

Somaclones of mandacaru (Cactaceae) with high morphological divergence may generate new varieties of ornamental cacti⁽¹⁾

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ABSTRACT

Somaclones of mandacaru cactus (*Cereus peruvianus* Mill.) with typical and atypical shoots were regenerated from a callus. Since the genetic relationship among regenerated somaclones has not been investigated, current study employed heterologous microsatellite primers to examine the molecular diversity within and among somaclones, showing typical and atypical phenotypes with high morphological divergence. Nei identity value calculated between the somaclones was high ($I = 0.929$) and AMOVA showed higher genetic variation within (96%) than among (4%) the samples of somaclones. The polymorphism in the microsatellite loci indicated high levels of mean observed and expected heterozygosity in atypical somaclones, presumably with high adaptive potential and as source of genotypes for generation of new varieties of ornamental cacti. On the other hand, low molecular divergence between typical and atypical morphologies of somaclones is a promising perspective for use of the atypical somaclones as source of chemical compounds of commercial and industrial interest. The somaclonal variations occurring in vitro callus culture has generated phenotypically differentiated subpopulations with low molecular divergence, however with high genetic variability, enough to be recommended as a source of genotypes to generate new varieties of ornamental cacti and of plants with new traits, necessary for breeding programs.

Keywords: micropropagation, cactus, Cactaceae, microsatellite transferability, genetic diversity.

RESUMO

Somaclones de mandacaru (Cactaceae) com alta divergência morfológica podem gerar novas variedades de cactos ornamentais

Somaclones de cactos mandacaru (*Cereus peruvianus* Mill.) com caules típicos e atípicos foram regenerados a partir do cultivo de calos. Uma vez que a relação genética entre estes somaclones ainda não foi investigada, o presente estudo empregou *primers* de microssatélites heterólogos para avaliar a diversidade genética dentro e entre os somaclones com fenótipos típicos e atípicos com alta divergência morfológica. O valor de identidade genética entre os somaclones foi alto ($I = 0,929$) e AMOVA mostrou maior variação genética dentro (96%) do que entre (4%) as amostras de somaclones. O polimorfismo nos locos microssatélites indicou altos níveis de heterozigosidades observada e esperada nos somaclones atípicos, presumivelmente com potencial alto de adaptação e como fonte de genótipos para a geração de novas variedades de cactos ornamentais. Por outro lado, a divergência genética baixa entre os somaclones típicos e atípicos é uma indicação promissora para o uso dos somaclones atípicos como fonte de compostos químicos de interesse comercial e industrial. As variações somaclonais que ocorreram no cultivo *in vitro* dos calos geraram subpopulações fenotipicamente diferenciadas com baixa divergência genética, porém com alta variabilidade genética, suficiente para recomendar os somaclones atípicos como fonte de genótipos para gerar novas variedades de cactos ornamentais e de plantas com novas características, necessários para a implementação de programas de melhoramento.

Palavras-chave: micropropagação, cactos, Cactaceae, transferabilidade de microssatélites, diversidade genética.

1. INTRODUCTION

Cacti are used as ornamental plants due to their beautiful flowers and particularly to their great variety and diversity. Pérez-Molphe-Balch et al. (2015) report tissue culture techniques in more than a hundred species from more than 40 genera of ornamental cacti with high demand in the international trade market. Barrel-shaped cacti are most frequently used as ornamental plants (BÁRCENAS, 2006), while column-shaped cacti are frequently employed

to embellish home gardens and public parks. However, private collectors form a specialized market that is primarily interested in rare or newly discovered species within the Cactaceae family, with high commercial value.

Somaclones of columnar cactus *Cereus peruvianus*, regenerated from callus tissues, showed somaclonal variations at morphological and molecular level (OLIVEIRA et al., 1995; MANGOLIN et al., 1997) and may be characterized as rare unique copies. Somaclonal variations, frequently evident in regenerated plants from in

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vitro callus culture (LARKIN and SCOWCROFT, 1981), may be used to obtain new varieties of ornamental interest. The following morphologies were observed in 20-year-old somaclones: *i*) typical or regular shoots (erect, with linear ribs) resembling those found in *C. peruvianus* plants growing from seeds in cultivated populations (62.5%); *ii*) atypical shoots, in which the areoles lie in broken ribs (with the formation of knobs, spaced regularly or irregularly), sometimes with tortuous areoles resembling spiraled ribs (23%); and *iii*) mixed shoots (14.5%) showing mixed morphological forms (typical and atypical parts).

Molecular markers (randomly amplified DNA-polymorphism and isozymes) were used to detect genetic divergence between somaclones and *C. peruvianus* plants grown from seeds in cultivated populations (RESENDE et al., 2010; SALA et al., 2011). However, the genetic relationship between regenerated somaclones from callus culture, with typical and atypical phenotypes, has yet to be investigated. Callus culture was induced from typical plants (obtained from seeds) and typical and atypical somaclones regenerated from calluses (in the same culture medium and conditions) represent morphologically divergent clonal subpopulations.

Since morphological divergence between typical and atypical somaclones is evident, our hypothesis is that the typical and atypical somaclones represent two clonal subpopulations genetically divergent at molecular level, and also that the atypical somaclones have higher molecular diversity than the typical ones. Current study employed heterologous microsatellite primers to examine the molecular diversity within and among somaclones, showing typical and atypical phenotypes. Microsatellites are simple sequence repeats (SSR) occurring in tandem and abundantly distributed in coding or non-coding regions of plant genomes (KALIA et al., 2011). Several studies have shown the possibility of microsatellite transferability in Cactaceae (OTERO-ARNAIZ et al., 2004; HARDESTY et al., 2008; CARUSO et al., 2010; MONTEIRO et al., 2015). Consequently, heterologous microsatellite primers were employed to assess the diversity and divergence between the typical and atypical somaclones at molecular level. The objective of the present study was to evaluate the diversity and divergence between the typical and atypical somaclones at molecular level. High levels of

molecular diversity in atypical somaclones may provide an opportunity to generate morphological variants to improve ornamental cacti.

2. MATERIALS AND METHODS

Somaclones were regenerated from induced callus culture using MS medium containing B5 vitamins, 0.8% agar, 3% sucrose, and a combination of growth regulators 2,4-dichlorophenoxyacetic acid (2,4-D) and N-(2-furanylmethyl)-1H-purine-6 amine (kinetin) according to the method of Oliveira et al. (1995). Callus tissue was formed from hypocotyl stalks of seedlings of *C. peruvianus*, which grew from seeds collected from a single adult plant with typical shoot. Callus subcultures were performed at 15-day intervals, and 18-20 weeks after culture initiation (total of 23 somaclonal generations) the friable calluses produced cactus shoots (somaclones). Seedlings (somaclones) with typical (areoles in erect ribs) and atypical (areoles in broken ribs forming alternate knobby) shoots were simultaneously regenerated from each callus. Each callus produced 10-50 seedlings (typical and atypical) which were removed from the callus and individualized after the root formation. After acclimatization and growing of the seedlings in to pots containing a mix of red earth (Paleudult) and river sand (1:1) at controlled conditions of laboratory (24 months) the somaclones were transferred and planted at the Experimental Botanic Garden, where they are maintained as a cultured population with about one hundred plants where have been maintained since 1997 (20 years).

Genomic DNA was extracted from shoots of seventeen somaclones showing typical or atypical shoot morphologies (Figure 1). Pieces of shoots of each typical and atypical somaclones were cut in random parts of the shoots. DNA was extracted from 200 mg of shoots using the methodology described by Resende et al. (2010). After DNA extraction, DNA quantity and quality were determined by 0.8% agarose gel electrophoresis buffered with 1X TAE (0.04 M Tris-Acetate and 0.001 M EDTA). A standard DNA ladder (λ phage, 50, 100 and 150 ng) was used as a marker. The gel was stained with 0.5 $\mu\text{g mL}^{-1}$ ethidium bromide, and the image was visualized with a Molecular Image LOCCUS L-PIX - HE (LOCCUS DO BRASIL LTDA., São Paulo, SP., Brazil) using the Picasa 3 software.



Figure 1. Somaclones of *Cereus peruvianus* maintained at Experimental Botanic Garden showing typical (I) and atypical (II) shoot morphologies.

The microsatellite primer transferability was analyzed by testing a total of 33 primer pairs from *Polaskia chichipe* (seven primer pairs), *Astrophytum asterias* (six primer pairs), *Ariocarpus bravoanus* (eight primer pairs), and *Echinocactus grusonii* (twelve primer pairs). The polymerase chain reaction (PCR) was performed using 0.2 mL microtubes in a Techne TC-512 thermocycler. A 20 μ L reaction was carried out using 20 ng of genomic DNA, 2.0 mL of 1X buffer containing Tris-HCl (10 mM Tris-HCl pH 8.3 and 50 mM KCl), 2.5 mM of $MgCl_2$, 0.1 μ M dNTPs, 1 U of Taq-DNA Polymerase (Invitrogen), 0.5 μ M of each of the forward and reverse primers, and Milli-Q water to make up to 20 μ L. Microsatellite amplification was initially performed by Touchdown PCR (DON et al., 1991). The products that were not clearly amplified by Touchdown PCR were amplified again with the program described by Albert and Schmitz (2002), and the annealing temperature was changed to between 50-59 $^{\circ}C$ to achieve clearer products. Electrophoresis was performed in a 50% Metaphor agarose gel using 0.5X TBE buffer (0.45 M Tris-borate and 0.001 M EDTA) at 60V for 4 h. Thereafter, the gels were stained with ethidium bromide at 0.5 μ g mL⁻¹, and the image was captured with a Molecular Image Loocus L-PIX - HE using the Picasa 3 software. The sizes of the fragments were determined using a 100 bp DNA Ladder (Invitrogen). The alleles were confirmed on polyacrylamide gel. The denaturing polyacrylamide gel was prepared at 12% with 8 M urea. After polymerization, the gel was placed in a vat containing 1X TBE buffer for vertical electrophoresis, and a pre-run was performed for 30 min at 200 volts. After the pre-run, samples were applied to wells. Samples were prepared by combining 10 μ L of a sample buffer containing formamide and 7 μ L of the amplified product. The sample buffer was prepared with 900 μ L bromophenol blue, 900 μ L xylene cyanol, 900 μ L TBE (10X), 4.5 μ L Ficol 30%

(diluted in distilled water), 1.8 mL EDTA 0.5 M pH 8.0 and 3.6 g sucrose. All components were mixed until the sucrose completely dissolved, and then, three volumes of formamide were added (formamide loading buffer ratio is 1:3). Migration was maintained at 200 volts until xylene cyanol run off was obtained from the gel. The polyacrylamide gel was stained with Sybr gold and viewed using the same system used for agarose gels.

The relation between the number of primers showing amplified products and total number of tested primers was used to estimate microsatellite transferability. For analysis of the relationship between the two types of somaclones at molecular level, typical and atypical somaclones were admitted as being two clonal subpopulations of species. The polymorphic microsatellite loci in the typical and atypical somaclones were used to estimate the average number of alleles per locus, the average observed heterozygosity (H_o), the expected heterozygosity (H_e) and the genetic divergence between the somaclones (F_{ST}) were estimated using the GenAlEx 6.2 software (PEAKALL and SMOUSE, 2006). For exploring the hierarchical partitioning of genetic variation within and between the samples of somaclones we performed an Analysis of Molecular Variance (AMOVA, GenAlEx 6.2) (PEAKALL and SMOUSE, 2006).

3. RESULTS AND DISCUSSION

Genomic DNA quantification indicated that the amount of DNA ranged from 5 to 80 ng μ L⁻¹. Nine microsatellite primers from the amplified DNA fragments of *Polaskia chichipe* (Pchi21, Pchi47, Pchi54), *Ariocarpus bravoanus* (mAbR28, mAbR42, mAbR77) and *Echinocactus grusonii* (mEgR02, mEgR76 and mEgR78) were polymorphic in the *Cereus peruvianus* somaclones (Table 1).

Table 1. Nucleotide sequences of the microsatellite primers, simple sequence repeats of each primer, microsatellite transferability from each cactus specie, number of alleles (Na) detected by each primer in the original specie of cactus, number of alleles detected by each primer in the *Cereus peruvianus* somaclones, variation in allele size (bp) detected in the somaclones.

Species	Primer	Nucleotide Sequence	Simple Sequence Repeated	Transferability	Na	Base Pair
<i>Polaskia chichipe</i>	Pchi21	CGTTTAGCCCCCTCTTTCTCC ^(F) GTTCCCAACTGACCGACAAC ^(R)	(CT) ₅ (AT) ₃ (GT) ₈ GA(GT) ₅		2	>100 bp
Otero-Arnaiz et al. (2004)	Pchi47	GTCCTTGTTGGCTAGCCCTTT ^(F) CCATTCTCTCGCCAATCTG ^(R)	(TG) ₁₅		3	136-172 bp
	Pchi54	CCTTGAGCTTTGACATTGAGA ^(F) GGAAGGTTTTTCATTGGATGAG ^(R)	(CA) ₅ CG(CA) ₅ TG(CA) ₂₂ (TA) ₃		2	446-464 bp
				42.86%		
<i>Ariocarpus bravoanus</i>	mAbR28	CCATAAGCTGTGGTGGGTCT ^(F) ATTTAAAGCTCCCCCTCCA ^(R)	(AG) ₇		4	103-176 bp
Hughes et al. (2008)	mAbR42	GGGCAATTCACATATGCACAA ^(F) TTGTCCACCTTCCCTATTG ^(R)	(TC) ₁₀		2	469-484 bp
	mAbR77	CGGGGAAGGAATAATCCAAG ^(F) ATGTGCCGTTTGCAATCTTT ^(R)	(AG) ₄ CG(AG) ₁₁		2	355-371 bp
				37.5%		
<i>Echinocactus grusonii</i>	mEgR02	TGGGTTGGAGAAGTGAAG ^(F) CGGTGTGAGGCTTCATTG ^(R)	(AG) ₁₀ AC(AG) ₂ AC(AG) ₇		3	>100 bp
Hardesty et al. (2008)	mEgR76	TCACAATTTGGAAGGAAGCA ^(F) GTGAGCAAAGGGCTGATTTC ^(R)	(AG) ₁₀ (AAG) ₂ C(CA) ₂		2	368-395 bp
	mEgR78	AGCCCAAAGCCCAACTTATT ^(F) TGCATGCAATCATAAGGTTTTTC ^(R)	(AG) ₁₃ GAG(CA) ₃		3	250-288 bp
				25%		
Total				27.27%	23	

The six primers from *Astrophytum asterias* showed no clear SSR-amplifiable products. The global microsatellite transferability was 27.27% (9 primers showed amplified products/total of 33 primers tested). Four alleles were

observed at the *mAbR28* locus, three alleles were observed at the *Pchi47*, *mEgR02*, and *mEgR78* loci and two alleles were detected at *Pchi21*, *Pchi54*, *mAbR42*, *mAbR77*, and *mEgR76* loci (Table 2).

Table 2. Number of somaclones (N), number of alleles (Na) and number of effective alleles (Ne) per polymorphic microsatellite locus, mean heterozygosity observed (H_o) and expected (H_e) in somaclones of *Cereus peruvianus* showing typical and atypical shoot morphologies.

Somaclone	N	Na	Ne	Ho	He	P(%)
Typical	17	2.556	2.152	0.335	0.501	100
Atypical	17	2.444	1.953	0.368	0.452	100
Total/Mean	34	2.5	2.052	0.352	0.477	100

A total of 23 alleles, which sets an average of 2.56 alleles per locus, were detected in *C. peruvianus* somaclones. The number of alleles in each microsatellite loci was confirmed by electrophoresis in polyacrylamide gel. The two variant alleles in agarose gel electrophoresis may migrate at the same gel position and consequently determine homozygote phenotypes. Thus, the use of polyacrylamide gel electrophoresis for analyzing somaclone shoots has actually been important in the identification of the heterozygote phenotypes and number of alleles. The number of alleles per locus on polyacrylamide gel was equal to the number of alleles per locus on agarose gel.

The polymorphisms in the *Pchi21*, *Pchi47*, *Pchi54*, *mAbR28*, *mAbR42*, *mAbR77*, *mEgR02*, *mEgR76*, and *mEgR78* loci was 100% in the plants with atypical and typical phenotypes. However, a smaller number of alleles and lower value of H_e in the microsatellite loci detected in the somaclones showing atypical shoots contrary our hypothesis that the atypical somaclones have higher molecular diversity than the typical somaclones. The number of alleles and effective number of alleles was lower in plants with atypical than in plants with typical morphologies (Table 2). The *mEgR02'* allele was not found in somaclones with atypical shoots.

The mean expected heterozygosity in plants with the atypical morphologies was also lower ($H_e = 0.452$) than the H_e detected in plants with the typical morphologies ($H_e = 0.501$). The value for mean expected heterozygosity ($H_e = 0.477$) of both types of somaclones (typical and atypical phenotypes) is near the value for H_e reported by Sala et al. (2011) in loci for esterase isozymes (0.497) for descendants (R1 generation) of the somaclones.

Supposedly, in vitro culture would decrease the ability of populations to adapt to abiotic and biotic environmental changes as well as reducing their flexibility in response to pathogens and herbivores (ALLEN DORF and LUIKART, 2007). However, genetic diversity was 20% higher in regenerated somaclones in vitro than in plants cultured in vivo (RESENDE et al., 2010) and the somaclones showed higher values of heterozygosity than *C. peruvianus* plants grown from seeds in nature. Lower values of H_e have been detected in 12 microsatellite loci ($H_e = 0.4012$) in 40 cultivated plants of *C. peruvianus* growing from seeds (unpublished results). The high heterozygosity detected in the somaclones (typical: $H_e = 0.501$ and atypical: $H_e = 0.452$) may be an important indicator that this plant population as well as their descendants has a substantial amount of genetic variation to adapt to abiotic and biotic environmental changes.

High heterozygosity in somaclones is also important because they may be target of selection processes for generation of new varieties. Different genotypes ("monstruosus", crested, and variegated forms) have been selected in ornamental breeding programs of *Cereus* (ZAPPI et al., 2010). These characteristics are more likely to occur from crossing certain clones. Some crossings have resulted in 100% "monstruosus" offspring. Thus, atypical somaclones with high heterozygosity represent promising genotypes to the *Cereus* ornamental plant breeding programs. The potential of the atypical somaclones for generate different "monstruosus" phenotypes (new morphological varieties) is evident in atypical somaclones kept in vases in home gardens (Figure 2), which are promising as ornamental specimens.

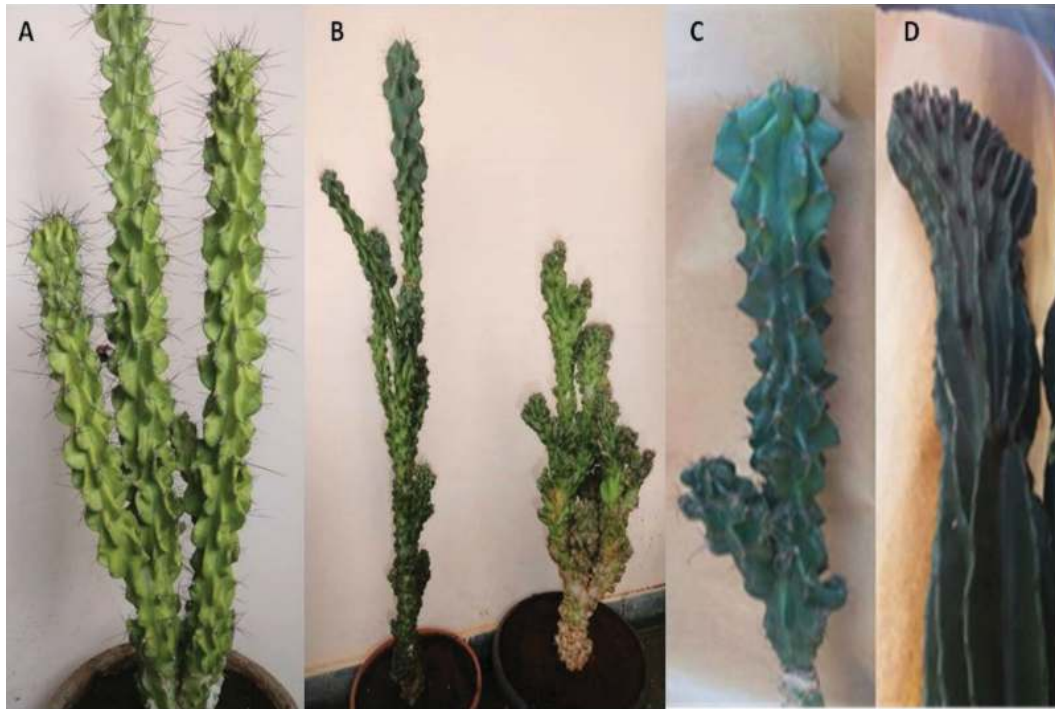


Figure 2. Somaclones of *Cereus peruvianus* in home gardens showing different “monstruosus” phenotypes (A-D).

Typical and atypical somaclones with high heterozygosity are also promising to broaden the genetic basis of the species. In addition to the ornamental value of this species, several studies have revealed the importance of the *C. peruvianus* species as fruit crop (WEISS et al., 1994; NERD et al., 2002; MIZRAHI, 2014), emphasizing the need to broaden this species's genetic base for breeding programs. The use of in vitro regenerated somaclones in cross-pollination with cultivated plants in vivo may be important as a source for new traits, necessary for breeding programs. Analyses of floral biology and microsporogenesis in somaclones showed that the floral and reproductive characteristics as well as the somaclones' meiotic characteristics are similar to the floral, reproductive, and meiotic characteristics of the *C. peruvianus* natural populations (RUVOLO-TAKASUSUKI et al., 2006; SILVA et al., 2006). Therefore, typical and atypical somaclones may be recommended for crosses with plants grown in vivo to broaden the species's genetic base.

Microsatellites as co-dominant markers revealed the appearance of new alleles results of genetic variation induced in the in vitro culture. Higher number of alleles in microsatellite loci (*mAbR28* and *mEgR78*) was also detected in somaclones ($N_a = 2.55$) than in the 40 cultivated plants ($N_a = 2.33$) (unpublished results). The new alleles were detected in typical and atypical somaclones simultaneously regenerated from callus tissues, and therefore, are not associated with morphological changes in atypical somaclones. The phenotypic changes in plants induced by environmental conditions have been frequently related with epigenetic variations (HIRSCH et al., 2012). The DNA methylation events evolved with phenotypic changes were described by Zhang and Hsieh (2013) as resulting from epigenetic changes. It is possible that DNA methylation

occurring in groups of cells of the callus tissues during the organogenesis may lead to gene silencing in crucial sequences for the most frequent erect pattern for the shoots of the *Cereus*. Gene silencing occurring in only some groups de cells may explain the origin of the atypical and typical (in absence of gene silencing) somaclones from the same callus. Cells of a callus near medium and far from it often suffer different intensity of environment stresses (medium of culture) and show different variability and unequal division speed. Therefore, it is possible that the responses of the cell groups are spatially variable in a callus. The polymorphism analysis in microsatellite loci of the typical and atypical somaclones was important to show the occurrence of genetic variation in vitro induced while the occurrence of epigenetic variations in the atypical somaclones should be investigated further.

Use of heterologous primers to assess molecular divergence in somaclones of *C. peruvianus* also was important to show that the remarkable morphological divergence in vitro induced in the atypical shoots of the somaclones was not followed by differences in the number of repeated units in tandem in the microsatellite loci. The nine microsatellite loci analyzed represent a portion of the genome in the somaclones that are not divergent in the typical and atypical morphologies of the regenerated plants from a callus culture at the same culture medium and equal culture conditions. Thus, until proven otherwise, the premise is that typical and atypical somaclones showing remarkable morphological divergence are examples of two clonal subpopulations with low genetic divergence at molecular level and high morphological divergence (23% with atypical shoots and 14.5% showing mixed morphological forms: typical and atypical). Analyses of the molecular divergence among the somaclones with

typical and atypical morphologies indicated a global value lower than 0.05 ($F_{ST} = 0.036 \pm 0.018$). According to Wright (1978), values of F_{ST} ranging from 0.01 to 0.05 indicate a minimal difference between populations. The changes in allele frequencies at the nine microsatellite loci were minimal compared to the phenotypic variation observed in the somaclones.

The Nei identity (NEI, 1972) values calculated between the somaclones with different shoot morphologies was high ($I = 0.929$), indicating less than 8% of molecular divergence between plants showing typical and atypical shoot morphologies. AMOVA also showed higher genetic variation within (96%) than among (4%) the samples of somaclones. The minimal molecular divergence observed between the typical and atypical somaclones provides evidence of the common origin of somaclones from callus tissues. The estimated value may represent the molecular diversity (in the nine microsatellite loci) among the morphological types of clones regenerated from the callus culture ($\approx 4\%$). While the morphological divergence in the somaclones corresponds to 37.5% (atypical shoots) the molecular divergence in microsatellite loci was almost 10 times lower. In somaclones of *C. peruvianus*, the somaclonal variation at morphological level is 10 times greater than somaclonal variation at molecular level. On the other hand, low molecular divergence between typical and atypical somaclones as well as the high value for Nei's identity ($I = 0.929$) may be preliminary evidences to justify investments for use of atypical somaclones in order to obtain the chemical composts usually obtained from plants with typical morphology. Industrial and economic importance has been attributed to *C. peruvianus* cultivated in southern Brazil. *C. peruvianus* plants have been used as sources of chemical compounds for the cosmetic, food and pharmaceutical industries (ALVAREZ et al., 1992, 1995; BARROS and NOZAKI, 2002; OLIVEIRA and MACHADO, 2003). Preliminary studies of hemicelluloses extracted from typical and atypical somaclones (as well as from naturally cultivated plants of *C. peruvianus*) by Fourier Transform Infrared Spectroscopy (FT-IR) showed a similar profile for the FT-IR spectra (unpublished results). Other investments in order to obtain chemical composts such as heteropolysaccharides, fatty acids, and alkaloids, from somaclones with atypical morphology may be recommended due the high molecular similarity between the morphologically divergent plants.

Although microsatellite loci are not necessarily linked to specific coding genes that would lead to the specific morphological changes, the polymorphism at the nine analyzed microsatellites in our study represents genome sequences which can be used as molecular markers to assess changes at DNA level occurring in regenerated plants from the same callus culture. The transferability of microsatellite primers for the *Cereus* somaclones was a strategy that allowed the estimation of polymorphism and heterozygosity levels as well as relevant information to ornamental cacti breeding programs. The use of the heterologous microsatellite primers in typical and atypical somaclones of *C. peruvianus* revealed the callus culture as a source of atypical phenotypes for ornamental





cacti breeding programs. Atypical somaclones may be considered of greater ornamental value since one of the aims of the breeding program of ornamental cacti reported by Assis et al. (2011) is the selection of suitable genotypes for obtaining variegated plants. The polymorphism in the microsatellite loci indicated: *i*) genetic variation at molecular level induced in the callus culture during organogenesis and regeneration of the somaclones; *ii*) low molecular divergence ($\approx 4\%$) for allele frequencies between typical and atypical morphologies of somaclones; *iii*) a promising perspective for use of the atypical somaclones as source of chemical compounds of commercial and industrial interest; *iv*) high levels of mean observed and expected heterozygosity in atypical somaclones, presumably with high adaptive potential and as source of genotypes for generation of new varieties of ornamental cacti.

The somaclonal variations occurring in vitro callus culture has generated phenotypically differentiated subpopulations with low molecular divergence, however with high genetic variability, enough to be recommended as a source of genotypes to generate new varieties of ornamental cacti and of plants with new traits, necessary for breeding programs.

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AUTHORS CONTRIBUTIONS

PGM 0000-0001-9302-3532, **JSFT** 0000-0003-3085-6398, and **CAM** 0000-0002-1653-3106; carried out the molecular analysis using heterologous primers for microsatellite loci to examine the molecular diversity within and among somaclones, showing typical and atypical phenotypes with high morphological divergence, and also performed the genetic data analysis. **PGM, CAM, and MFPSM** 0000-0001-7815-4606; participated in the initial discussion of this project, discussed results and participated in writing the manuscript. **MFPSM**: conceived and supervised the project, assisted in the data analysis, discussed the results and contributed to draft the manuscript. All authors have read and approved the final manuscript.

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