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Chapter 8
Genetic Diversity and Ecotypes of *Opuntia* spp.

Ahmad A. Omar, Abdelaleim I. ElSayed, and Azza H. Mohamed

Abstract The genus *Opuntia* belongs to the family Cactaceae, subfamily Opuntioideae, tribe Opuntieae. Many *Opuntia* is found on roadsides and routes due to usual ruderal behavior and it is planted for nourishment fruit production and border formation. The plant genetic background of any species plays an essential role in the improvement of plants by enriching the germplasm with a big pool of genetic variation for the breeders to create new cultivars. For many years *Opuntia* taxonomy was based on the morphological description and subsequently enriched with biochemical, physiological, and cytogenetic parameters. Molecular markers are widely used and overcome problems associated with genetic variation, genome mapping, phylogenetic, and evolutionary studies. *Opuntia* spp. has some individualities regarding molecular marker investigation, such as polysaccharides content and secondary metabolites, which makes DNA isolation extremely difficult. Besides, the ploidy level among *Opuntia* spp. cause several problems in the analysis of codominant markers. The polyploidy level in *Opuntia* can range from triploid (2n = 3x = 33) to octoploid (2n = 8x = 88). The ploidy level of *Opuntia* spp. depends on the population's origin. *Opuntia* spp. cultivated in regions that differ from the origin present a lower genetic diversity than that of the areas of origin. Due to the presence of cleistogamy (self-fertilization) and polyembryony and the lack of rain and low temperature, seed germination is difficult, which makes the extent of genetic diversity limited. To increase the genetic variability among *Opuntia* populations, it requires

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introducing germplasm and landraces from the center of origin and domestication besides adding different genotypes from other regions.

Keywords Origin of *Opuntia* spp. · Phenotypic diversity · Genome size · Ploidy levels · Apomixis · Molecular marker

Abbreviations

cpSSR	Chloroplast simple sequence repeats
GA	Gibberellic acid
ISSR	Inter-simple sequence repeats
nuSSR	Nuclear microsatellite
PCR	Polymerase chain reaction
QTL	Quantitative trait locus
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeats
UPGMA	Unweighted pair group method with arithmetic mean

1 Introduction

The *Opuntia* spp. (Cactaceae, Opuntioideae), is a xerophytic plant native to Mexico (Reyes-Agüero et al., 2005; Russell & Felker, 1987). It grows in many regions worldwide, such as; North and South America, Africa, India, Australia, and the Mediterranean countries (Piga, 2004). The species *O. ficus indica* is native to Mexico, where it was domesticated by the ancient Mexicans (Griffith, 2004; Kiesling, 1998). After discovering America, *O. ficus indica* was introduced into Spain by sailors because of its anti-scurvy properties. Afterwards, it was introduced to other parts of the world, particularly to the Mediterranean region (Kiesling, 1998). Generally, the *Opuntia* spp. are well adapted to grow in arid and semi-arid environments and tolerated poor soils (Gallegos-Vazquez et al., 2012). Furthermore, *Opuntia* spp. have been domesticated a long time ago throughout arid and semi-arid regions and constituted until today an essential crop in many countries' agricultural economy (Griffith, 2004). Particularly, *Opuntia* spp. is an alternative crop for the Mediterranean region's economy because of the increase in temperatures and the lack of rain during summer in this region. In many countries, *Opuntia* spp. was intensively cultivated for commercial purposes. *Opuntia* crop is popular in some countries due to its attractive taste, nutritional value, and effects on human health (Díaz et al., 2017; Zakynthinos & Varzakas, 2016). Its economic and ecological importance rises from the fact that it can be used as a forage crop for cattle and other

animals, especially when there is a shortage of fresh forage due to drought (Anaya-Perez, 2001; Mondragón-Jacobo & Pérez-Gonzalez, 2001; Nefzaoui & Ben Salem, 2001; Vigueras & Portillo, 2001) or as a medicinal plant (Griffith, 2004).

The edible parts of *Opuntia* plants are known as cladodes, pads, nopales, or pencas, as well as the tender young part of the cactus stem consumed as a vegetable in salads. Mexico and Italy are the main producer and consumers countries. From the approximately 590,000 ha cultivated worldwide, Mexico accounts for 70% and Italy for 3.3%. Under optimal conditions, Mexico's annual production can reach 350,000 tons (Reyes-Agüero & Rivera, 2011). The cultivated *Opuntia* spp. are grown in at least 30 countries worldwide (Díaz et al., 2017).

The taxonomic classification of the *Opuntia* genus has been reported as a complex, which explains many reports of misclassification of *Opuntia* spp. (Samah et al., 2016). Continuous morphological variation and limited morphological descriptions for cultivar discrimination are the most difficult obstacles to achieve a stable classification (Caruso et al., 2010; Labra et al., 2003; Samah et al., 2016; Valadez-Moctezuma et al., 2014). Thus, the difficulties in morphological interpretation have led to the publication of many binomials, many of which are synonyms, homonyms, or false attributions (Gibson & Nobel, 1986).

2 Geographical Origin of *Opuntia* spp. and History of the Movement

The genus *Opuntia* in the Cactaceae family (subfamily Opuntioideae), which included several species, widespread in different areas around the world such as North and South America, the Mediterranean basin, Middle-East, South Africa, India, Thailand, and Australia (Griffith, 2004). The genus *Opuntia* is one of the major recognized genus of the Opuntioideae, which includes over 181 species and 10 naturally occurring hybrids (Anderson, 2001). Several species of genus *Opuntia* become cultivated, such as *O. robusta*, *O. megacantha*, *O. hyptiacantha*, *O. cochenillifera O. streptacantha*, *O. albicarpa*, and *O. amyclaea*, (Kiesling, 1998; Mondragón-Jacobo & Pimienta-Barrios, 2001; Pimienta, 1990; Scheinvar, 1995). The most widespread and economically important species is *O. ficus indica*. This species, commonly called Indian fig, cactus pear, prickly pear, and barbary fig, which probably domesticated in central Mexico for 9000 years and spread in several warm regions of the world by European travelers beginning in the late fifteenth century (Griffith, 2004; Kiesling, 1998). *O. ficus-indica* specie was certainly known at the beginning of the sixteenth century (Casas & Barbera, 2002). Further, *O. ficus-indica* fruits and shoots were also reportedly consumed by the Maya of southeastern Mexico (Coe, 2015). There is also some evidence for the use of *O. ficus-indica* by the Nazca of Peru, placing these plants in South America at a very early date (Sejuro, 1990). Figure 8.1 explains how the *O. ficus-indica* species was found early in central Mexico and the cultivated plants then have been spread through trade

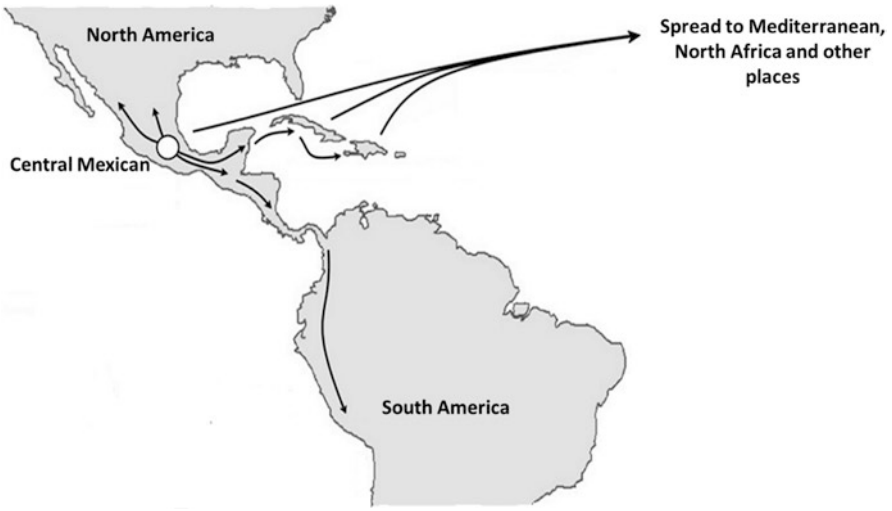

Fig. 8.1 A biogeographic model of dispersal of *Opuntia ficus-indica*. From central Mexico, the ancestors of *O. ficus-indica* were selected from arborescent, fleshy-fruited taxa (one or more species such as *O. leucotricha*, *O. hyptiacantha*, *O. megacantha*, and *O. streptacantha*). The cultivated plants then spread through trade throughout Mesoamerica and the Caribbean and possibly into South America. European travelers then spread these plants into Mediterranean Europe and North Africa and subsequently throughout the world's arid and semi-arid regions. The graph has been adapted and modified from Griffith (2004)

throughout Mesoamerica and the Caribbean and possibly into South America. Then the European travelers spread these plants into Mediterranean Europe and North Africa and subsequently throughout arid and semi-arid regions (Griffith, 2004). The biogeographic and evolutionary origins of *O. ficus-indica* species have been obscured through ancient and widespread cultivation and naturalization. Griffith (2004) studied the origin of *O. ficus-indica* species through Bayesian phylogenetic analyses of the internal transcribed spacer DNA sequences. The results of this study revealed that *O. ficus-indica* is a close relative of a group of arborescent, fleshy-fruited prickly pears from central and southern Mexico; that the center of domestication for this species is in central Mexico; and that the taxonomic concept of *O. ficus-indica* may include clones derived from multiple lineages and therefore be polyphyletic.

3 Morphological Taxonomy (Phenotypic Diversity) of *Opuntia* spp.

Based on vegetative and floral morphology, four major subfamilies have been classified in the Cactaceae family: Pereskioideae, Cactoideae, Maihuenioideae, and Opuntioideae (Anderson, 2001; Barthlott & Hunt, 1993; Gibson & Nobel, 1986).

Opuntia is one of the major recognized genus of the Opuntioideae, including over 181 species and 10 naturally occurring hybrids (Anderson, 2001). Amani et al. (2019) studied the morphological characters of four *Opuntia* genus species (*Opuntia tomentosa*, *Opuntia ficus-indica*, *Opuntia undulata*, and *Opuntia engelmannii*). As mentioned in Table 8.1, quantitative and qualitative parameters showed the most discriminant traits allowing separating among the four studied species. Small red-pink flowers and dark green cladodes with velvety texture are the important apomorphic traits found for the species "*Opuntia tomentosa*". Furthermore, *Opuntia undulata* seems to be one species with many apomorphic characters; the light green color of cladodes, light yellow flowers, and purple fruits are observed only in this species. The results also revealed a very high level of morphological variability and a remarkable similarity between *O. ficus-indica* and *O. engelmannii* (Amani et al., 2019). Gallegos-Vazquez et al. (2012) found that qualitative variables such as flesh and peel color proved useful to distinguish among potentially the 21 redundant accessions collected from Mexico. Gutiérrez-Acosta et al. (2000) mentioned that some other qualitative traits, such as fruit dimensions, are useful in separating cactus pear accessions (Gallegos-Vazquez et al., 2012).

Table 8.1 Quantitative and qualitative characters of four *Opuntia* spp. to discriminant morphological variability.

Quantitative and qualitative parametters	*Opuntia* spp.			
	O. ficus-indica	*O. engelmannii*	*O. undulata*	*O. tomentosa*
Number of seeds per fruit	Very low seeds number	Considerable number of seeds	Very low seeds number	Very low seeds number
Number of areolascladode	Very low number of areolas/cladodes	Considerable number of areolas/cladodes	Very low number of areolas/ cladodes	Considerable number of areolas/ cladodes
Fruit weight	Small fruits	Medium fruits	Big fruits	Small fruits
Cladode shape and color	Green cladodes ovoid, round or elongated cladodes Smooth cladodes	Green cladodes Ovoid, round or elongated cladodes Smooth cladodes	Light green cladodes Oblong curved cladodes Smooth cladodes	Dark green cladodes Ovoid, round or elongated cladodes Cladodes with a velvety texture
Flowers color	Yellow, light orange, orange, red-orange hermaphrodite flowers	Yellow, light orange, orange, red-orange hermaphrodite flowers	Light yellow hermaphrodite flowers	Red-pink hermaphrodite flowers
Fruit color/pulp color	Green, yellow, greenish-yellow, orange, or red fruits	Green, yellow, greenish-yellow, orange, or red fruits	Purple fruits	Green, yellow, greenish-yellow, orange, or red fruits

O. ficus indica reproduces sexually and propagates vegetative (Reyes-Agüero et al., 2005), where outcrossing (allogamous) is common among cacti family (Pimienta & del-Castillo, 2002). This phenomenon may lead to different genetic diversity among this species. Generally, the cactus is present in different climatic ecosystems in the world. The cactus family's highest diversity degree was found in Mexico, followed by Brazil, Bolivia, and Peru (Ortega-Baes et al., 2010). This diversity was positively associated with environmental factors such as temperature and precipitation (Mourelle & Ezcurra, 1997). The domestication process of *Opuntia* was begun by producing spineless cladodes with large, sweet fruits. The partial or total absence of the spine is the main diagnostic character of *O. ficus indica* (Reyes-Agüero et al., 2005), where it has been mistakenly related to *O. ameclya*, *O. megacantha*, and *O. streptacantha* (Kiesling, 1998; Labra et al., 2003). *O. ficus indica* was considered as a synonym of *O. megacantha* since the presence or absence of spines is insufficient for separating them (Benson & Walkington, 1965). Based on the combination of the differential vegetative and reproductive characters, Reyes-Agüero et al. (2005) considered that *O. ficus indica* constitutes a taxonomic entity that differs from *O. megacantha* and *O. streptacantha*. Moreover, (Kiesling, 1998) suggested that the spiny and spineless specimens are only forms of *O. ficus indica*.

The dominant color for flowers in *Opuntia* spp. is yellow, but there are also pink, orange, red, white, purple, mottled flowers (Anderson, 2001). Hermaphrodite flowers are the most common (Anderson, 2001; Gibson & Nobel, 1986). Stamens are numerous, being 265 in *O. polyacantha*, 358 in *O. phaeacantha* (Osborn et al., 1988), 450 in *O. viridirubra*, and 598 in *O. brunneogemmia* (Schlindwein & Wittmann, 1997). Stamens are generally yellow or green (Grant et al., 1979) with a circular or spiral arrangement around the style (Boke, 1980). In some cases, the stamens closest to the style are short and successively grow longer, with the longest occurring close to the tepals (Grant & Grant, 1981; Schlindwein & Wittmann, 1997).

4 Genome Size and Ploidy Levels in *Opuntia* spp.

Polyploidy is a usual situation throughout the tribe Opuntieae. Diploids (2n = 2x = 22) are uncommon in this tribe, according to the reported chromosome counts (Majure et al., 2012b). Polyploid taxa within *Opuntia* spp. range from triploid (2n = 3x = 33) to octoploid (2n = 8x = 88), and many species have multiple ploidy levels (Majure et al., 2012a; Pinkava, 2002). The ploidy level of the studied species is unclear since the information from the literature lacks concordance, particularly in the case of *O. dillenii* (2n = 12, 22, 26, 36, 40, 44, 66), *O. elata* (2n = 22, 44) and *O. robusta* (2n = 22, 44, 66, 88) (Majure et al., 2012b). The ploidy level of cultivated *O. ficus-indica* populations is 2n = 88 (Segura et al., 2007). However, penta, hexa, hepta, and diploid levels were also reported in these species, depending on the population origin (Majure et al., 2012a).

Polyploidy and asexual reproduction of zygotes and embryos have long been correlated (Stebbins, 1980). Different ploidy levels occur in the Cactaceae, and it has been suggested that both polyploidy and hybridization events have led to speciation in this family (Cota & Philbrick, 1994; Pinkava et al., 1985). Relative to the basic chromosome number for the family and *Opuntia*, x = 11, *O. spinosissima* is considered a hexaploid species (2n = 66; Cota, unpublished data; (Pinkava et al., 1985). According to J. H. Cota (personal communication), the chromosomes are very small and morphologically uniform in shape and size. Additionally, Cota suggested that this taxon is a consequence of a recent polyploidization event; therefore, low genetic diversity is expected. Electrophoretic data showed that this population is genetically monomorphic for 12 of 13 loci, and at the remaining locus (malate dehydrogenase-1), all individuals are heterozygous, suggesting this genotype is fixed (Hamrick & Godt, 1997). Evidence implies that these 13 extant plants are clones from a single lineage.

The 2C-DNA amount of the *Opuntia* spp. analyzed ranged from 4.17 pg for *O. incarnadilla* to 6.53 pg for the diploid *O. heliabravoana* (Segura et al., 2007). Four ploidy levels, diploid, tetraploid, hexaploid, and octoploid, were identified among the 23 species of *Opuntia* (Table 8.2). Of the 18 species from the series *Streptacanthae* as determined by Estrada-Galván et al. (2000), 15 were octoploid, 2 were hexaploids (*O. incarnadilla* and *O. matudae*), and 1 (*O. elizondoana)* produced tetraploid values (Table 8.2).

The mean 2C genome of the group of principal edible species of *Opuntia* analyzed here is 5.05 pg. In comparison to 2C-values for the cacti by Bennett and Leitch (2005), the mean of *Opuntia* spp. analyzed here exceeded those of *Escobaria bella* Britton et Rose (2C = 3.05 pg), *Pseudolobivia* sp. (2C = 3.25 pg), *Borzicactus aurivillus* K. Schum. (2C = 3.35 pg), *Cleistocactus smaragidifolius* (F.A.C. Weber) Speg. (2C = 3.35 pg), *Aporocactus flagelliformis* Lem. (2C = 3.80 pg), and *Trichocereus werdermannianus* Backeb. (2C = 3.90 pg), but less than most of *Mammillaria* species reported or *Weberbauerocereus winterianus* Anthony (2C = 14.20 pg). Variations of ploidy levels estimated for this *Opuntia* spp. are consistent with their assignment to series, except *O. heliabravoana*, *O. elizondoana*, *O. matudae*, and *O. zamudioi*.

Opportunities are already available to test the value of *Opuntia* genome size as a predictor of environment and evolution responses. The full value of nuclear DNA amounts will likely be realized only when these determinations can be applied in association with other measurable plant traits (Grime, 1998; Otto & Whitton, 2000). Variations in chromosome size and subsequent meiotic abnormalities have disrupt breeding programs. *O. ficus-indica* x *O. robusta* ssp. *robusta* has been used as the basis of recombinant populations, and such variation could explain some of the segregationally disturbances (Cota & Philbrick, 1994; Michael Powell & Weedin, 2001; Pinkava et al., 1973, 1985).

Table 8.2 Estimated DNA amounts, species, subspecies and varieties groups and ploidy levels as determined for *Opuntia* spp. by flow cytometry, according to Segura et al. (2007).

Species	2C-DNA amount estimated (pg)	Interpretation (ploidy level)
O. leucotricha	5.71	4X
O. spinulifera	5.51	4X
O. oligacantha	5.33	6X
O. Zamudioi	4.35	8X
O. lasiacantha	4.88	8X
O. hyptiacantha	4.84	8X
O. streptacantha ssp. *streptacantha*	4.64	8X
O. streptacantha ssp. *aguirrana*	4.43	8X
O. megacantha	5.01	8X
O. joconostle	4.70	8X
O. ficus-indica	4.90	8X
O. albicarpa	4.80	8X
O. amarilla	4.84	8X
O. chavena	4.70	8X
O. cochinera	5.10	8X
O. incarnadilla	4.17	6X
O. fuliginosa	4.64	8X
O. pachona	4.70	8X
O. matudae	5.25	6X
O. cretochaeta	4.35	8X
O. rzedowskii	4.77	8X
O. elizondoana	5.29	4X
O. heliabravoana	6.53	2X
O. robusta var. robusta	4.98	8X
O. robusta var. larreyi	5.96	4X
O. robusta var. guerrana	5.05	8X
Cylindropuntia imbricata	6.92	2X

5 Apomixis in *Opuntia* spp.

Apomixis frequently occurs in *Opuntia* (Mondragon-Jacobo & Perez-Gonzalez, 1996; Pimienta 1990). Apomixis is the production of seeds without previous fertilization (Mondragón-Jacobo & Bordelon, 2002). In *Opuntia*, the most common method is the development of adventitious embryos from nucellar tissue (sporofitic agamospermy) (Garcia-Aguilar & Pimienta-Barrios, 1996; Mondragón-Jacobo & Pimienta-Barrios, 2001; Vélez-Gutierrez & Rodríguez-Garay, 1996) or like in *O. streptacantha*, embryos can be developed from an unfertilized egg (diplospory-parthenogenesis) (Garcia-Aguilar & Pimienta-Barrios, 1996). *O. streptacantha*

flowers emasculated and isolated from exogenous pollination produce fruits with seeds. This result has been interpreted as evidence of apomixis (Pimienta & del-Castillo, 2002; Trujillo Argueta et al., 1986). Polyembryony has also been considered as proof of apomixis (Mondragón & Pimienta, 1995). Thus, apomixis is said to occur commonly in members of *Opuntia*. For example, 20 of the 23 most important fruit cultivars of *Opuntia* in the San Luis Potosi and Zacatecas highlands form polyembryonic seeds (Pimienta & del-Castillo, 2002), although only 3–4% of seeds per fruit are polyembryonic (Mondragón & Pimienta, 1995). Apomixis is more frequent in xenogamic cultivars (Mondragón-Jacobo & Pimienta-Barrios, 2001). Polyembryony is common in wild populations of *O. robusta*, *O. cochinera*, *O. leucotricha*, *O. rastrera*, *O. streptacantha* (Trujillo Argueta et al., 1986), *O. joconostle* (Sánchez, 1997), and *O. stricta* (Reinhardt et al., 1999). Thirty-three percent bi-embryony, 13% tri-embryony, and 4% tetra-embryony have been reported for *O. ficus-indica* (Nieddu & Chessa, 1996). Seedless fruits have been obtained experimentally by inducing male sterility with a chemical gametocide and gibberellic acid (GA) (Aguilar & Chávez, 1995; Gil & Espinoza, 1980). Emasculated flowers with an application of GA develop seedless normal-sized fruits. The most efficient treatments were: (1) a single application of 500 mL L^{-1} GA during anthesis and (2) the application of 100 mL L^{-1} GA 22 and 42 days after anthesis (Aguilar & Chávez, 1995). GA inhibits seed development and induces fruit growth, causes fruit development with a thin peel, little pulp, and a low total content of soluble solids (Nerd & Mizrahi, 1994). Since epidermal cells in the funicular cover will not differentiate without fertilization, Rosas and Pimienta (1986) stated that *Opuntia* could not produce parthenogenetic fruits.

O. monacantha is not an apomictic species. According to Reyes-Agüero et al. (2006), apomixis is rare in *Opuntia*, although it has been reported in *O. aurantiaca* Lindl., *O. dillenii* Haw., *O. leucantha* Link. & Otto, and *O. tortispina* Engelm. & J. M. Bigelow. In these species, the apomictic seeds are almost always of nuclear origin (sporophytic agamospermy) and do not demonstrate abnormalities (Mondragón-Jacobo & Bordelon, 2002). On the other hand, Osborn et al. (1988) reported that the seeds of *O. polyacantha* Haw. and *O. phaeacantha* Engelm. that arose through xenogamy demonstrated greater viability than those produced by other forms of pollination. The same situation appears to apply to *O. monacantha*, which demonstrated high seed production levels and germination arising from natural pollination regimes and manual crosspollination experiments. Additionally, most of the fruits generated from self-pollination experiments aborted, and these abortion rates were higher than those observed in cross-pollination. These results suggest that the abortion of fruits formed through spontaneous or manual self-pollination may represent situations of delayed incompatibility or endogamic depression, suggesting the predominance of endogenous pollen flux between flowers of different clones.

6 Techniques to Study the Genetic Diversity of *Opuntia* spp.

Molecular markers play an important role in the improvement of wild and cultivated plants. Isozymes and restriction fragment length polymorphism (RFLP) are molecular markers that have been largely used as reliable markers for plant genetic analyses (Wang et al. 1992, 1998). PCR-based techniques provide DNA markers scattered throughout the genome which are easier to analyze (Vos et al., 1995). Mainly, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) have been widely used for accession classification, cultivar identification, cereals and vegetables, and for diversity estimation or genetic variability of complex species too (Bendhifi et al., 2013; Shilpha et al., 2013; Xiao et al., 2004). Since these markers are very useful because of their simplicity, low cost and performance capacity (Waugh & Powell, 1992), particularly ISSR analysis, it continues to be used as an alternative tool for SSR diversity studies since they are repeatable (Zietkiewicz et al., 1994).

In the last decade, molecular markers have been applied to complement morphological characters in determining genetic diversity to address the taxonomic uncertainty regarding the delineation of the various species within *Opuntia* (Erre et al., 2010). Different molecular methods have been proven to be used in classifying the different species of *the Opuntia* plant (Gordon & Kubisiak, 1998; Labra et al., 2003; Wang et al., 1998). Among the molecular markers, RAPD, Chloroplast simple Sequence Repeats (cpSSR) (Chessa et al., 2008), and Simple Sequence Repeats (SSR) (Helsen et al., 2009) have been used to identify and characterize genetic diversity in *Opuntia* spp. Microsatellites have been used in germplasm diversity evaluation, phylogenetic and evolutionary studies, and genome mapping (Kalia et al., 2011). Microsatellites, also known as simple sequence repeats (SSRs), are non-coding, repetitive DNA regions consisting of tandem repeated small motifs (1–6 bp); they are present throughout the genome of an individual, both in coding and non-coding regions. In recent years, microsatellites have been demonstrated to have many important biological functions (e.g., the regulation of chromatin organization, DNA metabolic processes, gene activity, and RNA structure) (van der Knaap & Verrijzer, 2016).

In comparison to other molecular markers, microsatellites are the most informative due to their reliability and abundant multiallelic forms. They exhibit higher mutation rates than the rest of the genome (Gao et al., 2016) and can be easily analyzed using PCR-based methods, including fluorescent automated genotyping and multiplexing. Therefore, SSR markers have been the preferential choice for various applications, such as variety identification, genetic diversity evaluation, phylogenetic relationship analysis, genetic map construction, linkage/association mapping of gene/QTL, marker-assisted selection, and comparative mapping (Shilpha et al., 2013). Labra et al. (2003) reported that the combination of cpSSR and AFLP markers provides a quantitative estimation of genetic relationships among several *Opuntia* spp.

Molecular markers represent reliable tools for the characterization and genetic variability of the *Opuntia* varieties since the environment does not influence them. (Valadez-Moctezuma et al., 2015) assessed the genetic variation of 52 *Opuntia* cultivars using marker tools (RAPD and ISSR). This analysis revealed that *O. ficus-indica*, *O. albicarpa*, and *O. megacantha* species exhibited high genomic variation, while varieties of *O. xoconostle*, *O. robusta*, and *O. streptacantha* showed a higher level of association.

Other ambiguities in the taxonomic classification of *Opuntia* spp. emerged in a study that used microsatellite polymorphisms to try to discriminate between two morphologically distinct *O. echios* botanical varieties (echios and gigantea) native to the Galapagos islands (Helsen et al., 2009). Once again, the authors highlighted that molecular data did not support the current taxonomic differentiation between these taxa. Although these studies clarified some taxonomical aspects of the genus and were useful for cultivar fingerprinting, there is still a lack of knowledge regarding the level of genetic diversity among the most diffused cultivated genotypes throughout the world and the diversity of cultivars, wild genotypes, and species related to *O. ficus indica*. Garcia-Zambrano et al. (2009) used microsatellite markers (SSR) to investigate the genetic diversity among *O. ficus indica* cultivated varieties and some related species. They reported that SSR could analyze a greater number of individuals originating from controlled crosses with different parentals to assess the molecular evolution of polyploidy in *Opuntia* spp.

Additionally, microsatellites may use as fast and reliable techniques to discriminate *Opuntia* apomictic seedlings from zygotic ones (Mondragón-Jacobo & Bordelon, 2002; Reyes-Agüero et al., 2006). Therefore, molecular techniques are the most appropriate tools for assessing the evolution of genetic diversity in *Opuntia* germplasm collections; such analysis should be a prerequisite for planning breeding programs that capture most of the existing variability among prickly pears (Caruso et al., 2010). The use of these molecular markers is strongly suggested to reclassify the cactus pear cultivated accessions, which exhibit a high level of variation regardless of the current taxonomical classification and probably should be classified as the same species, as suggested by Kiesling (1998).

7 Genetic Diversity of *Opuntia* spp.

The molecular characterization of populations could assist plant breeders with a better knowledge of the existing genetic variability. Reis et al. (2018) investigated the genetic diversity of 19 Portuguese *Opuntia* spp. populations from the species *O. ficus-indica*, *O. elata*, *O. dillenii*, and *O. robusta* using nuclear microsatellite (nuSSR) markers. Also, they used the Italian cultivars 'Bianca', 'Gialla' and 'Rossa' for comparison purposes. The study of Reis et al. (2018) found no genetic differences between the inermis form, *O. ficus-indica* f. *ficus-indica*, and the rewilded spiny one, *O. ficus-indica* f. *amyclaea*. The UPGMA tree indicated that the clustering pattern was unrelated to the geographical origin. Furthermore, the results from

the same study showed a low level of genetic diversity among the Portuguese populations of *O. ficus-indica*.

Labra et al. (2003) used two molecular techniques: chloroplast simple sequence repeat (cpSSR) and amplified fragment length polymorphism (AFLP) to evaluate the characterization and genetic relationships among 11 *Opuntia* spp. The analysis of cpSSR and AFLP results in *O. ficus-indica* and *O. megacantha* species showed a common genomic constitution and a clear distinction from all other *Opuntia* accessions analyzed. Moreover, based on molecular analysis, morphological traits, and biogeographical distribution, Labra et al. (2003) suggested that *O. ficus-indica* and *O. megacantha* should be considered the same species.

Although most of the *Opuntia* wild species related to cactus pear, they are clustered in separate groups, the genotypes classified as *O. amyclaea*, *O. megacantha*, *O. fusicaulis*, *O. streptacantha*, and *O. albicarpa* are closely related to the *O. ficus indica* cultivated varieties. The analysis and previous work based on molecular variation (Griffith, 2004; Labra et al., 2003; Wang et al., 1998) support the fact that the present classification of cultivated varieties and wild genotypes based on morphological parameters is misleading. Consequently, molecular tools are the most appropriate tools for assessing the level of genetic diversity in *Opuntia* germplasm collections; such analysis should be a prerequisite for planning breeding programs that capture most of the existing variability among prickly pears. The use of these markers is strongly suggested to reclassify the cactus pear cultivated accessions, which exhibit a high level of variation regardless of the current taxonomical classification and probably should be classified as the same species, as suggested by Kiesling (1998).

Two important studies (Griffith, 2004; Labra et al., 2003) employed different molecular tools to elucidate taxonomical aspects of the genus, particularly the origin of *O. ficus indica*. Labra et al. (2003) used AFLP to verify the lack of genetic differentiation between *O. ficus indica* and *O. megacantha* populations and suggested that *O. ficus indica* should be considered as a domesticated form of the spiny *O. megacantha*. On the other hand, (Griffith, 2004) considered the species to be a group of different clones, selected for their low number of spines and their fleshy fruits derived from different parentals, most likely from other arborescent opuntias from central and southern Mexico. Most of these DNA-based analyses revealed discrepancies between molecular characterization and classical taxonomical classification.

Caruso et al. (2010) investigated 6 highly polymorphic simple sequence repeats (SSR) and 2 expressed sequence tag (EST)-SSR loci in 62 wild and cultivated genotypes belonging to 16 *Opuntia* spp., which collected from several areas throughout the world. The phylogenetic analyses separated the wild opuntias from the cultivated ones. However, the *O. ficus indica* accessions did not cluster separately from other arborescent cactus pear species, such as *O. amyclaea*, *O. megacantha*, *O. streptacantha*, *O. fusicaulis*, and *O. albicarpa*, indicating that their current taxonomical classifications do not fit with their genetic variability. In general, the genotypes cultivated in Mexico showed high diversity levels, whereas most of the spineless accessions collected in other countries had a very narrow genetic base.

Furthermore, Browne et al. (2003) evaluated the phylogenetic relationships among 8 of the 14 Galapagos *Opuntia* taxa (240 individuals), using 8 allozyme markers, but found no variation, probably due to conservatism in the allozyme markers they used. Browne et al. (2003) used a larger set of neutral and highly variable microsatellite markers (Goldstein & Schlotterer, 1999) to re-evaluate the genetic variability of two *O. echios* varieties. The ample genetic diversity uncovered by these markers is postulated to allow population structures to be revealed more accurately than by other markers (Liu et al., 2003).

The *Opuntia* genus includes important cultivated species that have been widely studied using different molecular markers, mostly nuclear ones. Although most of the wild *Opuntia* spp. related to cactus species exhibited differences in fruit color, flesh consistency, and thorniness, the results of Las. Casas et al. (2017) showed that genotypes classified as *O. amyclaea*, *O. megacantha*, *O. elizondoana*, *O. streptacantha*, *O. vulgaris*, *O. joconostle*, *O. undulata*, and *O. albicarpa* had the same chlorotype of the *O. ficus-indica* cultivated varieties, clustering into a unique group. More than two-thirds of the analyzed genotypes and 9 species among the 15 species showed the same maternal inheritance, attesting to the narrow genetic variability among the cultivated *Opuntia* genotypes and the above-supposed species. The results were mostly in agreement with those obtained by cpSSR analyses from Labra et al. (2003), who reported that *O. stricta* grouped independently from *O. ficus-indica*, *O. megacantha*, *O. amyclaea*, and *O. undulata*, which have the same chlorotype. However, in the dendrogram, *O. spinulifera* and *O. robusta* segregated separately, as well. Other works confirmed that *O. robusta*, *O. stricta*, *O. leucotricha*, and *O. cochenillifera* had different plastid genealogies (Bárcenas et al., 2011; Majure et al., 2012a; Realini et al., 2015). The maternal inheritance analysis results revealed that genotypes classified as *O. ficus-indica*, *O. albicarpa*, *O. streptacantha*, and *O. megacantha* are closely related and do not show species-specific chlorotype (Fig. 8.2). The result agrees with the previous studies based on nuclear SSRs, ISSRs, and RAPDs (Caruso et al., 2010; Griffith, 2004; Labra et al., 2003; Samah et al., 2016; Valadez-Moctezuma et al., 2015).

Generally, the molecular analysis of plastid genetic variability confirmed previous works based on nuclear marker analysis (Caruso et al., 2010; Griffith, 2004; Labra et al., 2003; Samah et al., 2016; Wang et al., 1998), clearly supporting the fact that *O. amyclaea*, *O. megacantha*, *O. elizondoana*, *O. streptacantha*, *O. vulgaris*, *O. joconostle*, *O. undulata*, and *O. albicarpa* are closely related to *O. ficus-indica*, and weakening the previous taxonomical classification based on the morphological parameters.

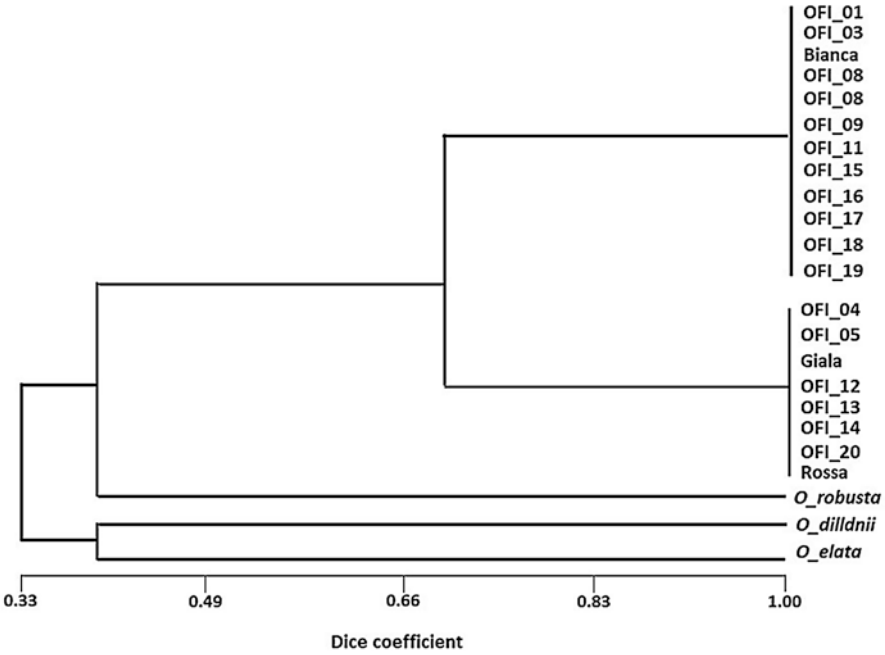

Fig. 8.2 Dendrogram of the 22 *Opuntia* spp. populations obtained from SSR markers based on Dice coefficient and using the unweighted pair group arithmetic mean method (UPGMA) as the clustering method. The data obtained from SSR markers and phylogenetic analysis revealed two major clusters contained all *O. ficus-indica* (OFI) populations, in addition to cvs. Rossa, Gialla, and Bianca. Regarding the species *O. dillenii*, *O. elata* and *O. robusta* were separately in different branches (Fig. 8.1). The graph has been adapted from the study of Reis et al. (2018)

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