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RESEARCH ARTICLE

Genetic diversity and differentiation of South African cactus pear cultivars (*Opuntia* spp.) based on simple sequence repeat (SSR) markers

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Abstract *Opuntia ficus-indica* (L.) Mill. is one of the most recognisable agricultural crops that can withstand harsh environmental conditions. South Africa is one of the few countries that hosts a large germplasm of *Opuntia* cultivars, which represent an *ex-situ* conservation population. However, little is known about the genetic diversity in this population. Additionally, some genotypes are morphologically indistinct, and therefore, making it a challenge for novice farmers and researchers to recognise specimens in the germplasm. The current study aimed to differentiate and measure the genetic diversity in 44 cultivars that represent the South African *Opuntia* germplasm using eight simple sequence repeat (SSR) markers. Evidently, the cultivars comprised moderate levels of diversity (average polymorphic information content, PIC = 0.37, Nei's unbiased gene diversity = 0.42) that discriminated 90% of the cultivars. Analysis of the cultivars with the unweighted pair-group method using arithmetic averages (UPGMA) method revealed three main clusters, whereas principal coordinate analysis (PCoA) showed indistinct clustering of cultivars based on their usage in the agricultural market. Overall, the used SSR markers were able to distinguish most cultivars in the South African *Opuntia* germplasm—a genetic resource that appears to hold sufficient genetic diversity to aid the conservation and breeding of novel cultivars for the agriculture market.

Keywords *Opuntia ficus-indica* · Cactus pear germplasm · Cultivars · *Ex-situ* conservation · Genetic resources

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Introduction

Cactus pear is a widely used and most economically valuable member of Cactaceae—a plant family that contains approximately 1600 species of cacti (Wallace and Gibson 2002). The family is currently classed into four subfamilies, and thus, Maihuenioideae, Pereskioideae, Cactoideae and Opuntioideae (Simpson 2010). The genus *Opuntia* (subfamily Opuntioideae) comprises taxa that are predominantly used in the agriculture industry as livestock feed (Dubeux et al. 2021; Pastorelli et al. 2022; Sipango et al. 2022). However, humans also consume cactus pear fruits (also called 'prickly pear') and young cladodes as vegetables (Barba et al. 2022). Fruit is highly nutritious as it contains numerous bioactive compounds (including betalains, carotenoids, flavonoids and phenols) along with proteins, minerals, vitamins, fatty acids, sterols, carbohydrates and fibres

(Daniloski et al. 2022). Apart from nutritional benefits, cactus pear may further serve an important socio-economic role in the livelihood of human societies (Moshobane et al. 2022) and, as such, make it a crop of agricultural significance.

The genus *Opuntia* is highly diverse and comprises up to 250 different species (da Silva et al. 2022a). Members of *Opuntia* show different levels of ploidy, ranging from $2n = 2\times$ to $2n = 8$x (Segura et al. 2007). Polyploidy in plants has been construed as the main driver of diversity in addition to supporting geographic expansion, climatic niche exchange and lineage longevity (Clark and Donoghue 2018). Further, polyploid genomes may have significant implications for plant breeding efforts by increasing allelic diversity, heterozygosity fixation, larger quantitative traits and genomic organisational changes at the genetic and epigenetic levels (van de Peer et al. 2017, 2021; da Silva et al. 2022a, b). Polyploidy in *Opuntia* facilitates survival in hostile environments (Granados-Aguilar et al. 2022). *Opuntia ficus-indica* (L.) Mill. and *O. robusta* J.C. Wendl. are, for instance, usually octoploids ($2n = 8x = 88$, Segura et al. 2007). Yet, *O. ficus-indica* can endure temperatures up to 70 °C (Granados-Aguilar et al. 2022), whereas *O. robusta* tolerates frost much better (Snyman et al. 2007). The capacity to withstand severe environments has made *Opuntia* species some of the important production crops in the South African agriculture industry (de Wit and Fouché 2021).

Opuntia breeding over the years has produced many cultivars and South Africa hosts one of the largest germplasm collections in the world (Chapman et al. 2002). However, cultivar identification tends to be challenging when plants are in a vegetative state due to inadequate distinctive features (Felker et al. 2006). Therefore, novel methods are required for differentiating *Opuntia* cultivars and species too. Modern high-throughput sequencing techniques have facilitated the discovery of genome-wide DNA markers that can be used to characterise germplasm pools and assess genetic diversity therein. To this end, simple sequence repeats (SSRs) have been established as molecular markers of choice owing to their abundance in plant genomes (Feng et al. 2016; Lu et al. 2019; Bhattarai et al. 2021).

As molecular markers, SSRs are more informative than the biallelic single nucleotide polymorphism (SNP) markers as the former tend to have many alleles per locus (Hamblin et al. 2007). Further, being co-dominant means that SSRs are valuable over dominant molecular markers as they will be relatively variable and may easily differentiate between homozygous and heterozygous individuals (Csencsics et al. 2010; Dutta et al. 2011). In the past, SSRs have been used to assess the genetic diversity in many diploid (Wen et al. 2010; Lin et al. 2015; Feng et al. 2016) and polyploid (Pfeiffer et al. 2011; Ali et al. 2019; Cusaro et al. 2022) plant species. Additionally, SSRs can discern morphologically indistinct cultivars (Caruso et al. 2010) and even determine gene flow incidents among individuals of different populations or closely related species (Fava et al. 2020). Then, SSRs are ideal markers for discerning and assessing the diversity in species and cultivars that form the South African *Opuntia* germplasm.

In this study, we aimed to distinguish and evaluate the genetic diversity in cultivars that make up the South African *Opuntia* germplasm. This was done using SSR markers that were previously identified in the *Opuntia ficus-indica* genome (Maleka et al., unpublished data). Similar to other genotypes all over the world (Caruso et al. 2010), some cultivars in the South African germplasm are phenotypically indistinguishable. Therefore, the current study may assist in genetically resolving the local germplasm in addition to enabling assessments of genetic diversity within breeding pools across South Africa.

Materials and methods

Plant material and DNA extraction

In 2015, an orchard including 44 *Opuntia* cultivars was established at the University of the Free State in Bloemfontein, Free State Province, Republic of South Africa (29°6′27.08″S; 26°11′32.90″E). The plants were propagated clonally from a 10-year-old evaluation trial that was planted at the Waterkloof farm, which is located roughly 30 km outside Bloemfontein. The Waterkloof orchard was established in 2005 using cladodes collected from other conservation sites located all over South Africa.

For the current study, a single flower was harvested from each of the 44 *Opuntia* cultivars (Table 1) and used for extracting genomic DNA. Specifically, genomic DNA was extracted from 50–100 mg

Table 1 A list showing the 44 *Opuntia* cultivars studied herein and their main use in the agriculture industry. All cultivars, barring Monterey and Robusta (*Opuntia robusta*) are samples of *O. ficus-indica*. The quantity (DNA concentration) and purity (UV absorbance at 260/280 nm) of genomic DNA isolated from each cultivar are also indicated

Sample ID	Main use[a]	DNA conc. (ng/µL)	$A_{260/280}$	Sample ID	Main use[a]	DNA conc. (ng/µL)	$A_{260/280}$
1. Algerian	Fruit	93.60	1.36	23. Nepgen	Fruit	19.00	1.74
2. American Giant	Fruit	88.67	1.27	24. Nudosa	Fruit	43.49	1.68
3. Amersfoort	Forage	53.74	1.54	25. Ofer	Forage	16.86	1.79
4. Arbiter	Forage	186.14	1.41	26. Postmasburg	Fruit/Forage	48.08	1.64
5. Berg x Mexican	Forage	56.92	1.51	27. R1251	Fruit	197.08	1.38
6. Blue Motto	Fruit	106.55	1.35	28. R1259	Forage	57.96	1.53
7. Botswana	Unknown	138.69	1.74	29. R1260	Forage	161.57	1.40
8. Clocolan	Unknown	122.14	1.44	30. Robusta	Forage	60.96	1.54
9. Corfu	Forage	42.08	1.46	31. Robusta x Castillo	Forage	43.21	1.59
10. Cross X	Fruit	161.20	1.52	32. Roedtan	Fruit	38.98	1.44
11. Direkteur	Fruit	139.60	1.44	33. Roly Poly	Forage	93.20	1.52
12. Ficus Indica	Forage	48.91	1.25	34. Rossa	Fruit	156.05	1.50
13. Fresno	Forage	193.23	1.36	35. Santa Rosa	Fruit	61.35	1.56
14. Fusicaulis	Fruit	13.90	1.52	36. Schagen	Forage	188.09	1.50
15. Gymno Carpo	Fruit	69.79	1.49	37. Sharsheret	Forage	92.72	1.58
16. Malta	Fruit	43.27	1.54	38. Sicilian Indian Fig	Forage	149.55	1.41
17. Messina	Forage	77.40	1.58	39. Skinners Court	Forage	207.09	1.46
18. Mexican	Fruit	37.59	1.75	40. Tormentosa	Forage	79.47	1.48
19. Meyers	Fruit	22.17	1.87	41. Turpin	Fruit	121.29	1.39
20. Monterey	Unknown	58.17	1.43	42. Van As	Fruit	168.47	1.45
21. Morado	Fruit	54.14	1.55	43. Vryheid	Forage/Fruit	52.62	1.54
22. Muscatel	Forage	120.92	1.51	44. Zastron	Forage/Fruit	52.73	1.43

[a]The main use of cactus pear cultivars in the agriculture industry as per Mashope (2007)

of liquid nitrogen-powdered samples following the NucleoSpin Plant II Kit (Macherey–Nagel, Düren, Germany). However, cell lysis was completed at 65 °C for 30 min. DNA samples were quantified with a spectrophotometer (NanoDrop ND-1000, Thermo Fisher Scientific, Waltham, USA), whereas DNA quality was analysed by electrophoresis via 1% (w/v) TAE agarose gels. Gels were stained with the GelRed Nucleic Acid Gel Stain (Biotium, Hayward, USA) and visualised under UV light using a G:Box Gel Documentation System (Syngene, Cambridge, UK).

Polymerase Chain Reaction (PCR) amplification and genotyping of SSR loci

In the past, we identified many SSR loci in the *Opuntia ficus-indica* genome after Illumina sequencing (Maleka et al., unpublished data). In this study, the 5′ ends of forward primers for eight SSR loci were labelled with the 6-FAM fluorescent dye and used during PCR. PCR was carried out with 40–50 ng template DNA, 0.2 µM of each primer, 1×EmeraldAmp GT PCR Master Mix (Takara Bio Inc., Shiga, Japan) and filled to 10 µL volumes per reaction with nuclease-free water. Cycling conditions included the primary denaturation at 98 °C for 3 min, followed by 30 cycles of denaturation at 98 °C for 10 s, primer annealing at 55.7 °C or 62.5 °C (Table 2) for 20 s and primer extension at 72 °C for 20 s. The final extension was done at 72 °C for 1 min. All reactions were effected on an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Post-PCR, selected products were stained with GelRed (Biotium) and electrophoresed on 3% (w/v) TBE agarose gels that contained a 100–3000 bp PCR DNA ladder (Ampliqon, Odense, Denmark). Once verified by gel visualisation (G:Box Gel Doc. System), amplicons were analysed by capillary electrophoresis on an

Table 2 Primer information and statistics of the polymorphisms observed at the eight simple sequence repeat (SSR) loci

Locus	Sequence (5′→3′)	T_a [#]	N_A [a]	Allele frequencies	λ [b]	Hexp [c]	Evenness	PIC [d]	χ^2 [e]	*p*-value
L5354	For: TTAGGACTCGCCAATCTTCTGG Rev: TTCCATTAGCCTCCTCCATCAA	62.5 °C	1	1.00	0.0	0.0	NaN	0	61.67	2.589e−13
L10464	For: GGTGGCTCAAGGAAGTGTATGG Rev: TGCATGTTTGGCAGCTCAGTAT	62.5 °C	4	0.08–0.62	0.54	0.54	0.67	0.52	164.26	0.000e+00
L23031	For: GAGTGATGAATGTTGGTGGTGCT Rev: ACCATCTCCTCCTTCTGGTTGAC	62.5 °C	4	0.06–0.55	0.62	0.62	0.75	0.56	697.93	0.000e+00
L28477	For: TGCGAGGTAGACGTGTTGGA Rev: TGCTCTCGACTCTCCCCACT	62.5 °C	2	0.38–0.62	0.47	0.47	0.94	0.36	73.47	7.159e−11
L37320	For: CAGCCAACGACCCAACATCTAT Rev: CAATGTCCTCCCTTCCACTGTC	62.5 °C	2	0.36–0.64	0.46	0.46	0.92	0.35	108.04	0.000e+00
L38909	For: AGGTCCGTCATAGTCCCTCC Rev: TCATCGGTTGAGAATGGGCT	55.7 °C	4	0.04–0.39	0.66	0.66	0.89	0.62	115.24	1.266e−11
L86067	For: CCACATCACCATGCAAACCATT Rev: TGTTGTTGCGCCTGCTCTATG	62.5 °C	1	1.00	0.0	0.0	NaN	0	73.33	7.772e−16
L161092	For: TGCAAGGGTAAGACTGCCTAC Rev: AAAGCCGACTCAAGCACGA	55.7 °C	6	0.01–0.51	0.61	0.62	0.79	0.56	18,082.95	0.000e+00
Mean			3			0.422		0.37		
Total					0.973		0.959			

[#]Annealing temperature

[a]Number of alleles

[b]Simpson's index

[c]Nei's unbiased genetic diversity

[d]Polymorphic Information Content

[e]Chi-square statistic (HWE)

ABI3500xl Genetic Analyzer (Applied Biosystems). The polymer POP-7 (Thermo Fisher Sci.) was applied as a separation matrix, while fragment sizes were determined with a GeneScan 500 LIZ Size Standard (Applied Biosystems) during these analyses.

Allele scoring and genetic diversity estimation

SSR alleles at the eight loci were scored with the software package GeneMarker v1.6 (BioGene Ltd., Kimbolton, UK). After scoring, allelic data were exported into the R packages *polysat* v1.7–6 (Clark and Jasieniuk 2011) and *poppr* v2.9.2 (Kamvar et al. 2014) for calculating different genetic diversity statistics. Statistics included the numbers of alleles (N_A), allelic frequencies, the number of multilocus genotypes (MLG, which signifies the unique combination of alleles at two or more loci), the expected MLG (eMLG) and the Hardy–Weinberg equilibrium (HWE). Moreover, the polymorphic information content (PIC) for each locus was calculated with PIC_Calc 0.6 (http://www.biosoft.net/dna/pic.htm).

Genotypic diversity was computed using various indices, and these included the Simpson's index (λ; Simpson 1949), the Evenness index (E.5; Grünwald et al. 2003) and Nei's unbiased gene diversity (Hexp; Nei 1978). The software *poppr* was also used to create a genotype accumulation curve, which shows the power of random loci to discern unique individuals. Linkage disequilibrium (LD) between pairs of loci was determined via the standardized index of association test (rbarD; Agapow and Burt 2001). Contrary to the traditional index of association (I_A), calculations for the rbarD are independent of the number of loci included in the test. The programme DARwin v6.0.021 (https://darwin.cirad.fr/) was used to do principal coordinate analysis (PCoA) and make a dendrogram based on the unweighted pair-group method using arithmetic averages (UPGMA).

Results

DNA quantity and quality

The amount of DNA extracted from the 44 *Opuntia* flower tissues ranged from 13.90–207.09 ng/uL (Table 1), with an average yield of 91.79 ng/uL. Three cultivars (Fusicaulis, Nepgen and Ofer) yielded DNA amounts < 20 ng/µL, while another three (Mexican, Meyers, and Roedtan) produced < 40 ng/µL. Regarding DNA quality, the A260/280 ratios ranged from 1.25–1.87 (Table 1), with an average ratio of 1.51. Curiously, cultivars that produced low DNA concentrations presented DNA of ideal quality, with Ofer and Meyers having the highest A260/280 ratios of 1.79 and 1.87, respectively.

SSR genotyping and genetic diversity estimates

Barring some loci in particular individuals, the eight loci generally yielded reproducible profiles that permitted allele scoring. Six of the eight SSR loci were polymorphic (thus, 75% polymorphism rate) and two (L5354 and L86067) were monomorphic. In total, the eight loci consisted of 24 alleles in the 44 *Opuntia* samples, with a mean number of three alleles per locus (Table 2). The 24 alleles occurred at frequencies ranging from 0.01–1.00. With six alleles, locus L161092 yielded the highest number of alleles per locus, and this corresponded to a PIC of 0.56. The most informative locus in this study, however, was L38909 (PIC = 0.62), and L37320 (PIC = 0.35) was the least polymorphic. The mean PIC value across all eight loci was 0.37 (Table 2), implying that the studied loci are fairly informative.

According to the *poppr* package, a total of 40 MLGs were expected (SE = 0.0) and realised based on the current data. The genotype accumulation curve, however, showed that five loci were sufficient to distinguish 90% of the 40 MLGs (Fig. 1). Each of the four remaining MLGs (10%) occurred twice in the samples (data not shown). The Simpson's index was high, and this indicated that most genotypes are unique (λ = 0.973), while the high Evenness score (E.5 = 0.959) suggested that the population has equally abundant genotypes. Genetic variation in *Opuntia* cultivars was assessed with Nei's unbiased genetic diversity index. To this end, locus L38909 emerged as the most diverse (Hexp = 0.66) in the study, followed by both L23031 (0.62) and L161092 (0.62). The least diverse polymorphic locus was L37320, with Hexp = 0.46 (Table 2). Across all loci, the Hexp index = 0.422 and this shows that the South African *Opuntia* germplasm harbours modest levels of genetic variation. Indices of the two LD tests deviated significantly from the null hypothesis of individuals in the population mating randomly (I_A = 0.763,

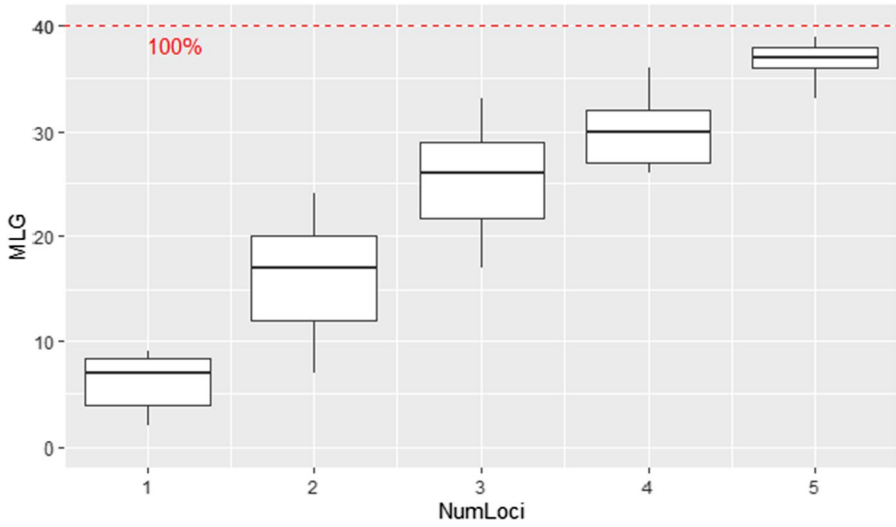

Fig. 1 A genotype accumulation curve illustrating the number of loci required to differentiate the identified multilocus genotypes (MLGs)

p-value = 0.02; rbarD = 0.173, *p*-value = 0.02). Hence, the null hypothesis is rejected and the alternative hypothesis (that the germplasm is asexual or clonal) is deemed to be valid. Four of the eight loci diverged from the HWE significantly (Table 2).

Genetic relationships among *Opuntia* cultivars

Relationships among the 44 *Opuntia* cultivars were analysed via clustering (UPGMA) and ordination (PCoA) methods. Clustering analysis produced a dendrogram with three main clades (Fig. 2). Overall, cultivars in clade I tended to be genetically farther distant from each other than those within clades II and III. However, the two cultivars of *O. robusta* (Monterey and Robusta) in clade I were genetically closer to each other as expected. Cultivars that historically came from the same country, surprisingly, did not cluster together. Also, the three cultivars from Botswana (R1251, R1259 and R1260) occurred in each of the three clades, while those from Israel (Ofer, Messina and Sharsheret) grouped into clades II and III. Clades II and III each comprised two branched tips relating to the four shared MLGs defined earlier. One of the divided tips showed Roedtan to be genetically related to Rossa, a cultivar of Italian origin (Fig. 2). As with the dendrogram, PCoA (Fig. 3) did not show any distinctive clustering, even when considering the commercial usage of cultivars as depicted in Table 1. Overall, the SSR loci in this study contain sufficient polymorphisms that effectively segregated cultivars in the South African *Opuntia* germplasm.

Discussion

Members of the Cactaceae attract research interest globally because of their specialised physiological traits that allow survival in severe habitats (Nobel 2002). Among such interests is the requirement to genetically characterise and differentiate species and cultivars, particularly within the genus *Opuntia*. Studies have been done on many ecotypes from diverse germplasms using various molecular markers (Omar et al. 2021). *Opuntia* cultivars forming the South African germplasm have, however, received little molecular characterisation despite this being one of the key genetic pools (Chapman et al. 2002). Notable studies include those of Oelofse (2002) and Mashope et al. (2011) that both applied amplified fragment length polymorphisms (AFLPs) as molecular markers to discern cultivars in the germplasm. Similarly, the current study sought to differentiate and measure the genetic diversity within the South African *Opuntia* germplasm using eight genomic SSR markers. Information on the relationships and genetic diversity in cultivars will assist in the selection of superior genotypes toward the development of new cultivars and preservation of cultivars as distinct genotypes. Overall, the studied SSR markers were able to distinguish 90% of the *Opuntia* cultivars, and evidently,

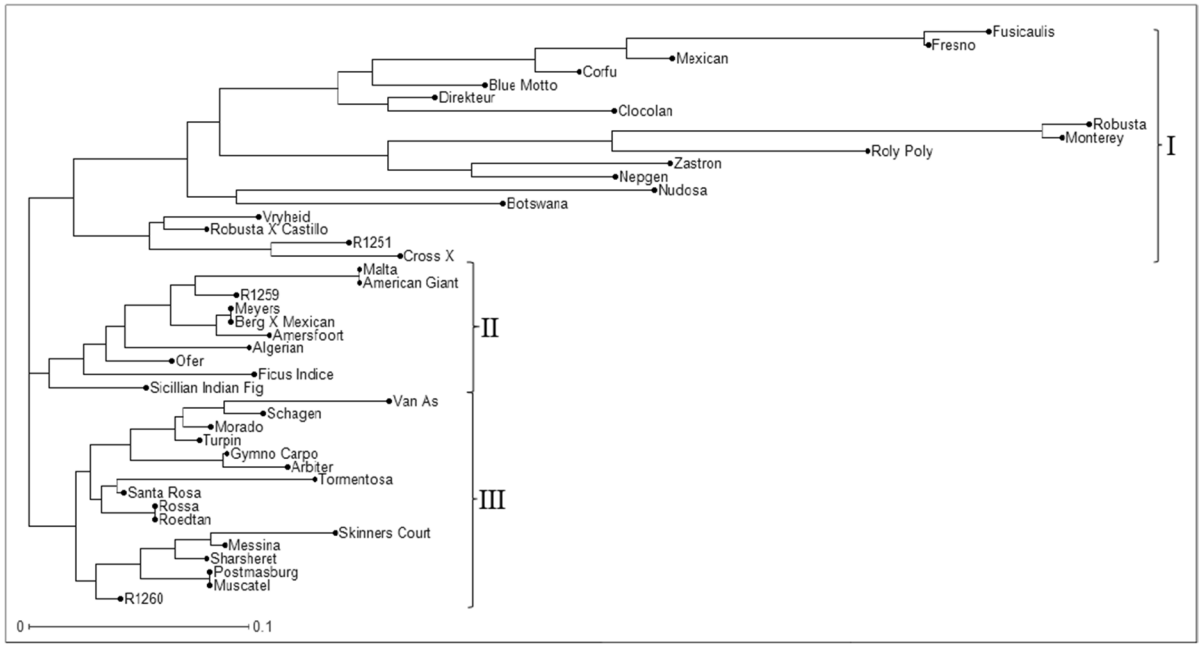

Fig. 2 A UPGMA tree demonstrating the relationships among the 44 cultivars of *Opuntia ficus-indica* and *O. robusta* (Robusta and Monterey). The tree reveals three main clusters referred to as clades I–III. Clade II shows two branched tips that clustered Malta with American Giant and Meyers with Berg x Mexican. Rossa and Roedtan along with Postmasburg and Muscatel occupy the branched tips in clade III

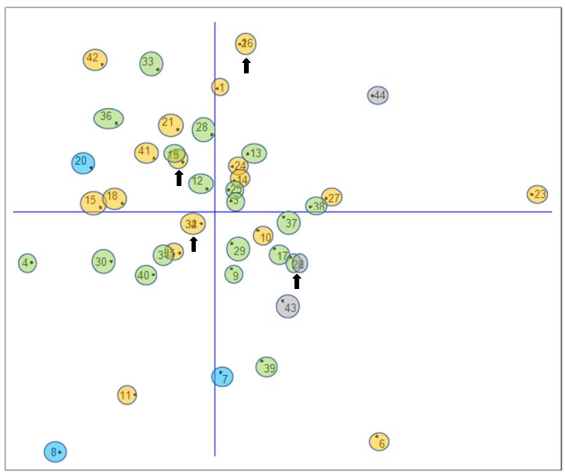

Fig. 3 Principal Coordinate Analysis (PCoA) showing the relationships among the 44 cultivars of *Opuntia ficus-indica* and *O. robusta* (Robusta and Monterey). The numbers refer to the cultivars as per Table 1. Overlaid numbers, marked with black arrows, relate to the branched tips in Fig. 2 as follows: 2 + 16, 5 + 19, 22 + 26 as well as 32 + 34. Coloured circles indicate cultivar usage as follows: orange (fruit), green (forage), grey (fruit/forage) and blue (unknown)

the genotypes harbour moderate levels of genetic variability.

DNA extraction from cacti is infamously challenging due to the large quantity of mucilage, secondary metabolites and polysaccharides (Nobel et al. 1992). As such, several protocols have been developed and improved over the years to try overcoming this challenge (De La Cruz et al. 1997; Mondragon-Jacobo et al. 2000; Fehlberg et al. 2013; Martínez-González et al. 2017). Although the protocols have been effective, they tend to be laborious. Moreover, some protocols need large amounts (ca. 5–8 g) of tissue material to begin with (Mondragon-Jacobo et al. 2000). Interestingly, Fehlberg et al. (2013) developed a novel method that is based on relatively small amounts (ca. 40–100 mg) of cactus pear spines. Our study also sought to simplify DNA isolation from cactus pear by using small amounts of flower tissues because they are soft and amenable to crushing with minimal effort. While the amount of DNA extracted from flower tissues was below that obtained from cladodes using current protocols, the quality was, however, similar

(Martínez-González et al. 2017). In retrospect, we suppose that the flower tissues were not crushed sufficiently, and this impacted DNA recovery. Even so, our approach establishes flowers as an alternative source of quality DNA for molecular studies in *Opuntia*. Tissue sampling, however, should be planned around the limited annual flowering season.

Of the eight SSR markers used in the current study, five were able to discriminate 90% of the 44 *O. ficus-indica* samples—meaning that ~ 20% of the samples (four MLGs) remained undifferentiated. In European plum (*Prunus domestica* L.), however, four genomic SSRs were sufficient to differentiate 44 MLGs and this was attributed to the high number of alleles detected at the loci (averaging 19.2 per locus; Manco et al. 2020). Elsewhere, Fiore et al. (2022) were able to distinguish 30 hazelnut (*Corylus avellana* L.) genotypes via nine SSR markers that had an average of 7.67 alleles per locus. Given that the *Opuntia* genotypes in the current study revealed low to moderate allelic diversity per locus, it can, therefore, be asserted that the genetic diversity in the germplasm was most likely underestimated. The use of additional markers to further distinguish the overlapped genotypes will offer an opportunity to better estimate the genetic diversity in this germplasm.

To our knowledge, this is the first study to describe estimates of genetic diversity in the South African *Opuntia* germplasm via SSRs. The moderate levels of polymorphism (average PIC = 0.37) observed in the studied loci were similar to those described by Mashope et al. 2011 (average PIC = 0.29) based on AFLP markers. PIC indicates the ability of a marker to identify polymorphisms among individuals in a population, and this depends on the number of alleles detected at each locus and the corresponding allelic distribution frequencies (Serrote et al. 2020). Markers with PIC values ≥ 0.50 are usually said to be very informative, while values in the range 0.25–0.50 are moderately informative. Under 0.25, the markers are deemed not to be very informative. Therefore, the current SSR markers and the AFLP markers used on *Opuntia* samples in the past (Mashope et al. 2011) are moderately informative.

Prior to this study, SSRs have been used several times to assess the genetic diversity in global *Opuntia* specimens (Helsen et al. 2009; Caruso et al. 2010; Erre et al. 2011; Chessa et al. 2013; Samah et al. 2016; Reis et al. 2018). Astonishingly, the *Opuntia* germplasm from Mexico presented low levels of polymorphisms (average PIC = 0.246; Samah et al. 2016), as did a global group of wild and cultivated genotypes that represented 16 different species of *Opuntia* (average PIC = 0.191; Caruso et al. 2010). Asserted reasons for the low polymorphic rate included the background of researched germplasms as well as the choice and number of tested SSR loci (Samah et al. 2016). Overall, that the South African *Opuntia* germplasm is more polymorphic than specimens used by Caruso et al. (2010) and Samah et al. (2016) was remarkable and encouraging.

In contrast to the above studies, Chessa et al. (2013) and Bendhifi Zarroug et al. (2015) reported high levels of polymorphism (average PIC > 0.71) within global genotypes of *Opuntia* species and cultivars having diverse histories. Similarly, Reis et al. (2018) found high levels of polymorphisms (average PIC = 0.711) in *Opuntia* populations from Portugal. In other plants, the average PIC from genomic SSRs ranged from 0.25 in global accessions of *Spinacia oleracea* L. (Bhattarai et al. 2021); 0.29–0.43 in cultivars of five *Prunus* species (Dettori et al. 2015); 0.61 in worldwide accessions of *Olea europaea* L. (Li et al. 2020) and 0.75 in Chinese genotypes of *Citrus sinesis* L. Osbeck (Biswas et al. 2014). In addition, Ali et al. (2019) reported an average PIC of 0.92 in species and hybrids of *Saccharum* and *Erianthus*. Nevertheless, the high levels of polymorphisms and diversity observed in *Opuntia* samples were attributed to the varied origins of cultivars along with the prevalence of asexual propagation and limited artificial selection (Chessa et al. 2013; Bendhifi Zarroug et al. 2015). The *Opuntia* germplasm in South Africa seemingly derive from the Burbank collection, which was produced through extensive crossings and selections among accessions from Mexico, Africa, Australia and several other countries (Chapman et al. 2002). Thus, their histories are convoluted. Also, the modest levels of polymorphisms and diversity in the South African *Opuntia* germplasm may suggest the adaptation of cultivars to the local climate. Increased genetic diversity and divergence are known indicators of organisms adapting to new or extreme environments (Chen et al. 2018; Wang et al. 2018). Indeed, the *Opuntia* population at the Waterkloof orchard was founded recently in relation to other cactus pear conservation sites in South Africa (Potgieter and

Mashope 2009). Cultivars in this study may, therefore, be adapting to the local environment—a notion that can only be confirmed by analysing the genetic diversity in *Opuntia* cultivars grown at other sites in South Africa. Overall, our findings affirm the South African *Opuntia* germplasm as one of the diverse and key breeding pools of cactus pear in the world.

The 44 *Opuntia* cultivars differentiated into three main clades, unlike the nine identified by Mashope et al. (2011) using AFLPs on 38 cultivars. Moreover, there was no distinct grouping of cultivars based on their main usage (either as fruit or fodder) in the agriculture industry. Such grouping was, however, noted when using randomly amplified polymorphic DNA (RAPD) markers to describe a few *Opuntia* samples (Wang et al. 1998). Mashope et al. (2011) also discovered some clustering in relation to plant growth habit (upright or bushy), whereas *Opuntia ficus-indica* samples from Portugal clustered based on fruit pulp colour (Reis et al. 2018). While it would have been interesting to detect some reasonable clustering in the South African *Opuntia* germplasm, the lack of it is also significant as it suggests the presence of sufficient levels of diversity in the cultivars. Future studies can try to identify and develop molecular markers associating with traits desired in the agriculture market (e.g., fruit quality, de Wit et al. 2010; Coetzer et al. 2019). Similar studies have already been done on fruit traits in sweet cherry (Ganopoulos et al. 2011) and kiwifruit (Liao et al. 2019). The South African *Opuntia* germplasm, in essence, appears to not only support the *ex-situ* conservation of cactus pear, but also holds the genetic capacity for breeding novel cultivars that will benefit the agriculture industry.

Conclusions

South Africa hosts one of the major germplasms of *Opuntia ficus-indica* in the world. The germplasm understandably derives from the Burbank collection that was established using material sourced from all over the world. Consequently, the diverse history and possibly adaptation to the local environment over many years may have influenced the moderate genetic variation found in South African cultivars. Genetic variability in the South African *Opuntia* cultivars means that the germplasm may be used for breeding new cultivars for the agriculture industry. Alternatively, the cultivars may serve as a source population for deriving new alleles that can rescue less diverse *Opuntia* breeding programmes around the world. Last, the SSR markers used in this study will be useful toward distinguishing and verifying unknown samples used by farmers and researchers all over South Africa.

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Declarations

Competing interests The authors declare that no external funding was received to help with the execution of this study or preparation of this manuscript.

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