Plant Genetic Resources: Characterization and Utilization

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Morphological and molecular divergence in ornamental variants of cactus which may be useful to generate new variants

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Research Article

Cite this article: das Neves AF, Mangolin CA, Fernandes VNdeA, Monteiro ER, Machado MdeFPS (2022). Morphological and molecular divergence in ornamental variants of cactus which may be useful to generate new variants. *Plant Genetic Resources: Characterization and Utilization* **20**, 290–296. https://doi.org/10.1017/S1479262123000163

Received: 21 March 2022
Revised: 25 February 2023
Accepted: 27 February 2023
First published online: 24 March 2023

Keywords:
Cactaceae; *Cereus*; microsatellite transferability; ornamental varieties; SSR loci

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Abstract

Although morphological plasticity has been widely known in various cactus genera, few studies have investigated the origin and molecular relationship between morphological variants from cacti. Morphological variants are relevant specimens because atypical, exotic and generally unique forms are preferred by cactus traders and collectors. The current study investigates the molecular relationship between the *tortuosus* and *monstruosus* ornamental variants of *Cereus peruvianus* used in landscapes. Polymorphisms in loci of simple-sequence repeats in DNA were used as molecular markers. The variants *tortuosus* and *monstruosus*, and plants with typically columnar and erect shoots cultivated in southern Brazil were retrieved from public parks and home gardens. High polymorphism, an indicative of vegetative propagation, and a moderate genetic divergence were detected at the molecular level in *monstruosus* and *tortuosus* plants. Artificial selection and vegetative propagation of the ornamental variants of *Cereus* may be inducing a moderate genetic divergence and formation of two heterologous groups with conservative genetic diversity. Polymorphism in *Cereus* variants revealed groups with contrasting genes among the variants *tortuosus* and *monstruosus* which may be useful for breeding to generate new and different new variants.

Introduction

The ornamental potential of cacti is known worldwide. Diversity of forms, different from other plants, beautiful flowers, tolerance to drought and/or cold, and minimum nutritional requirements are some remarkable characteristics that confer cacti their ornamental potential (Anderson, 2001; Ortega-Baes *et al.*, 2010). More than 300 species of cacti are cultivated as ornamental plants besides many variants available only in commercial nurseries involved with the conservation of rare cacti or maintained by collectors (Pérez-Molphe-Balch *et al.*, 2015; Cavalcante *et al.*, 2017; Gomes *et al.*, 2020; Gdaniec *et al.*, 2022). Morphological plasticity has also been widely known in various cactus genera. Morphological variants are relevant specimens since atypical, exotic and generally unique forms are preferred by cactus traders and collectors. However, few studies are available that investigated the origin and molecular relationship between morphological variants of cacti. Studies have shown that morphological and functional cacti variations have been influenced by anthropic activities and by environmental variables associated with different microhabitats (Barbosa *et al.*, 2018; Octavio-Aguilar *et al.*, 2019). High temperatures, even in the absence of strong direct solar UV radiation, have also been indicated as a possible factor which causes aberrations and variegations in the Cactaceae family (Ortega-Baes *et al.*, 2010; Basiuk, 2014).

The *tortuosus* and *monstruosus* variants of *Cereus* [P. Miller, 1754] are the genus's preferred plants ornamental use. The *tortuosus* and *monstruosus* variants are cultivated beside plants with typically erect shoots. The *tortuosus* variant with its spiral shoots (also known as 'screw cactus') has been selected and propagated in breeding programmes for the development of ornamental plants (Assis *et al.*, 2011). Plants with misshapen shoots (*monstruosus*) in which the areoles are found in broken ribs (forming alternate regular- or irregular-spaced knobs), crested shoots (*cristate*, forming alternate regularly spaced knobs), spiral (*tortuosus*) and variegated shoots (with combined characteristics) have greater ornamental value. The *monstruosus* and *cristate* phenotypes are observed in several cactus genera and species (Basiuk, 2014). Although the several phenotypic changes within an organism are the product of epigenetic changes (Zhang and Hsieh, 2013), one of the aims of the breeding programme of ornamental cacti reported by Assis *et al.* (2011) is the selection of genotypes suitable for obtaining variegated plants.

While plants with typically erect shoots of *Cereus peruvianus* Mill. are growth as a fruit-crop, marketed mainly in Israel under the name 'Koubo' (Mizrahi, 2014), the *tortuosus* and *monstruosus* variants with marked morphological divergence as well as plants with typically erect shoots are frequently cultivated in home gardens and public parks and squares in the southern region of Brazil. This species is also known under the name 'Princess of the Night'. The beautiful flowers of *C. peruvianus* open at early in the night, for only one night, closing daily the next morning (Mizrahi, 2014). According to Assis *et al.* (2011) *C. peruvianus* is a synonymy of *Cereus hildmannianus* K. Schumann in the southern region of Brazil. However, to date, there is no information in the literature on the molecular divergence between *tortuosus* and *monstruosus* variants. Molecular relationship between morphological variants of the *C. peruvianus* has been reported particularly in *in vitro* regenerated plants from callus tissues (Eloi *et al.*, 2017; Martin *et al.*, 2018). In spite of marked morphological divergence in *in vitro* regenerated plants, high genetic identity and low genetic divergence were reported among typical and atypical (*monstruosus*) regenerated plants of *Cereus*.

The current study investigates the molecular relations between *in vivo* cultivated *tortuosus* and *monstruosus* variants of *C. peruvianus*. The authors hypothesize that artificial selection and vegetative propagation as the predominant form of specimen multiplication of *C. peruvianus* may lead to genetic divergence and formation of genetically structured varieties. Polymorphisms in simple-sequence repeats (SSR) loci in the DNA (also called microsatellites) were used as molecular markers to evaluate whether these morphological variants of *C. peruvianus* are genetically structured.

Materials and methods

Morphological variants of C. peruvianus *and microsatellite transferability*

Samples of *C. peruvianus tortuosus* (26 plants) and *C. peruvianus monstruosus* (47 plants) as well as plants with typically columnar and erect shoots (19 plants), cultivated in southern Brazil (Fig. 1) were collected in several isolated urban areas, in different public parks and home gardens, and have been maintained for more than 5 years in the *Cereus*'s active germplasm bank in our Institution (latitude 23°25′38″, longitude 51°56′15″). Plants with erect shoots were collected in the several isolated urban areas and in different public parks and home gardens, while the *tortuosus* and *monstruosus* phenotypes were collected only in different home gardens. A greater number of variants *monstruosus* was collected since they have been more frequently found than *tortuosus*.

Genomic DNA was isolated from shoot pieces (200 mg) of each plant with erect, *tortuosus* and *monstruosus* morphologies by a procedure described by Martin *et al.* (2018). The DNA concentration was estimated by comparison to known concentrations of lambda phage DNA (50, 100 and 150 ng) on 0.8% agarose gel buffered with 1× TAE (0.04 M Tris-Acetate and 0.001 M EDTA). Ethidium bromide (0.5 µg/ml) was used to staining of gel which was visualized with a Molecular Image LOCCUS L-PIX – HE (Loccus do Brasil Ltda., São Paulo, SP, Brazil) using Picasa 3 software.

The microsatellite loci were analysed by transferability using the Pchi47 primer pair from *Polaskia chichipe* (Otero-Arnaiz *et al.*, 2004), the AaB6, AaD9 and AaH11 primer pairs from *Astrophytum asterias* (Terry *et al.*, 2006), the mAbR28 and mAbR86 primer pairs from *Ariocarpus bravoanus* (Hughes *et al.*, 2008) and mEgR78 primer pair from *Echinocactus grusonii* (Hardesty *et al.*, 2008) (online Supplementary Table S1). SSR amplification was performed using 20 µl of polymerase chain reaction (PCR) mixture solution containing 2.0 µl of 1× buffer containing Tris-HCl (10 mM Tris-HCl (pH 8.3) and 50 mM KCl), 2.5 mM of $MgCl_2$, 0.1 µM dNTPs, 1 U of Taq-DNA polymerase (Invitrogen), 0.5 µM each of the forward and reverse primers, 20 ng genomic DNA templates and Milli-Q water to make up to 20 µl. PCR amplification was run using a Techne TC-512 thermocycler.

Microsatellite amplification was initially performed according to the temperature originally described for each primer (Otero-Arnaiz *et al.*, 2004; Terry *et al.*, 2006; Hardesty *et al.*, 2008; Hughes *et al.*, 2008). The amplification condition described by Martin *et al.* (2018) and annealing temperatures between 53.8 and 64°C was employed to amplify again the products that were not clearly amplified by original procedures for each primer.

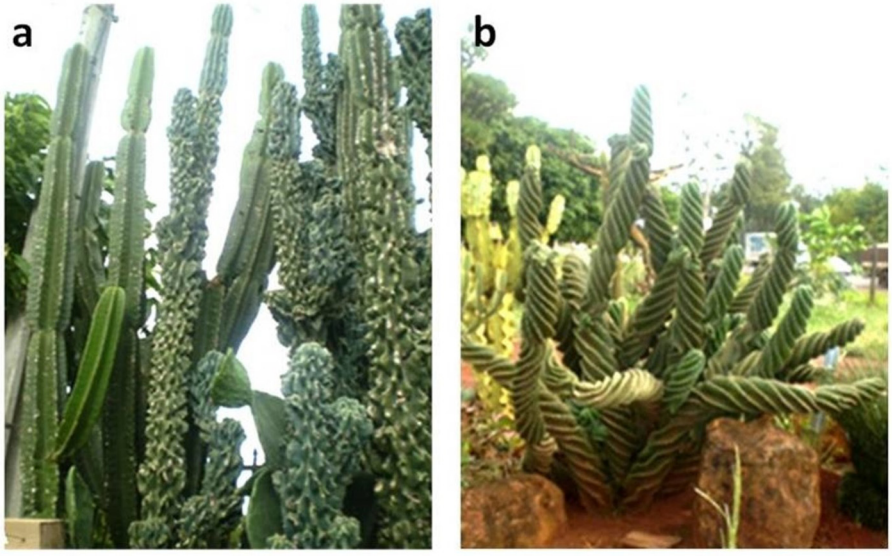

Fig. 1. Plants of the *C. peruvianus* cactus with erect shoots and plants of the variants *monstruosus* (a) and *tortuosus* (b) growing in home gardens in urban areas of Paraná state (south region, Brazil).

https://doi.org/10.1017/S1479262123000163 Published online by Cambridge University Press

Table 1. Number of alleles (Na) and number of effective alleles (Ne) per polymorphic SSR locus

	Erect				*Tortuosus*				*Monstruosus*			
Loci	N_a	N_e	H_o	H_e	N_a	N_e	H_o	H_e	N_a	N_e	H_o	H_e
AaB6	3.0	2.44	0.368	0.607	2.0	1.89	0.153	0.482	3.0	1.848	0.170	0.464
AaD9	2.0	1.87	0.736	0.478	2.0	1.45	0.384	0.316	2.0	1.999	0.595	0.505
AaH11	2.0	1.11	0.000	0.102	2.0	1.40	0.038	0.291	2.0	1.967	0.276	0.497
mAbR28	3.0	2.93	0.526	0.677	3.0	1.77	0.461	0.445	3.0	2.297	0.361	0.570
mAbR86	2.0	1.97	0.263	0.507	2.0	1.89	0.615	0.482	2.0	1.956	0.680	0.494
mEgR78	3.0	1.92	0.631	0.493	3.0	1.54	0.269	0.358	3.0	1.953	0.085	0.493
Pchi47	1.0	1.00	0.000	0.000	1.0	1.00	0.000	0.000	2.0	1.531	0.319	0.350
Mean	2.28	1.89	0.360	0.409	2.14	1.56	0.274	0.339	2.42	1.936	0.355	0.482

Mean observed heterozygosity (H_o) and expected heterozygosity (H_e) in the erect, *tortuosus* and *monstruosus* variants of *C. peruvianus* at each *AaB6*, *AaD9*, *AaH11*, *mAbR28*, *mAbR86*, *mEgR78* and *Pchi47* loci.

PCR products were separated on 4% metaphor agarose gel using 0.5× TBE buffer (0.45 M Tris-borate and 0.001 M EDTA) at 60 V for 4 h, stained with ethidium bromide at 0.5 µg/ml and photographed under UV light with a Molecular Image Loccus L-PIX – HE and Picasa 3 program. A 100 bp DNA Ladder (Invitrogen) was used to estimate the sizes of the DNA fragments obtained. Electrophoresis for 30 min at 200 V in polyacrylamide gel prepared at 12% with 8 M urea also was performed to confirm the number of alleles at each SSR loci. The amplified products (7 µl) were prepared with 10 µl of buffer prepared with 900 µl bromophenol blue, 900 µl xylenecyanol, 900 µl TBE (10×), 4.5 ml Ficol 30% (diluted in distilled water), 1.8 ml EDTA (0.5 M, pH 8.0) and 3.6 g sucrose. After complete dissolution of sucrose was added three volumes of formamide. After electrophoresis, the SYBR® Gold dye was used to stain the polyacrylamide gels and to identify the alleles in the SSR loci.

Polymorphism analysis

Polymorphisms from SSR loci were analysed with POPGENE 1.32 to estimate the average number of alleles per locus, the frequency of allele in each locus, the deficit of heterozygotes and the genetic divergence between the erect, *tortuosus* and *monstruosus* variants of *C. peruvianus*. Similarity matrix was computed with UPGMA, followed by Nei's clustering method, with resampling analysis, using 1000 replications. A dendrogram was constructed and drawn from a reference tree by using R Development Core Team program (R Core Team – R, 2019) using the adegenet package (Jombart, 2018). Principal coordinate analysis (PCoA) was also performed as an alternative means of detecting and visualizing the structure of the three morphological variants of *Cereus*.

Polymorphisms from SSR loci were also analysed using STRUCTURE software 2.0 to evaluate the level of genetic admixture between the 92 phenotypic variants of the *C. peruvianus*. The STRUCTURE software 2.0 was implemented using a burn-in period of 5000 repeats followed by 50,000 Markov chain Monte Carlo repeats. The *K*-values tested ranged from 2 to 8. To find the best *K* we used *K* statistics in Structure Harvester. The graphical representation was displayed with the Evanno method. An analysis of molecular variance (AMOVA) in GenAlEx 6.2 was performed to explore the hierarchical partitioning of genetic variation within and between the phenotypic variants of *Cereus*.

Results

Genomic DNA quantification indicated that the amount of DNA ranged from 10 to 80 ng/µl. Three alleles were observed at each *AaB6*, *mAbR28* and *mEgR78* loci, and two alleles at each *Pchi47*, *AaD9*, *AaH11* and *mAbR86* loci (Table 1). A total of 17 alleles, which makes an average of 2.28, 2.14 and 2.42 alleles per locus, were detected in the phenotypic variants erect, *tortuosus* and *monstruosus* shoots, respectively, of *C. peruvianus* (Table 1). Polyacrylamide gel showed equal number of alleles per SSR locus as detected on agarose gel. Polymorphism at the microsatellite loci was higher (100%) in the *monstruosus* variant than in the *tortuosus* and erect cacti (85.7%).

Mean expected heterozygosity (H_e) was higher than mean observed heterozygosity (H_o) in the erect, *tortuosus* and *monstruosus* variants (Table 1). Highest H_e rate were estimated in the *monstruosus* ($H_e = 0.4822$) and erect ($H_e = 0.4095$) plants, whereas the lowest rates was detected in the *tortuosus* shoots ($H_e = 0.3397$).

A moderate genetic divergence ($0.05 < F_{ST} < 0.15$) was observed between the erect, *tortuosus* and *monstruosus* variants. Genetic divergence among the three morphological variants resulted in $F_{ST} = 0.0962$. AMOVA showed higher genetic variation within (87%) than among (13%) the three phenotypic variants of *Cereus*. The Nei identity values calculated for the samples from the three phenotypic variants varied from 0.8456 (between *tortuosus* and *monstruosus*) to 0.9329 (between *tortuosus* and the cacti with erect shoots). Genetic divergence among the *tortuosus* and *monstruosus* variants resulted in $F_{ST} = 0.1075$.

The dendrogram generated by Nei's coefficient from the analysis of individual plants (constructed from seven microsatellite primers data using the adegenet package from R Core Team – R (2019)) identified two well-defined larger groups and smaller subgroups within each group formed by plants of the erect, *tortuosus* and *monstruosus* variants (Fig. 2). Dendrogram showed heterogeneous groups and sub-groups formed by mixture of erect, *tortuosus* and *monstruosus* plants. However, the highest proportion of *monstruous* plants (70%) was observed in group 1 whereas in group 2 a larger proportion of plants with *tortuosus* morphology (85%) was observed. The highest proportion of erect plants (58%) was observed in group 2. Genetic identity ($I = 1.0$) in the dendrogram was evident between 17% of the *monstruosus* plants and 50% of the *tortuosus* plants in groups 1 and 2, respectively.

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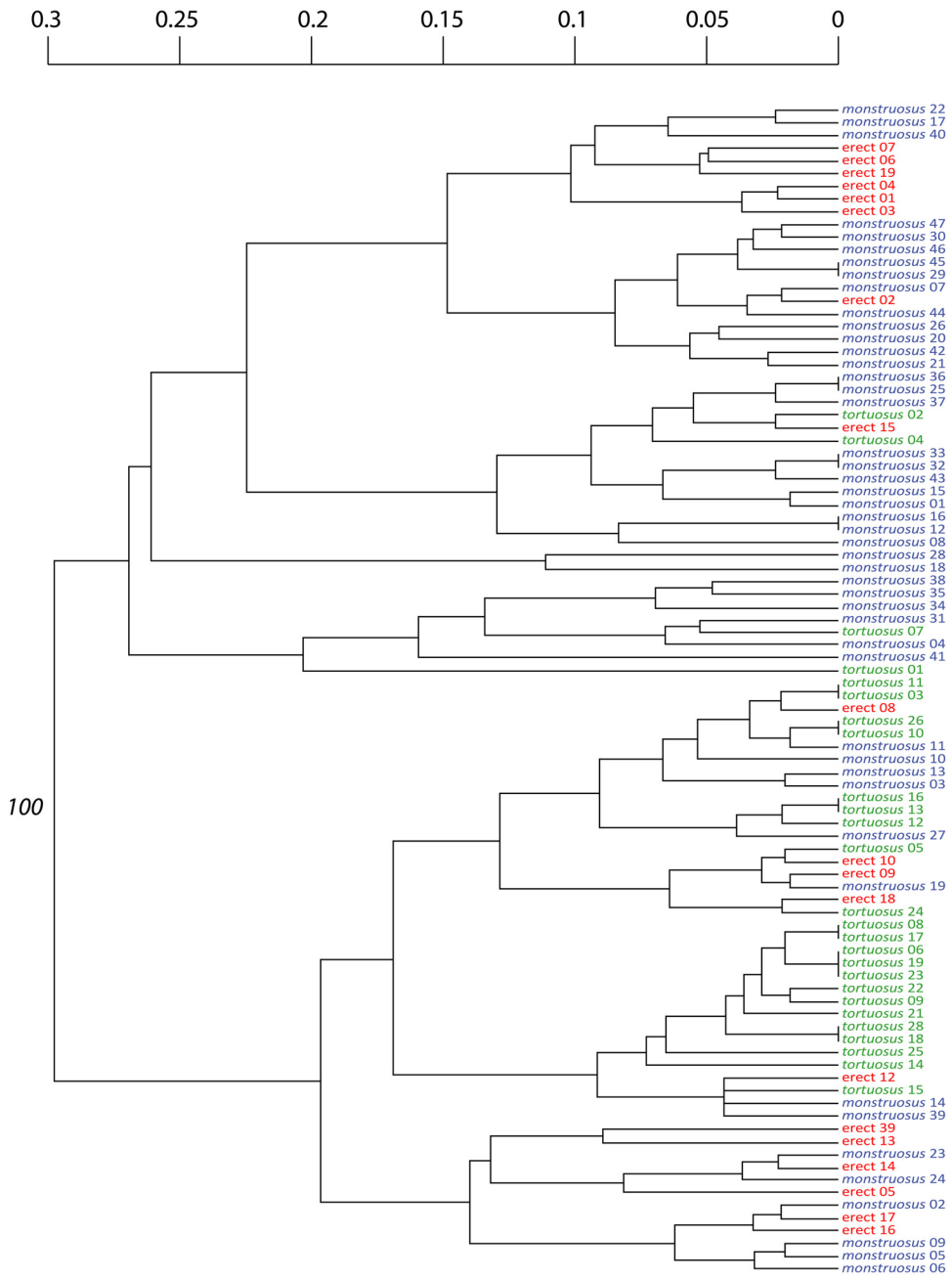

Fig. 2. Dendrogram generated by Nei's coefficient from analysis of individual plants of the variants erect, *monstruosus* and *tortuosus* of *C. peruvianus* constructed from seven microsatellite primers (*Pchi47*, *AaB6*, *AaD9*, *AaH11*, *mAbR28*, *mAbR86* and *mEgR78*) data using the adegenet package from R Development Core Team (2019). Number beside node indicates relative bootstrap frequency (%).

The PCoA revealed the 92 cactus plants into three groups formed by isolated plants of *tortuosus* and *monstruosus* morphologies and by the mixture of plants from the three different morphologies (Fig. 3), which match the dendrogram constructed according to the Jaccard coefficient (Fig. 2).

The clustering of the 92 samples of the three phenotypic variants of *C. peruvianus* according to a model-based Bayesian algorithm is shown in Fig. 4. Each bar in the graph represents a plant and the colours represent different proportions of plants in each group. The 92 plants of the three phenotypic variants were grouped into three subpopulations ($\Delta K2 = 0.00$; $\Delta K3 = 2.58$; $\Delta K4 = 2.05$; $\Delta K5 = 0.00$). The bar plot obtained for the K value ($K = 3$; $\Delta K = 2.58$), and the results were consistent with the evidence that 48.2% of the plants with erect shoots are in the red group, 57.5% of the plants with spiralled shoots (*tortuosus* variant) are in the blue group, while 62.9% of the *monstruosus* plants are in the green group (Table 2).

The bar plot graph (Fig. 4) shows individual plants sharing genomes of different ancestral groups among the plants with erect shoots and also among plants of the *tortuosus* and *monstruosus* variants. However, the large proportion of *tortuosus* and *monstruosus* plants within the blue and green groups, respectively, illustrates how artificial selection is leading to the formation of genetically structured populations, morphologically and genetically divergent for the analysed microsatellite loci.

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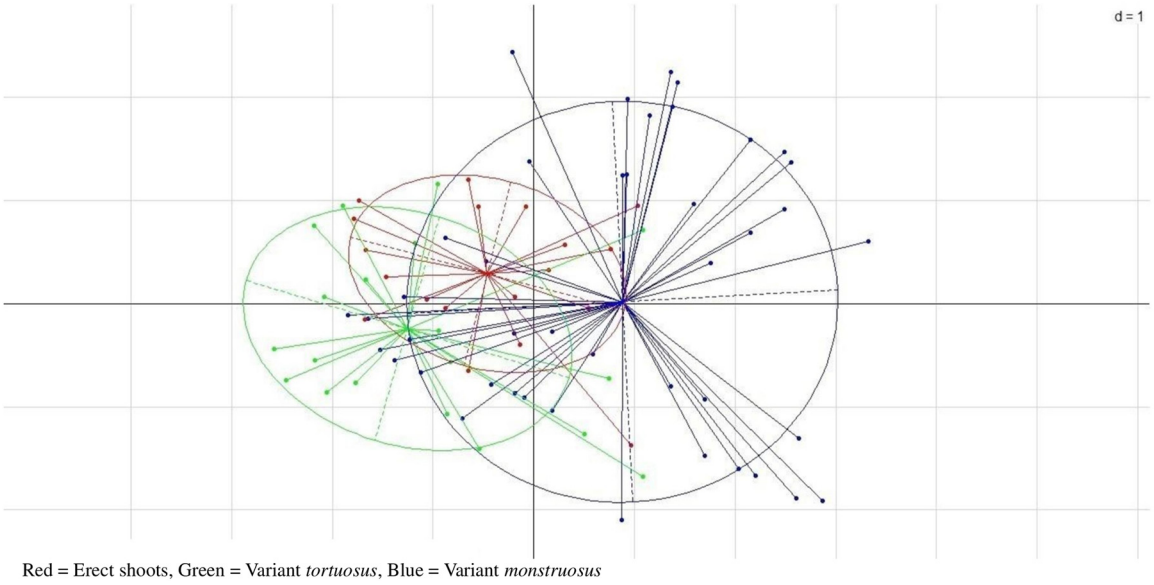

Fig. 3. Three-dimensional projection obtained for plants of the variants erect (red), *monstruosus* (blue) and *tortuosus* (green) of *C. peruvianus* constructed from seven microsatellite primers (*Pchi47*, *AaB6*, *AaD9*, *AaH11*, *mAbR28*, *mAbR86* and *mEgR78*) data.

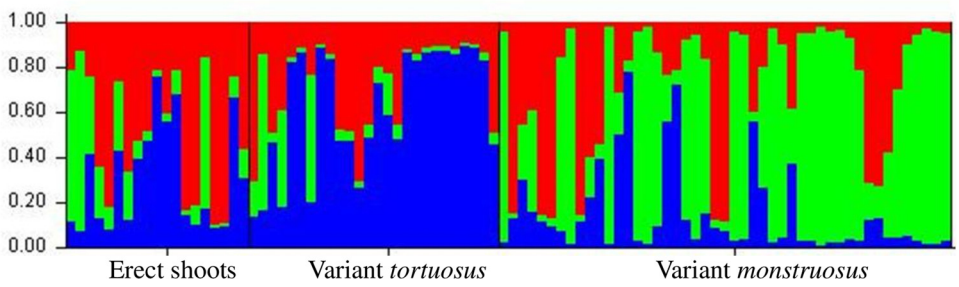

Fig. 4. Bar plot-like population structure based on microsatellite markers for plants of the *Cereus* genus with erect shoots and for the variants *tortuosus* and *monstruosus* within the *K* clusters. Each plant is represented by a single vertical bar broken in *K* coloured segments ($K = 3$), with lengths proportional to each of the *K* inferred clusters. Each colour represents the proportion of DNA segments for each plant, represented by a vertical bar, in each group.

Discussion

Despite marked morphological divergence between *tortuosus* and *monstruosus*, a moderate genetic divergence has been detected at the molecular level in *Pchi47*, *AaB6*, *AaD9*, *AaH11*, *mAbR28*, *mAbR86* and *mEgR78* microsatellite loci. A moderate genetic divergence has also been detected at the molecular level among erect *tortuosus* and *monstruosus* variants of the *Cereus* plants. Moderate genetic divergence suggests that *tortuosus* and *monstruosus* variants may have a common ancestry. Several studies have shown that morphological variants in cactus species are the result of environmental variables of different microhabitats (Basiuk, 2014; Barbosa *et al.*, 2018; Octavio-Aguilar *et al.*, 2019) and selection occurring under human management (Casas *et al.*, 2007). The pattern of morphological variations and germination behaviour in columnar cacti was significantly influenced by human management.

According to Casas *et al.* (2007) the degree of differentiation between wild and domesticated populations is associated with the intensity of artificial selection. Artificial selection by silvicultural and cultural management of *Stenocereus pruinosus* promoted different levels of genetic diversity. Higher genetic diversity has been reported in silvicultural than in wild and cultivated populations of *S. pruinosus* (Parra *et al.*, 2010). According to Parra *et al.* (2012), artificial selection in favour of high-quality fruit promotes morphological variation and high divergence because of the continual replacement of plant material propagated and introduction of propagules from different regions. Morphological divergence and moderate genetic structure between wild and managed populations of *S. pruinosus* have been reported as a result of artificial selection (Parra *et al.*, 2012). Studies with other columnar cacti species with different degrees of domestication and managed with low and high intensity also showed that artificial selection influenced the susceptibility of these cacti to xeric environments (Guillén *et al.*, 2011, 2013, 2015). Guillén *et al.* (2015) identified some patterns that were associated with artificial selection and identified others associated with natural selection.

Genetic diversity in plant of *C. peruvianus* from natural populations (wild populations) was not evaluated in the present study. The *tortuosus* and *monstruosus* variants were not found in our expeditions by natural reserves so far. Thus, we suspected that the *tortuosus* and *monstruosus* variants may have emerged from erect plants cultivated in urban areas (public and/or home

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Table 2. Proportion of the 92 phenotypic variants of the *C. peruvianus* in each group ($K = 3$) analysed using STRUCTURE software 2.0

Variant	Number of plants	Group		
		Red	Green	Blue
Columnar erect	19	0.482	0.267	0.277
Tortuosus	26	0.211	0.104	0.575
Monstruosus	47	0.307	0.629	0.148

Source: Pritchard and Wen (2003).

gardens) and the atypical morphologies were then distributed to people fond of ornamental plants. Genetic identity ($I = 1.0$) observed in 50% of the *tortuosus* and 17% of the *monstruosus* plants (Fig. 2) indicated selection and vegetative propagation of the ornamental variants of *Cereus*. *C. peruvianus* is self-incompatible and propagation from seeds is used for breeding. However, the propagation from cuttings is the easiest and quick way (Mizrahi, 2014). Vegetative propagation of *tortuosus* and *monstruosus* plants may be inducing a moderate genetic divergence and formation of two heterologous groups with conservative genetic diversity. Although vegetative propagation is a multiplication form of *Cereus* variants, high genetic diversity has been estimated in *monstruosus* (100%) and *tortuosus* (85.4%) plants. High molecular polymorphism is relevant to the conservation of the genetic diversity and to obtain a diversity of atypical, exotic and unique forms in breeding programmes, preferred by cactus traders and collectors.

Microsatellite loci are usually considered as evolutionary neutral as DNA markers. However, different allele frequencies in *Pchi47*, *AaB6*, *AaD9*, *AaH11*, *mAbR28*, *mAbR86* and *mEgR78* loci may be targets of artificial selection and vegetative propagation during the formation of populations of the *C. peruvianus* variants *tortuosus* and *monstruosus*. The *Pchi47*, *AaB6*, *AaD9*, *AaH11*, *mAbR28*, *mAbR86* and *mEgR78* loci may be promising in the evaluation of the morphological plasticity at the molecular level in other cactus genera and species. The functional significance of a substantial part of microsatellite loci has been proven by rigorous experiments examining various biological phenomena (see review by Li *et al.*, 2002), and studies by Subirana and Messeguer (2007) have indicated that microsatellites with different repeated motifs may be structurally related and involved in the determination of chromosome structure. Microsatellites are abundantly distributed in coding or non-coding regions of plant genomes so that alterations in microsatellite loci located in a coding region or in introns due to the SSR expansion or contraction within gene can ultimately lead to phenotypic changes (Li *et al.*, 2002). Despite the known functional significance of the apparent selection detected in some microsatellite loci, the *tortuosus* and *monstruosus* variants of *C. peruvianus* may be considered an important reservoir of contrasting genes (ancestral genes prevalent of two different groups: blue and green) for the generation of different new variants.

The microsatellite loci examined in our study showed that the Nei identity values between the variants *tortuosus* and *monstruosus* ($I = 0.8456$) was lower than between the variants *tortuosus* and the cacti with erect shoots ($I = 0.9329$) and between the variants *monstruosus* and the erect cacti ($I = 0.9314$). The smallest identity between the variants *tortuosus* and *monstruosus* raise a hypothesis that *tortuosus* and *monstruosus* variants may be being led to form two species according with the estimates given by Thorpe and Solé-Cava (1984). According to the authors, levels of genetic identity lower than 0.85 are frequently detected between geographically isolated populations, populations in the process of speciation or for co-generic species. The similarities among the *tortuosus* and *monstruosus* plants were slightly lower than 0.85.

The phenotypes *tortuosus* and *monstruosus* should be resultant from changes in the molecules of proteins associated with division plane which contribute to the establishment and maintenance of the division plane. The evidences are that the position of the cell division plane during cellular division significantly contributes to cell shape and plant morphology (see review by Müller, 2012). It is suggested that the possible alterations that leaded to form the *tortuosus* and *monstruosus* phenotypes must be different, since the alteration that forms the *tortuosus* phenotype follows a spiral ordered pattern while the *monstruosus* phenotype is atypical and seems have an unpredictable orientation. In the genus *Cereus*, plants with different phenotypes, but with defined standards (erect or spiralled) were more genetically similar for the *Pchi47*, *AaB6*, *AaD9*, *AaH11*, *mAbR28*, *mAbR86* and *mEgR78* microsatellite loci.

The phenotypic variations also may be associated with epigenetic variations occurring at the DNA level (Lele *et al.*, 2018; Banerjee *et al.*, 2019; Xu *et al.*, 2019) and the repeat sequences in microsatellites loci such as CA repetitions (e.g. *AaH11* and *mEgR78* loci; online Supplementary Table S1) are strong candidates for methylation activity causing epigenetic variations.

The predominant form of vegetative propagation (as ornamental plants) led to the cultivation of the two varieties with differential morphological pattern of shoots, and the generation of distinct ancestral groups (blue and green groups) at the molecular level. A practical aspect of our study is that the polymorphism in microsatellite loci in the variants of *C. peruvianus* revealed groups with contrasting genes among the variants *tortuosus* and *monstruosus* which may be useful for breeding aiming the generation of different new variants.

Although genetically structured varieties of *C. peruvianus* have been not observed from analysis of microsatellite loci ($0.05 < F_{ST} < 0.15$; and dendrogram showing heterogeneous groups and subgroups formed by mixture of erect, *tortuosus* and *monstruosus* plants), the genetic identity ($I = 1.0$) observed in plants of *tortuosus* (50%) and in plants of *monstruosus* (17%) has indicated the artificial selection and vegetative propagation as the predominant form of specimen multiplication of the *tortuosus* and *monstruosus* varieties. Artificial selection and vegetative propagation of *tortuosus* and *monstruosus* plants may have contributed to generate moderate genetic divergence between plant populations with *monstruosus* and *tortuosus* morphologies and may lead in the long term to high genetic divergence and formation of genetically structured populations.

Conclusions

The genetic identity observed in plants of *tortuosus* (50%) and in plants of *monstruosus* (17%) has indicated the artificial selection and vegetative propagation as the predominant form of specimen multiplication of the *tortuosus* and *monstruosus* varieties. The ornamental *tortuosus* and *monstruosus* variants of the *Cereus* genus with marked morphological divergence showed high polymorphism at SSR loci and formation of two heterologous groups with contrasting genes which may be useful for breeding to generate new different variants.

https://doi.org/10.1017/S1479262123000163 Published online by Cambridge University Press

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262123000163.

Acknowledgements. The authors would like to acknowledge CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasília DF Brazil) for financial support (Finance Code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brasília DF Brazil) (grant number 304447/2016-1) and Fundação Araucária (Curitiba, PR, Brazil) (grant number 002/2017).

Author contributions. C. A. M. and M. F. P. S. M. conceived and designed research. A. F. N., V. N. A. F. and E. R. M. conducted experiments. A. F. N., C. A. M. and M. F. P. S. M. analysed data. M. F. P. S. M. wrote the manuscript. All authors read and approved the manuscript.

Conflict of interest. The authors declare no conflict of interest.

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