polymorphic banding patterns. Substantial variation was observed, with both shared and rare alleles detected; accession 1281 demonstrated distinctiveness through the presence of unique alleles. Cluster analysis grouped most accessions by geographical origin and breeding background, while 1281 formed a separate divergent cluster. Brazilian forage types (IPA, COPENA series) exhibited strong similarity, whereas Italian and Mexican accessions showed notable divergence. The results confirm ISSR markers as effective tools for dissecting genetic variability in cactus germplasm, highlighting redundancy in some breeding lines and the presence of unique genetic resources. These findings provide a molecular framework for conservation and breeding, underscoring the importance of rare alleles in enhancing stress tolerance, fruit quality and adaptability in arid agriculture.

Keywords: *Opuntia ficus-indica*, Genetic diversity, ISSR markers, Phylogenetic relationship, Spineless cactus.

Introduction

Opuntia ficus-indica, also known as prickly pear or Indian fig opuntia, is a perennial, trunk-forming cactus in the family Cactaceae. *Opuntia ficus-indica* has fleshy cladodes that function as photosynthetic organs, and utility water storages and it has fruit that is nutritionally dense and sought for nutrition and medicinal values.

Opuntia ficus-indica is native to Mexico and has been cultivated greatly through arid and semi-arid zones of the world due to its productivity and drought resistance (El-Mostafa *et al*., 2014). Cactaceae, with over 1,800 species, delivers an especially notable membership in *O. ficus-indica* given its ecological and economic impacts (Majure *et al*., 2012).

Central Mexico is recognized as the location of *O. ficus-indica* domestication, with phylogenetic research confirming that these fleshy-fruited prickly pears were closely related to the *O. ficus-indica* found in this region (Griffith, 2004). Indigenous populations utilized prickly pears in agrosystems for food, livestock feed and ecological management (Flores-Benítez *et al*., 2023). A global spread began in the 16th century, as they were exploited for cochineal dye, which rapidly increased its value across Europe, Africa and Asia (Sáenz *et al*., 2013). Over the years they emerged as a multi-purpose crop, contributing animal feed, soil stabilization and rehabilitation of degraded land in many arid regions, such as South Africa, India and Tunisia.

The morphological and physiological adaptations *of O. ficus-indica*, including succulent stems, waxy cuticles, shallow fibrous roots and areoles that produce spines and flowers, lead to its remarkable success (Mauseth, 2006). Fruits and floral structures are brightly colored and made to attract nocturnal pollinators that cause seed scatter. Drought tolerance is further increased by anatomical adaptations such as crystalline inclusions, mucilage cells and lignified vascular tissues (Arba and Hebbache, 2017). In terms of nutritional value, the fruits and cladodes are a source of antioxidants, dietary fiber, vitamins and minerals that are beneficial to cardiovascular and metabolic health (El-Mostafa *et al*., 2014).

Cladodes are able to help regulate diabetes and obesity because they are hypoglycemic and hypolipidemic (Stintzing and Carle, 2005). Bouaziz *et al*. (2010) reported that seeds produce oil containing high levels of linoleic acid, tocopherols and phytosterols, which support heart health and add value to recipes. Medicinal studies confirm antioxidant, antidiabetic, hepatoprotective, neuroprotective, anti-inflammatory and anticancer activities (Butera *et al*., 2002; Oliveira and Cabral, 2022). Biotechnological advancements over the past few decades have led to an increase in the uses of *O. ficus-indica.*

Elite, stress-tolerant clones generated in tissue culture and micro-propagation may be produced in large amounts (Inglese *et al*., 2017). Pharmaceutical and nutraceutical industries may take advantage of paths to production of secondary metabolites previously obscured by methods that utilize omics (Nam *et al*., 2023). Cladode mucilage is used for water purification and the development of eco-friendly biomaterials (Nharingo and Moyo, 2016) and seed oil may be extracted using supercritical CO_2, which enhances productivity and quality (Ciriminna *et al*., 2017). With modern tools and traditional applications, recent research demonstrates its potential in food and pharmaceutical applications and even environmental biotechnology (Bentchikou *et al*., 2024; Kheloufi *et al*., 2025). The amount of research on *O. ficus-indica* is limited in India despite its wide usage and knowledge. Therefore, the current work used ISSR markers to study the molecular diversity of the available opuntia gene pool.

Materials and Methods

Plant Material

Thirty genotypes of *Opuntia spp*. (mainly *O. ficus-indica*) were collected from different origins including Italy, Brazil, Mexico, Chile and the USA. The material was procured from the AICRP on Forage Crops field, MPKV, Rahuri. Healthy, mature cladodes (3–6 months old) were selected, cut with sterile blades in early morning hours and transported to the laboratory under sterile conditions. Only green, turgid and disease-free cladodes were used for DNA extraction.

Isolation of DNA

With slight modifications, the CTAB approach (Doyle & Doyle, 1990) was used to recover genomic DNA from cladodes. The second cladode layer was crushed in liquid nitrogen, dispersed in a preheated (65 °C) CTAB solution (2% CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl), and incubated for one hour. The extraction process of chloroform:isoamyl alcohol (24:1) was used to remove proteins and polysaccharides. DNA was precipitated using a 70% ethanol cold isopropanol wash, allowed to air dry, and then suspended in TE buffer. The NanoDrop spectrophotometer was used to quantify the purity, which was evaluated using spectrophotometry (OD 260/280 ratio). Values between 1.8 and 2.0 were deemed appropriate.

ISSR Amplification and Electrophoresis

The ISSR primers (UBC and ISSR series, 20 primers), and the appropriate annealing temperatures (48–60 °C) were used for PCR amplification. Each 10 µL of reaction includes primers, $MgCl_2$, Taq DNA polymerase, dNTPs, template DNA, Taq buffer, and water free of nucleases. Among the cycling conditions were initial denaturation (94 °C, 5 min), 35 cycles of denaturation (94 °C, 45 s), annealing (primer-specific Tm), extension (72 °C, 1 min), and final extension (72 °C, 5 min). A UV trans illuminator was used to evaluate the PCR products after they had been resolved on 2% agarose gels in 1X TBE buffer and stained with ethidium bromide. The size marker employed was a 100 bp ladder. A gel documentation system was used to record the banding patterns.

Data Analysis and Scoring

Amplified segments were recorded as existing (1) or not existing (0). Binary data matrices were utilized to assess genetic similarity using Jaccard's similarity coefficient. Cluster analysis utilized the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to create dendrograms, thus evaluating genetic diversity and phylogenetic connections among the opuntia genotypes.

Results and discussion

High-quality genomic DNA was isolated from 30 spineless opuntia genotypes using a modified CTAB technique. Concentrations were within 114-425 ng/µl. The agarose gel electrophoresis confirmed intact DNA with no signs of degradation, while Nanodrop measurement showed good purity and were ready for PCR amplification. The consistent quality of DNA among samples guaranteed decent reliability of molecular marker analysis, which was the basis of diversity estimation. A total of 20 ISSR primers were employed and all primers produced amplification across the 30 genotypes. Collectively, 55 alleles were scored, with fragment sizes spanning 160–800 bp. The number of alleles per primer varied from 3 (UBC 811, ISSR 857, ISSR 895) to 7 (ISSR 847), demonstrating variable discriminatory capacity among primers.

The Polymorphism Information Content (PIC) values ranged from 0.32 to 0.39, with primers ISSR 827, ISSR 847, ISSR 850, ISSR 861 and ISSR 887 recording the highest values, thereby proving most informative. These findings show that ISSR markers, which target short sequence repeats in the genome, are efficient in identifying both common and unusual alleles within opuntia germplasm. Numerous primers exhibited significant diversity patterns. For instance, ISSR 827 yielded five alleles (210–550 bp), featuring bands at 290 bp and 360 bp that were broadly distributed, whereas the less common alleles at 210 bp and 550 bp were specific to certain genotypes. ISSR 847 generated seven alleles (220–700 bp), featuring highly conserved bands at 300 bp and unique, genotype-specific fragments at 600–700 bp, validating its strong discriminatory capacity. Likewise, ISSR 887 displayed six alleles (290–800 bp), showing strong amplification at 600 bp and infrequent upper bands at 690 and 800 bp, suggesting considerable polymorphism. UBC 812 enhanced five alleles (200–600 bp), featuring a prominent 400 bp band and an infrequent 600 bp allele, emphasizing both conserved and distinct diversity.

Cluster analysis based on Jaccard's similarity coefficients (0.54–0.82) clearly demonstrated moderate to high genetic diversity. The UPGMA dendrogram divided the genotypes into five clusters. Cluster I was the largest, containing 29 accessions and was subdivided into six subclusters (IA–IF). Subcluster IA grouped 'Gialla Rocca Palumba' with 'Trunzara Bianca San Cono' (very high similarity), suggesting clonal origin or common domestication. Forage-type genotypes like "COPENA F1" and "Mexico Fodder 1278," which were closely related and reflected breeding selection for drought resistance and fodder quality, were grouped under Subcluster IE. The Brazilian IPA series (IPA-90-111, IPA-90-115, IPA-90-156) clustered within IF, consistent with their shared breeding background. Genotype 1281, on the other hand, independently established Cluster II, identifying it as a genetically isolated accession with distinct alleles not seen in other groups.

The genetic similarity matrix further revealed relationships of interest. High similarity values (>0.80) were recorded between IPA-90-111 and IPA-90-115, as well as between 1308 and 1278, suggesting common ancestry or vegetative propagation. Moderate similarity (0.52–0.72) was found among pairs such as 'Trunzara Gialla San Cono' and 'Gialla San Cono', indicating relatedness but with enough divergence to be useful for hybridization. Low similarity (<0.50) was observed between divergent pairs such as 'Bianca Rocca Palumba' and '1308', or 'Palma grande' and '1308', reflecting long-term geographic isolation or distinct evolutionary lineages. Such divergent accessions are particularly important as reservoirs of novel alleles for breeding programs. Comparisons with earlier studies support these findings. Souto Alves *et al*. (2009) and Valadez-

Moctezuma *et al*. (2014) reported moderate ISSR-based polymorphism in opuntia (PIC ~0.33–0.38), consistent with the present values. Bezerra *et al*. (2022) noted that ISSR primers from the 800 series generated multiple alleles across 200–700 bp, again in agreement with our results. The clustering of COPENA and IPA genotypes also mirrors earlier reports of breeding program effectiveness in maintaining cultivar identity. The detection of outliers like genotype 1281 underlines the hidden variability within the opuntia gene pool, as also highlighted by El Kharrassi *et al*. (2017) in Moroccan collections.

These findings highlight that spineless opuntia genotypes possess considerable molecular diversity. ISSR markers successfully distinguished both fine-scale differences (e.g., tight clustering within the IPA and COPENA series) and significant divergence (e.g., isolation of 1281). The noted variety is extremely important for the conservation and use of germplasm. Highly similar genotypes might help preserve clonal uniformity, whereas diverse accessions can introduce new alleles for stress resilience, forage production and adaptability. These results provide a molecular foundation for upcoming breeding and conservation approaches in opuntia, securing its promise as a durable crop for dry and semi-dry regions.

Conclusion

The current research on "Genetic variation and phylogenetic connections among spineless cactus species accessions (*Opuntia ficus-indica)* identified by ISSR markers" assessed 30 genotypes from various areas such as Italy, Brazil, Mexico, Chile and the USA, encompassing fodder, forage and fruit varieties. DNA profiling conducted with 20 ISSR primers yielded 55 consistent alleles (150–800 bp), with PIC values between 0.32 and 0.44, reflecting moderate to high genetic variation. Although several primers noted unique alleles or were able to identify unique alleles including ISSR 847, ISSR 887, ISSR 892, and UBC 827, ISSR 861 presented the highest degree of polymorphism. Also, while the accessions from Mexico with red fruited accession 1 (1281) was distinctive and isolated its own cluster, the UPGMA clustering aid highlighted the genetic pathway and breeding histories of most of the accessions, with several similarities noted in the IPA and COPENA series of accessions, and significant variation within COPENA 2, Orelha de elefante mexicana, Orelha de elefante africana, and accession 1281.

These results confirm the feasibility of using ISSR markers for assessing genetic diversity and identifying cultivars of spineless cacti. The grouping of lines related to forage suggests they have a common breeding history, while recognizing genetically unique accessions demonstrates they had potential as sources of new variability. Preserving these rare alleles is essential for characteristics associated with stress tolerance, fruit quality and disease resistance. The research provides a robust molecular foundation for the conservation, use and genetic enhancement of *Opuntia ficus-indica* and its related species, thus aiding the sustainable improvement of productivity, adaptability and resilience of cactus pear in arid and semi-arid farming.

Tables and figures

Table 1. List of opuntia genotypes used in the present a study

Sr. No	Opuntia genotypes	Species	Source/ origin	Characteristics
1	Gaialla Rocca Palumba	*Opuntia ficus-indica*	Italy	Spineless, fodder
2	Trunzara Bianca San Cono	*Opuntia ficus-indica*	Italy	Spineless, fodder
3	Bianca Rocca Palumba	*Opuntia ficus-indica*	Italy	Spineless, fodder
4	Seedless Santa Margherita Belice	*Opuntia ficus-indica*	Italy	Spineless, fodder
5	Trunzara Rossa Bronte	*Opuntia ficus-indica*	Italy	Spineless, fodder
6	Trunzara Gialla Bronte	*Opuntia ficus-indica*	Italy	Spineless, fodder
7	Trunzara Bianca Bronte	*Opuntia ficus-indica*	Italy	Spineless, fodder
8	Trunzara Gialla San Cono	*Opuntia ficus-indica*	Italy	Spineless, fodder
9	Gialla San Cono	*Opuntia ficus-indica*	Italy	Spineless, fodder
10	Bianca San Cono	*Opuntia ficus-indica*	Italy	Spineless, fodder
11	Palma grande	*Opuntia ficus-indica*	Brazil	Spineless, fodder
12	Palma redonda	*Opuntia spp.*	Brazil	Spineless, fodder
13	Orelha de elefante mexicana	*Opuntia stricta Haw.*	Brazil	Spineless, fodder
14	Orelha de elefante africana	*Opuntia undulata Griffiths*	Brazil	Spineless, fodder
15	COPENA F1	*Opuntia ficus-indica*	Brazil	Spineless, fodder
16	COPENA V1	*Opuntia ficus-indica*	Brazil	Spineless, fodder
17	IPA-90-92	*Opuntia ficus-indica*	Brazil	Spineless, fodder
18	IPA-90-111	*Opuntia ficus-indica*	Brazil	Spineless, fodder
19	IPA-90-115	*Opuntia ficus-indica*	Brazil	Spineless, fodder
20	IPA-90-156	*Opuntia ficus-indica*	Brazil	Spineless, fodder
21	Additional-1258	*Opuntia ficus-indica*	Brazil	Spineless, fodder
22	Mexico Fodder-1278	*Opuntia ficus-indica*	Brazil	Spineless, fodder
23	Mexico sp	*Opuntia ficus-indica*	Brazil	Spineless, fodder
24	1294	*Opuntia ficus-indica*	Mexico	Spineless, fodder

25	1248	*Opuntia ficus-indica*	Arizona,USA	Spineless, fodder
26	1282	*Opuntia ficus-indica*	Mexico	Spineless, vegetable
27	1278	*Opuntia ficus-indica*	Mexico	Spineless, fodder
28	1308	*Nopslea cochenillifero*	Mexico	Spineless, vegetable
29	1315	*Opuntia ficus-indica*	Chile	Spineless, fruit
30	1281	*Opuntia spp*	Mexico	Spineless, red fruit

Table 2. List of gene specific primers used in the present study

Sr. No	Primer	Sequence (5'-3')	Annealing Temp (°C)
1	UBC 811	GAG AGA GAG AGA GAG AC	51.50
2	UBC 812	GAG AGA GAG AGA GAG AA	50.30
3	UBC 827	ACA CAC ACA CAC ACA CG	55.77
4	UBC 840	GAG AGA GAG AGA GAG AYT	51.27
5	UBC 880	GGA GAG GAG AGG AGA	52.00
6	UBC 891	HVH TGT GTG TGT GTG TG	55.00
7	ISSR 827	ACA CAC ACA CAC ACA CG	48.00
8	ISSR 847	CAC ACA CAC ACA CAC ARC	48.00
9	ISSR 848	CAC ACA CAC ACA CAC ARG	47.00
10	ISSR 850	GTG TGT GTG TGT GTG TYC	53.00
11	ISSR 855	ACA CAC ACA CAC ACA CYT	49.00
12	ISSR 856	ACA CAC ACA CAC ACA CYA	54.00
13	ISSR 857	ACA CAC ACA CAC ACA CYG	49.00
14	ISSR 861	ACC ACC ACC ACC ACC ACC	61.00
15	ISSR 884	HBH AGA GAG AGA GAG AG	51.30
16	ISSR 885	BHB GAG AGA GAG AGA GA	51.93
17	ISSR 886	VDV CTC TCT CTC TCT CT	51.93
18	ISSR 887	DVD TCT CTC TCT CTC TC	51.93
19	ISSR 892	TAG ATC TGA TAT CTG AAT TC	49.10
20	ISSR 895	AGA GTT GGT AGC TCT TGA TC	55.25

Table 3. Quantification of DNA

Sr. No.	Genotypes	OD (Optical Density)	Concentration (ng/µl)
1	Gaialla Rocca Palumba	1.79	243
2	Trunzara Bianca San Cono	1.75	351
3	Bianca Rocca Palumba	1.79	156
4	Seedless Santa Margherita Belice	1.80	284
5	Trunzara Rossa Bronte	1.89	247
6	Trunzara Gialla Bronte	1.75	223
7	Trunzara Bianca Bronte	1.81	170
8	Trunzara Gialla San Cono	1.84	114
9	Gialla San Cono	1.80	185
10	Blanca San Cono	1.84	270
11	Palma grande	1.90	425
12	Palma redonda	1.82	262
13	Orelha de elefante mexicana	1.86	250
14	Orelha de elefante africana	1.79	334
15	COPENA F1	1.81	248
16	COPENA V1	1.79	166
17	IPA-90-92	1.79	187
18	IPA- 90-111	1.86	241
19	IPA-90-115	1.81	255
20	IPA-90-156	1.79	259
21	Additional-1258	1.82	252
22	Mexico Fodder- 1278	1.78	219
23	Mexico sp	1.80	256
24	1294	1.86	228
25	1248	1.83	266
26	1282	1.78	285
27	1278	1.79	216
28	1308	1.81	297
29	1315	1.81	298
30	1281	1.83	287

Table 4. Allele-specific marker profile of 20 ISSR primers

Primer	Genotypes Analyzed	Amplicon Size Range (bp)	Total Alleles	Band Clarity	Polymorphism Level	Notable Allele Observations
UBC 811	30	200–400	3	Good, minor noise	Moderate	~300 bp allele highly conserved; ~200 bp allele genotype-specific (lanes 10, 11, 24, 25).
ISSR 827	30	210–550	5	Mostly clear, minor smearing	High	~360 bp band strong and polymorphic; ~210 bp allele genotype-specific (lanes 8, 19, 22, 28).
ISSR 847	30	220–700	7	Mixed (faint to sharp)	Moderate–High	~300 bp allele very frequent; ~600 bp allele bright in lanes 2, 11, 12.
UBC 827	30	400–680	6	Clear	Moderate–High	~500 bp allele very common; ~680 bp allele rare, genotype-specific.
UBC 891	30	220–400	4	Moderate to faint	Moderate	~300 bp allele bright and consistent; ~220 and ~350 bp alleles faint, less frequent.
UBC 880	30	220–380	4	Moderate	Moderate	~320 bp allele bright and consistent; ~220 bp faint.
ISSR 850	30	290–700	5	Moderate–Strong	Moderate–High	~400 bp and ~500 bp bands strong and frequent; ~700 bp allele rare.
ISSR 856	30	220–400	4	Moderate–Strong	Moderate	~300 bp allele strong and dominant; ~220 bp faint but consistent.
ISSR 848	30	220–600	5	Moderate–Strong	Moderate–High	~400 bp strong in most genotypes; ~600 bp allele rare.
ISSR 892	30	300–700	5	Moderate–Strong	Moderate–High	~400 bp allele dominant; ~700 bp allele bright but infrequent.
ISSR 885	30	300–600	5	Clear, minimal smearing	Moderate–High	~300 bp allele strong in lanes 20–30; ~600 bp allele rare.
ISSR 887	30	290–800	6	Mostly clear	High	~600 bp allele strong in multiple lanes; ~800 bp allele distinct but rare.
ISSR 884	30	250–400	4	Moderate–Strong	Moderate	~400 bp allele strong and frequent; ~250 bp allele faint.
ISSR 855	30	300–600	4	Moderate–Strong	Moderate	~500 bp allele strong and frequent; ~600 bp allele less common.
UBC 812	30	200–600	5	Moderate–Strong	Moderate–High	~400 bp allele strong and frequent; ~200 bp allele faint.
ISSR 886	30	200–500	4	Moderate–Strong	Moderate	~400 bp allele most intense; ~500 bp allele less common.
ISSR 857	30	200–400	3	Moderate	Moderate	~300 bp allele moderate and widespread; ~200 bp allele faint but consistent.

ISSR 895	30	200–500	4	Moderate–Strong	Moderate	~400 bp allele strong and frequent; ~200 bp faint but consistent.
UBC 840	30	200–500	4	Moderate–Strong	Moderate	~300–400 bp alleles widespread; ~500 bp allele strong in some genotypes.
ISSR 861	30	150–400	5	Moderate–Strong	Moderate–High	~400 bp allele strong; ~150 bp faint but present.

Table 5. Consensus tree showing clustering of opuntia genotypes using the maker data

Main Cluster	Subcluster	Sub-subcluster	Genotype Names	Remarks
Cluster I	IA	IA1	Gialla Rocca Palumba, Trunzara Bianca San Cono	Very closely related genotypes (shortest linkage).
		IA2	Verde San Margherita, Trunzara Gialla Bronte	Genetically adjacent to IA1.
	IB	IB1	Trunzara Bianca Bronte, Trunzara Gialla San Cono	Moderate similarity with IA; form a sister group.
		IB2	Gialla San Cono	Slightly diverged from IB1.
	IC	IC1	Blanca San Cono	Unique but close to IC2.
		IC2	Palma grande, Palma redonda	Likely share morphological features.
	ID	ID1	Orelha de elefante mexicana, Blanca Rocca Palumba	Moderate similarity; elephant ear type.
		ID2	Trunzara Rocca Bronte, Orelha de elefante africana	Genetically close to ID1 group.
	IE	IE1	COPENA F1, Mexico Fodder - 1278	High forage type similarity.
		IE2	1248, 1315	Strong pairwise similarity.
		IE3	1282, 1278, 1308	Share a common base with other IE sub-subclusters.
	IF	IF1	COPENA V1, IPA-90-111, IPA-90-115	Brazilian IPA series; tightly linked.
		IF2	Additional-1258, IPA-90-156	Diverged from IF1 but part of same IPA lineage.
		IF3	Mexico sp, 1294	Mexican hybrids with moderate divergence.
Cluster II	—	—	**1281**	Highly distinct genotype; genetically isolated from all others.

Figure 1. Dendrogram of opuntia genotypes generated using NTSYS-pc software based on ISSR marker.

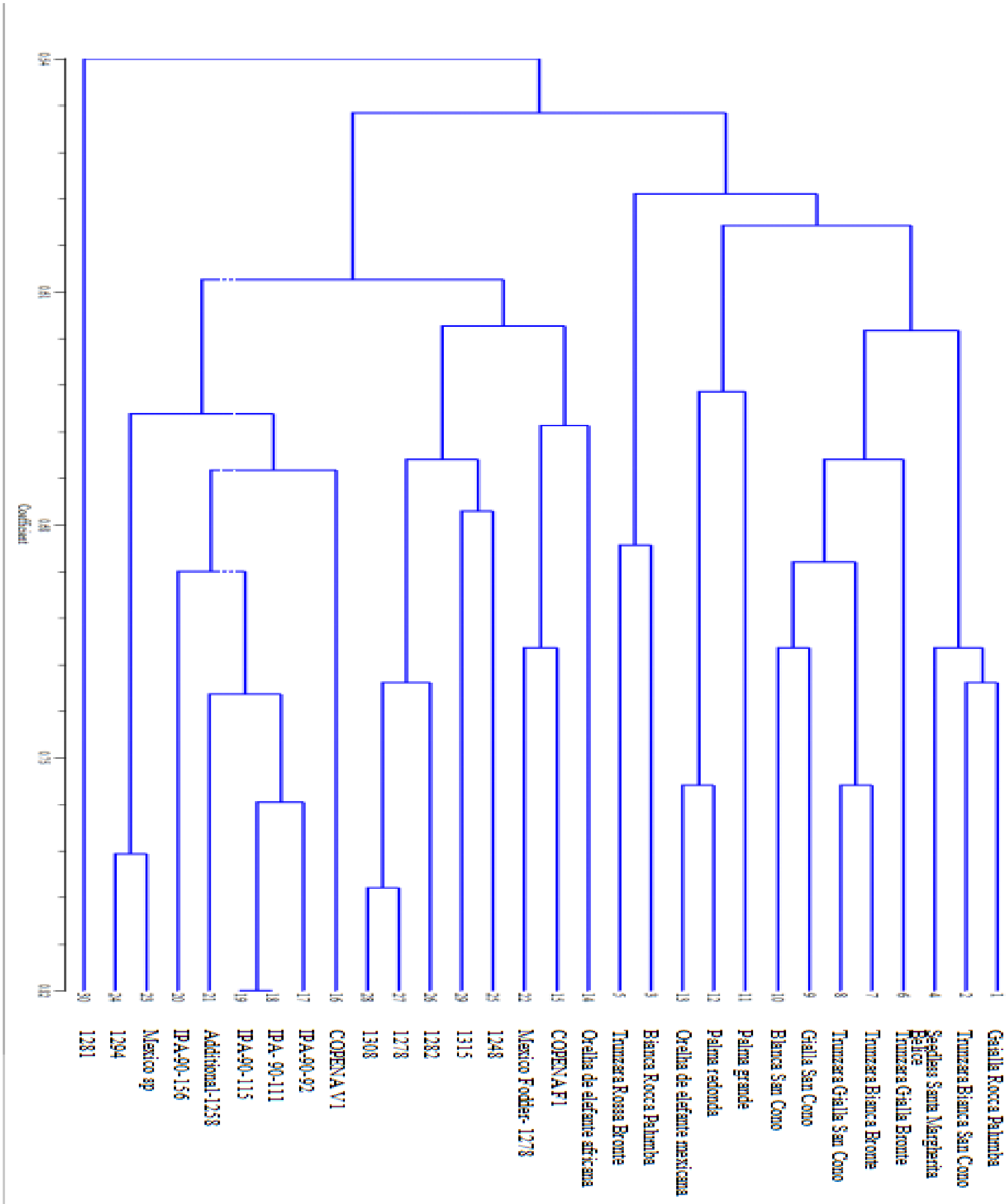

Fig. 2. Jaccard's coefficient similarity analysis of opuntia genotypes using ISSR markers

	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	26	27	28	29	30
1	1.0000																													
2	0.7291	1.0000																												
3	0.5416	0.6041	1.0000																											
4	0.7083	0.7291	0.5416	1.0000																										
5	0.5416	0.5625	0.6875	0.6666	1.0000																									
6	0.5416	0.5833	0.5625	0.6250	0.6875	1.0000																								
7	0.6562	0.5937	0.5312	0.6770	0.5520	0.6562	1.0000																							
8	0.5625	0.6041	0.5833	0.6041	0.5625	0.6875	0.7604	1.0000																						
9	0.6145	0.6770	0.5312	0.7187	0.6145	0.6562	0.6666	0.6979	1.0000																					
10	0.5833	0.6458	0.5625	0.6458	0.6250	0.6458	0.6770	0.7291	0.7187	1.0000																				
11	0.5000	0.5625	0.6458	0.5625	0.5416	0.5208	0.4895	0.5833	0.5312	0.6458	1.0000																			
12	0.6458	0.6250	0.5416	0.6875	0.5625	0.5625	0.6354	0.5625	0.6770	0.6458	0.6041	1.0000																		
13	0.5729	0.5312	0.6354	0.5729	0.5729	0.6354	0.5833	0.5937	0.5625	0.6770	0.6770	0.7604	1.0000																	
14	0.4687	0.4895	0.4063	0.6145	0.5520	0.5937	0.4791	0.4062	0.4791	0.4479	0.4687	0.6562	0.5625	1.0000																
15	0.5937	0.5729	0.5729	0.6563	0.5937	0.6562	0.5833	0.5104	0.5416	0.6354	0.6354	0.6770	0.6875	0.6666	1.0000															
16	0.6354	0.6562	0.5104	0.5938	0.4479	0.4687	0.5625	0.5104	0.5416	0.5729	0.5312	0.6562	0.5416	0.6250	0.6458	1.0000														
17	0.6562	0.6979	0.5312	0.6563	0.4895	0.5104	0.5625	0.5312	0.6875	0.6562	0.5937	0.7395	0.6250	0.5833	0.6875	0.6875	1.0000													
18	0.6979	0.6562	0.5312	0.6770	0.4479	0.5729	0.6041	0.5729	0.6041	0.6145	0.5729	0.6562	0.6041	0.5208	0.6458	0.6875	0.7916	1.0000												
19	0.6666	0.6041	0.5208	0.6458	0.5000	0.5416	0.6145	0.4791	0.6354	0.5625	0.4791	0.6875	0.6145	0.5312	0.6145	0.6145	0.7395	0.8229	1.0000											
20	0.6354	0.6145	0.5312	0.6145	0.5104	0.5312	0.5625	0.4479	0.6041	0.5520	0.5104	0.6562	0.5833	0.5416	0.6458	0.6250	0.7083	0.6875	0.7395	1.0000										
21	0.5937	0.5937	0.5104	0.6145	0.4687	0.5729	0.6458	0.5520	0.6666	0.5729	0.4895	0.6354	0.5833	0.6041	0.6250	0.7083	0.7083	0.7291	0.7604	0.6458	1.0000									
22	0.5208	0.5208	0.5416	0.5625	0.5833	0.5833	0.5729	0.4791	0.5312	0.5000	0.5208	0.5416	0.5312	0.6354	0.7187	0.5937	0.6145	0.6145	0.6666	0.5520	0.6770	1.0000								
23	0.4895	0.5729	0.5104	0.5729	0.4895	0.5312	0.5416	0.5104	0.6041	0.4895	0.5937	0.5729	0.5416	0.4166	0.5416	0.5416	0.6250	0.6458	0.6979	0.5833	0.6458	0.6562	1.0000							
24	0.5833	0.5625	0.4791	0.5833	0.5208	0.5416	0.5937	0.5000	0.5520	0.5625	0.5416	0.6041	0.5729	0.5520	0.6145	0.6562	0.6979	0.6562	0.6666	0.6562	0.6979	0.6666	0.7812	1.0000						
25	0.5416	0.6041	0.4791	0.6250	0.5000	0.5208	0.4687	0.4583	0.5312	0.4791	0.4791	0.6458	0.5104	0.6562	0.6354	0.6145	0.6354	0.6562	0.6875	0.5104	0.6562	0.7083	0.6979	0.6250	1.0000					
26	0.4895	0.5729	0.4270	0.6145	0.4895	0.5520	0.5833	0.6145	0.6250	0.5729	0.4687	0.6145	0.5416	0.6458	0.6458	0.5833	0.6875	0.5833	0.6562	0.6458	0.6458	0.6145	0.6458	0.6770	0.6979	1.0000				
27	0.5416	0.5000	0.4375	0.6458	0.5000	0.5208	0.5520	0.5000	0.5729	0.5208	0.4375	0.5625	0.4895	0.5937	0.5729	0.5937	0.5937	0.5729	0.6041	0.6145	0.6145	0.6041	0.6145	0.6458	0.6875	0.7604	1.0000			
28	0.5416	0.5416	0.3750	0.6250	0.5000	0.5208	0.5312	0.4583	0.5937	0.4791	0.3958	0.5000	0.4270	0.5937	0.5520	0.5104	0.5312	0.5520	0.5833	0.5520	0.6145	0.6666	0.5937	0.5833	0.6250	0.6979	0.7916	1.0000		
29	0.5729	0.6354	0.5520	0.5937	0.5729	0.5729	0.5208	0.4895	0.5625	0.5104	0.5520	0.5729	0.5208	0.6041	0.5833	0.5416	0.5833	0.6458	0.6145	0.5833	0.6041	0.6354	0.6044	0.6354	0.6770	0.6041	0.6562	0.6979	1.0000	
30	0.4895	0.5729	0.5520	0.5416	0.4687	0.5208	0.5312	0.5416	0.5104	0.5208	0.4895	0.5520	0.5208	0.5208	0.5208	0.5520	0.5208	0.5729	0.5625	0.5312	0.5937	0.5416	0.5729	0.5729	0.5520	0.5520	0.5416	0.5416	0.5833	1.0000

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