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Genetic and chemical diversity in seeds of cactus mandacaru (*Cereus* sp.) from two edaphoclimatic regions contrasting

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ABSTRACT

The purpose of this study was to evaluate the chemical, physiological and genetic differences in seeds of cactus of the *Cereus* genus (mandacaru) cultivated in the Northeast (Picos, State of Piauí) and Southern (Maringá, State of Paraná) regions of Brazil. Over a period of eight days, temperatures of 25°C and 30°C were equally efficient for the germination of all the seeds. Oleic acid (C18:1) was the most common fatty acid found in the seeds collected in the Southern (41%) and Northeast (45.5%) regions. The analysis of lipases indicated that seeds from Maringá have high mean observed and expected heterozygosities and that seeds from Picos have a higher number of alleles per loci. Therefore, the seeds of mandacaru from the semiarid region of Northeast as well as the seeds from the South (the two contrasting regions of Brazil) are promising with regards to the preservation of the biodiversity in the genome of mandacaru. The low genetic identity between mandacaru seeds from Maringá and Picos at *Lipase-5* locus analysis (I = 0.77) suggests that the mandacaru plants from Maringá and Picos may correspond to two species: *C. peruvianus* and *C. jamacaru*, respectively.

Key words: Cactus, *Cereus peruvianus, Cereus jamacaru,* seed germination, fatty acids, lipases, genetic diversity.

INTRODUCTION

The cactus species *Cereus peruvianus* is known in Brazil as "mandacaru". It is grown in gardens, has nocturnal flowers and requires cross-pollination, which is generally carried out by insects such as moths and bees (Silva and Sazima 1995, Ruvolo-Takasusuki et al. 2006). Fruits are produced approximately two to three years after propagation if the plant was developed from a cutting, or three to five years after propagation if it was germinated from a seed. The fruits are large and have smooth skin that vary in colour, ranging from yellow to red, and a white pulp that contains numerous small black seeds. They have

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an ellipsoid or round shape and are approximately 20 cm in length and 12 cm in diameter (Morton 1987, Mizrahi et al. 1997).

Plants of this species are considered important because, in addition to being ornamental, they also possess a series of characteristics that are of economic, commercial, industrial and medicinal interest. They produce amine alkaloids (Vries et al. 1971, Oliveira and Machado 2003), wax esters that have a potential application as an impermeable barrier (Rezanka and Dembitsky 1998), and a viscous gum that has several industrial applications (Alvarez et al. 1992, 1995, Nozaki et al. 1993, Barros and Nozaki 2002). Additionally, heteropolysaccharides that behave as polyelectrolytes can be extracted from the pulp of *C. peruvianus*. They can be used in the treatment of industrial waters because they are able to flocculate colloidal particles without altering the pH and thus act as primary coagulants (Kirchmer et al. 1965). Arabinogalactan polysaccharides extracted from the stems of *C. peruvianus* have been used as phytotherapeutic agents in the treatment of gastric ulcers (Tanaka et al. 2010). In Israel, this plant species is cultivated and domesticated for the production of fruit that are larger, better tasting, and free from splits (Mizrahi and Nerd 1999). In Europe, the fruit of *C. peruvianus* is widely accepted by the population and is considered an exotic fruit of commercial value.

The origin of this species of cactus has not yet been clarified, but some authors believe it originated in Brazil (Mizrahi and Nerd 1999). In the Southern region of Brazil, "mandacaru" plants are called *C. peruvianus*, but in the semi-arid region of Northeast, they are referred to as *C. jamacaru*. In addition to being ornamental plants, they are mainly used as forage plants to feed animals in the dry season and are also used in popular medicine (Andrade et al. 2006).

The remarkable economic and industrial importance of mandacaru plants leads to a system of extractivism, and consequent concern with the cultivation of plants that are destined for conservation and/or improvement programs, where maintaining genetic diversity is an important factor. Plants of mandacaru are predominantly clonally propagated, and as all members of a clone are products of mitosis, they are genetically uniform. *In vitro* regeneration of *C. peruvianus* plants has contributed to the increased genetic diversity of the species (Mangolin et al. 1997, Machado et al. 2000, Resende et al. 2007, Sala et al. 2011), but obtaining plants from seeds is also a safe way to ensure biodiversity. As with most asexually propagated crops, the seed produced through sexual reproduction by a clone shows considerable reduction in germination performance (Chahal and Gosal 2002). Low germination of the *C. peruvianus* seeds was overcome when the seeds were soaked in sterilized water for 24h, and when previously exposed to 0 and 50°C, for 2 min (Carvalho et al. 2008). The main factor affecting seed germination from plants of *C. peruvianus* appeared to be related to pre-soaking in water.

Studies using molecular markers (microsatellite loci) have showed that the plants of mandacaru from the Northeast region of Brazil have a larger number of alleles (unpublished results) indicating that these plants have higher genetic diversity than the mandacaru plants from the South region of Brazil. Seed germination of plants with high genetic diversity as mandacaru plants from the Northeast is important for the preservation of the biodiversity of the biome of the Cactaceae family. We suspect that the procedure used to germinate seeds of *C. peruvianus* from the South region of Brazil may be also used to seed germination of the mandacaru plants from Northeast. Therefore, one of our aims on this study was to analyze and compare the germination rates of seeds from mandacaru plants maintained in the two contrasting edaphoclimatic regions (Northeast and South of Brazil), and moreover investigate differences in the concentration and chemical composition

of fatty acids extracted from the lipid fraction of seeds collected from mandacaru plants cultivated in the Northeast and South regions of Brazil. Lipids are the most abundant reserve compounds in dry seeds of mandacaru plants cultivated in the Northeast region of Brazil (Alencar et al. 2012). The pattern of isozymes related with the fatty acids metabolism in seeds, such as lipases, was also analyzed. The composition of fatty acid and the expression of lipases in seeds may be used as parameters to evaluate the level of polymorphism in mandacaru plants from the two regions of Brazil and may indicate the potential of the seed from plants maintained in the South and the Northeast for preserving the biodiversity of the specie.

Evidence for environmental factors that determine the germination of *C. peruvianus* seeds was described by Carvalho et al. (2008) and Meiado et al. (2010), but intrinsic factors have not been related in the literature. Studies that demonstrate the role of the lipid reserves in germinating seeds of different species has been made since the 1970s to today (Shewry et al. 1972, Megat-Rusydi et al. 2011, Weitbrecht et al. 2011). The lipid reserves are responsible by the seeds metabolism and are intrinsic factors that may be associated with seed germination. Lipids were the main reserve mobilized during seed germination in *C. jamacaru* because their levels were strongly reduced after seed germination (Alencar et al. 2012). Thus, the chemical composition of fatty acids may be used to explain an equal or differential rate of germination. There are no records in the literature of studies on the chemical composition of fatty acids extracted from the lipid fraction of seeds of mandacaru. It is possible that the types and/or quantity of fatty acids are related to increased or decreased dynamics of the germination process in different genotypes of the species. Moreover, lipid reserves stored in seeds in the form of triglycerides (TAGs) are degraded by lipases in order to produce a carbon source that will stimulate growth of the embryo during the post-germination period. The search for knowledge of lipases associated with the breakdown of triglycerides that act during the post-germination process and seedling growth started long ago, but the molecular identity and physiological function of some lipase genes have only been obtained recently (Quettier and Eastmond 2009). There are no records in the literature of studies on the expression and/or number of lipases in mandacaru seeds. It is possible that the expression, types and/or number of lipases may be related to increased or decreased dynamics of the germination process in different genotypes of the species.

Considering the importance of mandacaru plants in the context of biotechnology and preservation of the biodiversity of the biome of the Cactaceae family, the objectives of this study were *a)* to analyze and compare the germination rates of seeds from two populations of mandacaru plants maintained in contrasting edaphoclimatic regions (Northeast and South of Brazil); *b)* to estimate and evaluate differences in the concentration and chemical composition of fatty acids extracted from the lipid fraction of seeds collected from mandacaru plants cultivated in Picos (Northeast of Brazil) and Maringá (South of Brazil); *c)* to genetically characterize lipases in mandacaru seeds by determining the number of loci and alleles involved in the expression of these enzymes; *d)* to estimate the genetic diversity of lipases from the seeds of the genotypes present in the two locations, and *e)* indicate the potential of seeds from plants maintained in the South and in the Northeast for the preservation of the biodiversity in the specie.

MATERIALS AND METHODS

For the development of this study, the seeds of mandacaru were collected from two plants in Picos in the state of Piauí in the Northeast of Brazil and from two plants in Maringá in the South of Brazil (Fig. 1). Maringá city is located in the state of Paraná in the Southern region of Brazil at a latitude

(South) of 23°25', a longitude (West) of 51°57', and an altitude of 596 m. The soil of this region consists of dystrophic purple Latosol. Rainfall (precipitation) is at a minimum in March, June, July and August and at a maximum in November, December and January with a mean annual rainfall of 1,500 mm. The mean annual temperature is 21.95°C, the minimum is 10.3°C and the maximum is 33.6°C. The mean relative humidity is 66%. The climate is classified as subtropical temperate. The city of Picos is located in the state of Piauí in the Northeast of Brazil at a latitude (South) of 7°, 04' 54", a longitude (West) of 41°, 28' 14" and an altitude of 250 m. This region is characterized by a gently undulating terrain. The soil is clayey with litholics and quartz sand. This region has characteristic vegetation with shrubby caatinga, caatinga forest, and cerrado vegetation. This vegetation reflects the climate, which is characterized as tropical semiarid and hot with a maximum temperature of 39°C, a minimum of 22°C, and an average annual temperature of 30°C. The months of February and March have the highest rainfall. The driest period occurs over seven to eight months.

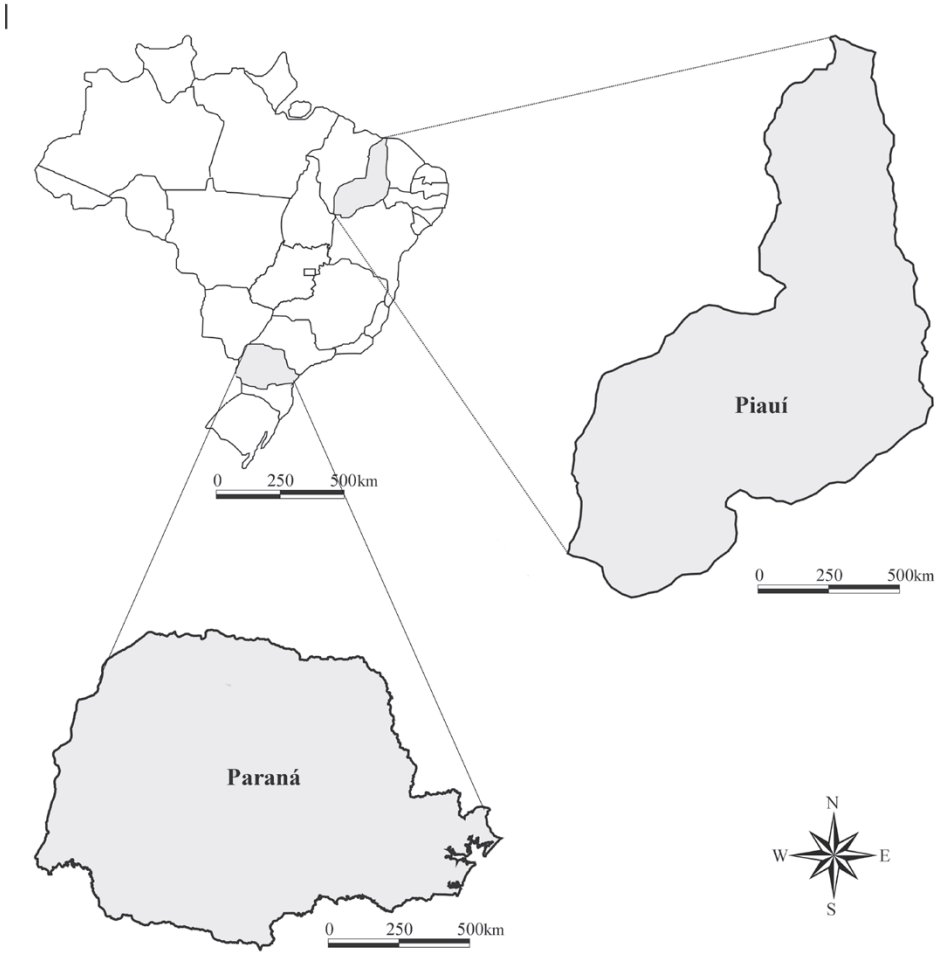

Figure 1 - Map of Brazil highlighting the states of Paraná and Piauí in the South and Northeast, respectively.

Germination Tests

To analyze germination potential, seeds were distributed over two sheets of Germitest® paper moistened with distilled and deionised water and placed in plastic Petri dishes. Germination was evaluated at three temperatures: 20, 25 and 30°C. For each temperature, 10 repetitions were performed with forty seeds each for a total of 400 seeds from each plant from Maringá and Picos. The plates were maintained in a BOD growth chamber with a 16-hr photoperiod; the number of germinated seeds was counted daily.

On the fourth day after soaking the seeds, the germination process was started and evaluation was performed until the eighth day after soaking the seeds. Analysis of the germination rates was performed using SAS software (SAS 9.1 System for Windows Copyright© 2002-2003 by SAS Institute Inc., Cary, NC, USA). The rate of seed germination after the fourth and eighth days for samples from Maringá and Picos were considered to be from different treatment groups. The experiment was conducted in a completely randomized design, and the data were analyzed using analysis of variance (ANOVA) with 5% significance.

Isolation and Identification of Lipids

For the identification of lipids, three repetitions were performed with 300 mg of seeds collected from mandacaru plants from Maringá and Picos. The seeds were crushed after freezing with liquid nitrogen, and after the maceration process, the material was diluted in hexane (75 ml) and kept in a reflux system for an hour. The material was subsequently filtered and placed in flasks to complete the evaporation process.

The samples went through a process of saponification, which consists of subjecting the samples to a new reflux step. In this process, exposure to a solution of 2% KOH diluted in methanol was used for 30 min. (50-ml). Subsequently, the methanol was evaporated with the aid of a rotary evaporator until the volume was reduced to 10 ml. After evaporation, the volume was increased to 25 ml with water. In the next step, the samples were desaponified. In this procedure, 25 ml of ethyl ether was used to extract the fatty acids from the alkaline mixture. The alkaline mixture was washed three times with ethyl ether, and a two-phase separation was carried out with a filtering funnel after each wash. The ethyl ether fraction that contained the fatty acids of interest was subjected to the methylation process.

The ethyl ether fraction was subjected to the methylation process and was acidified to pH 2.0 using 10% HCl. After acidification of the solution, fatty acids were again extracted using ethyl ether (3 x 25 ml). Residual water present in the solution was removed by adding anhydrous magnesium sulphate, and the ether was evaporated. Subsequently, free fatty acids were refluxed for 10 min. with five drops of concentrated hydrochloric acid of anhydrous methanol (25 ml). Fifty millilitres of water were then added, and the methyl esters were extracted three times with hexane. Residual water was removed with anhydrous magnesium sulphate. Fatty acids diluted in hexane were transferred to a previously weighed flask to determine the mass of free fatty acids obtained for each sample. Samples were weighed after the evaporation of hexane (Matos et al. 1992). Composition of the extracted fatty acids was evaluated using Gas Chromatography–Mass Spectrometry (GC-MS). The analyses were performed using a Varian 3800 gas chromatograph and 4000MS detector (ion trap) equipped with a 30 m × 0.25 mm low bleed/MS capillary column (VF-1ms). The track temperature was as follows: injector 250°C; the initial temperature of 50°C was maintained for 2 min., 90°C (20°C $min.^{-1}$ for 1 min.), and then 280°C (5°C $min.^{-1,}$ maintained for 2 min.). The electron impact (EI) spectra were obtained with 70 eV at 200°C. The volume injected was 1 ml. The post-test analyses were performed using Saturn Workstation 5.1.

Polyacrylamide Gel Electrophoresis (PAGE) for Analysis of Isozymes Lipase

Lipase isozymes from mandacaru seeds were analyzed by polyacrylamide gel electrophoresis and identified after comparing the samples on the gel with the α- and β-esterase isozymes previously defined by Sala et al. (2011) in the stems of *C. peruvianus* plants. Lipases were analyzed in dry seeds and in seeds exposed to six different periods of soaking: 24, 48, 72, 96, 120 and 144 hrs. The enzymes were extracted from individual seeds, crushed and homogenized in a microcentrifuge tube with 20 µl of cold extraction solution containing 1.0 M phosphate buffer pH 7.0, 5% PVP-40, 1.0 mM ethylenediaminetetraacetic acid (EDTA), 0.5% β-mercaptoethanol and 10% glycerol. The entire homogenization procedure was performed in an ice bath. After homogenization, the samples were centrifuged in a Jouan MR23i centrifuge for 30 min. at 14,000 rpm and 4°C. The supernatants were used as samples for polyacrylamide gel electrophoresis. The polyacrylamide gel (12%) was prepared with 0.375 M Tris-HCl at pH 8.8. Electrophoresis was performed for 5.5 hrs at 4°C with a constant voltage of 200 V. A 0.1 M Tris glycine buffer at pH 8.3 was used in the chamber.

To identify the α- and β-esterases with lipase activity, the gel was divided into two halves after enzyme migration. One half was incubated with a substrate and dye that are specific for the α- and β-esterase enzymes (Sala et al. 2011). The other half of the gel was incubated with a substrate and dye that are specific for lipases (glycerol acyl-hydrolase; EC 3.1.1.3) using the protocol described by Alfenas (1998). To identify lipases, the gel was incubated with 50 ml of a solution containing 10% SDS, 0.12 M α-naphthyl acetate and 5 mg · ml^{-1} Fast Garnet GBS. The gel was kept in the dark for 30 min. at 37°C or until the emergence of enzyme bands. To detect the α- and β-esterases, the gel was incubated in 60 ml of a solution containing 0.1 M sodium phosphate pH 6.2, 60 mg of α-naphthyl acetate, 60 mg of β-naphthyl acetate, and 60 mg of Fast Blue RR Salt dye dissolved in 1 ml of N-propanol (Sala et al. 2011). After staining, the gels were fixed at room temperature for 1-24 hrs in a solution of 7.5% acetic acid and 10% glycerol. After fixation, the gel was dried using 5% gelatin on its surface and placed between two stretched sheets of cellophane paper for 24-48 hrs.

The frequency of alleles in a lipase locus and the observed mean heterozygosity (H_o) and expected mean heterozygosity (H_e) were used to analyze the genetic diversity of lipases in mandacaru seeds from Maringá and Picos. A sample of 74 seeds from Maringá and 55 seeds from Picos was used to estimate the genetic diversity using the programme POPGENE 1.32 (Yeh et al. 1999).

RESULTS AND DISCUSSION

Analysis of the germination rates of seeds from plants found in Maringá and Picos showed that the seeds collected in Maringá and Picos did not germinate after four days at 20°C (Tables I and II). Germination was only observed after 8 days at 20°C, and the germination rate was lower than that of seeds maintained at 25°C and 30°C. Over a period of four days, the germination rate of seeds maintained at 30°C was greater than the germination rate of seeds maintained at 25°C (Table II). This observation was true for seeds collected from plants from both Maringá and Picos. At 25°C and 30°C, there were no significant differences in the seed germination rates between the two regions. A temperature of 30°C was most suitable for the germination of seeds from both evaluated regions over a period of 4 days. Over a period of 8 days, temperatures of 25°C and 30°C were equally effective for the germination of seeds collected from plants from Maringá and Picos. Therefore, the 30°C temperature equally stimulated the germination of seeds collected from Maringá and Picos over a period of 4 days, and 25°C and 30°C were equally effective for the germination of seeds over a period of 8 days.

TABLE I
Number of germinated seeds of mandacaru collected in Maringá (Southern region of Brazil) and Picos (Northeastern region of Brazil) at 20, 25 and 30°C after four and eight days.

	4 days			8 days		
Temperature	20°C	25°C	30°C	20°C	25°C	30°C
Maringá	0	147 (36.8%)	210 (52.5%)	125 (31.3%)	352 (88%)	371 (92.7%)
Picos	0	113 (28.3%)	246 (61.5%)	243 (61%)	306 (76.5%)	341 (85.3%)

TABLE II
Effect of temperatures on the mean number of germinated seeds (40 seeds for repetition; 10 repetitions) from Maringá (Southern region of Brazil) and Picos (Northeastern region of Brazil).

Temperature	4 days	8 days
20°C	-	18.40[b]
25°C	13.05[b]	32.90[a]
30°C	22.80[a]	35.60[a]
Maringá	17.85[a]	28.67[a]
Picos	18.00[a]	29.66[a]

Means following by same letters[a,b] are not significantly different at 5% probability by Tukey-Kramer Multiple Comparison Test.

There were no differential effects on the germination rates of seeds collected in Maringá and Picos and maintained at 20, 25 and 30°C. A positive effect of temperatures around 25°C has been indicated in the germination of *C. jamacaru* seeds (Meiado et al. 2010) and other species of cactus (Nobel 1998). The previous seeds germination of *C. peruvianus* was made at 27 ± 2°C by Carvalho et al. (2008). However, evidence in the present study showed that 30°C may be a more suitable and effective temperature than 25°C to obtain a greater germination rate in mandacaru seeds from both Northeast and South regions in a shorter time (4 days).

Analysis of 100% of the mass of free fatty acids extracted from mandacaru seeds collected from plants from Maringá and Picos showed that oleic acid (C18:1) was the most common fatty acid found in the seeds collected in Maringá (41%) and Picos (45.5%). Palmitic acid (C16) was also detected in large proportion in the seeds and was higher in the seeds collected in Maringá (Table III). Stearic acid (C18), linoleic acid (C18:2), myristic acid (C14), decanoic acid (C10), palmitoleic acid (C16:1) and arachidonic acid (C20) were all detected in smaller amounts of approximately 10% or less in the seeds from Maringá and Picos (Table III).

TABLE III
Fatty acid percentage in seeds of mandacaru from Maringá and Picos plants.

Fatty acid	Maringá-PR	Picos-PI
Decanoic acid (C10)	0.8	6.5
Myristic acid (C14)	5.2	2.8
Palmitic acid (C16)	38.8	23.6
Palmitoleic acid (C16:1)	1.3	1.5
Stearic acid (C18)	8.6	10.5
Oleic acid (C18:1)	41.0	45.5
Linoleic acid (C18:2)	0.7	6.4
Arachidonic acid (C20)	3.6	3.2
TOTAL	100	100

The increased percentage of palmitic acid (C16) observed in seeds from Maringá (38.8%) has also been observed in the stems of adult *C. peruvianus* plants (35%) (Machado et al. 2006). Ramadan and Mörsel (2003) studied the ratio of fatty acids in the seeds and pulp of *Opuntia ficus-indica* L. They found that palmitic acid and oleic acid were present in greater proportion in both the seeds and pulp. However, oleic acid (C18:1), which was found at a greater percentage in the seeds from Maringá and Picos, was described as being found in trace quantities in the stems of adult *C. peruvianus* plants (Machado et al. 2006). This result indicates that the oleic acid (C18:1) that is prevalent in *C. peruvianus*

seeds must be the type of fatty acid that is mainly used (consumed) to promote the processes of germination and seedling development. Oleic acid was also the most common monounsaturated fatty acid found in samples of several species of vegetables and cereals, and their amount was decreased in most of the germinated samples (Megat-Rusydi et al. 2011). Furthermore, it is possible that the mobilization and reduction of the lipids during seed germination of *C. jamacaru* related by Alencar et al. (2012) may be due to the consumption of oleic acid during the processes of germination and formation of the mandacaru seedlings. Thus, the oleic acid that is found in larger quantities in the seeds of mandacaru of both localities Maringá and Picos may explain the non significant difference in the rate of seed germination from the South and Northeast regions of Brazil.

The percentage of unsaturated fatty acids (palmitoleic, oleic, and linoleic acids) was 43.0% in seeds collected from Maringá and 53.4% in seeds collected from Picos. Unsaturated fatty acids percentages greater than 60% have been described in seeds of *Opuntia ficus-indica* cultivated in Tunisia (El-Mannoubi et al. 2009) and in cactus pear (Labuschagne and Hugo 2010). The increased amount of unsaturated fatty acids, especially linoleic acid, (C18:2) in seeds from Picos is an economically significant factor because linoleic acid has been used in the synthesis of conjugated linoleic acid, which has a potential application in the pharmaceutical industry (Guo and Sun 2004, Yang and Liu 2004). The action of conjugated linoleic fatty acid has been associated with tumour growth inhibition effects (antiproliferative effects in tumour cells) in studies by Agatha et al. (2004) and Wang et al. (2004).

Different percentages of saturated and unsaturated fatty acids in the seeds from Maringá and Picos do not seem to be related to the seed germination process as there was no difference in the germination rate of seeds from Maringá and Picos at low temperatures (20°C) or at higher temperatures (25 and 30°C). However, it is possible that the increased amount of unsaturated fatty acids in seeds from Picos may be related to the general mobilization of TAGs and to the specific lipases activity.

No specific lipase was evident in seeds that were dried and imbibed during the germination period (8 days), but analysis of the electrophoretic pattern of these enzymes showed specific alleles for lipases in seeds from Picos. The lipases were identified from the electrophoretic pattern of α- and β-esterases in seeds and seedlings of *C. peruvianus* previously determined by Sala et al. (2011). The electrophoretic patterns of α- and β-esterases in the seeds of mandacaru plants from Maringá and Picos were monitored in order to identify which esterases corresponded to lipases (EC 3.1.1.3). This analysis showed that of the 14 loci for α- and β-esterases determined by Sala et al. (2011), those encoded by loci *Est-5*, *Est-6*, *Est-7*, *Est-8*, *Est-10*, and *Est-14* were characterized as lipases. The difference between lipases and other hydrolases such as esterases is that lipases do not hydrolyze substrates that are below a minimum concentration in solution. The activity of lipases is stimulated when the substrate concentration is close to or exceeds its solubility limit (Costa and Amorin 1999).

Polymorphism of lipases from seeds was observed in the *Est-5* and *Est-14* loci but only *Est-5* was evaluated in our study as it had a well-defined electrophoretic pattern with enzymes showing dimeric structure (Sala et al. 2011). Although the expression patterns of lipases during the seeds germination (24, 48, 72, 96, 120 and 144 hrs after soaking) were the same for seeds from plants cultivated in Maringá and Picos, the polymorphism analysis of the lipase encoded by locus *Est-5* (= *Lipase-5*) showed the presence of a third allele (allele $Est\text{-}5^3$= $Lipase\text{-}5^3$), which was only observed in seeds

from plants cultivated in Picos (Fig. 2). Alleles *Est-5*^{1} (= *Lipase-5*^{1}) and *Est-5*^{2} (= *Lipase-5*^{2}) were observed in the seeds from Maringá, and alleles *Est-5*^{1}(= *Lipase-5*^{1}), *Est-5*^{2} (= *Lipase-5*^{2}) and *Est-5*^{3} (= *Lipase-5*^{3}) were observed in the seeds from Picos. The frequency of these alleles in 74 seeds collected in Maringá and 55 collected in Picos is shown in Table IV.

TABLE IV
Allele frequency values for the lipase from *Est-5* locus evaluated in mandacaru seeds collected in Picos-PI and Maringá-PR and the expected (H_e) and observed (H_0) heterozygosity values.

	N	*Lipase-5*^{1}	*Lipase -5*^{2}	*Lipase -5*^{3}	H_0	H_e
Picos	55	0.0091	0.9091	0.0818	0.1091	0.1668
Maringá	74	0.5203	0.4797	-	0.2838	0.4992
Mean Frequency	-	0.3023	0.6628	0.3490	-	-

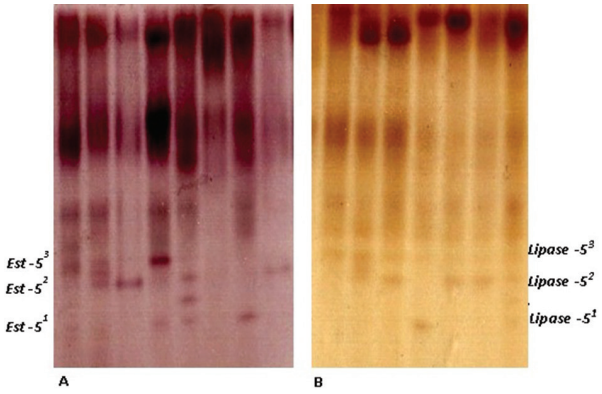

Figure 2 - Polymorphism evaluated at locus *Est-5* (*Lipase-5*) for mandacaru seeds collected in Picos; the alleles *Est-5*^{1}, *Est-5*^{2} and *Est-5*^{3} (**A**) were observed, which correspond to *Lipase-5*^{1}, *Lipase-5*^{2} and *Lipase-5*^{3} (**B**), respectively.

The methodology used in this study does not allow for the conclusive deduction that the specific lipase (LIPASE-5^3) detected in seeds from Picos is directly related to the different types of fatty acids found in these seeds. However, frequently, ecological interactions of plants with their environment may make a random genetic change occurring in an individual and if this change is adaptive, the selection increases the proportion of individuals who carry the new allele with trivial differences in adaptive value (Pichersky 2011). The fact that some authors have reported that seeds have only one predominant molecular class of TAG seems to indicate that selective pressure has chosen a lipase with a suitable specificity to hydrolyze TAG during the germination process (Hellyer et al. 1999). Therefore, the observation that seeds collected in Maringá contained higher amounts of palmitic acid than the seeds from Picos (Table II) leads to the suspicion that the specificity of lipase alleles *(Lipase-5*^{1} *and Lipase-5*^{2}*)* may be associated with the formation of these predominant fatty acids from specific TAGs.

Although the number of alleles found for the lipases of locus *Lipase-5* (3 alleles) in seeds from Picos is greater than the number of alleles detected in seeds from Maringá (2 alleles), the estimated values of the observed mean heterozygosity (Ho) and expected mean heterozygosity (He) were greater in the sample of seeds from Maringá (Table IV). The He value of the sample of seeds from Maringá (0.4974) was very close to the He value (0.4977) estimated in the analysis of 6 esterase loci including locus *Est-5* in 14 populations of plants cultivated in the region of Maringá and other cities in the South of Brazil (Sala et al. 2011). The high level of genetic diversity detected in the 14 populations of *C. peruvianus* plants cultivated in the South of the country was also seen in the analysis of the lipases of locus *Est-5* of *C. peruvianus*

seeds in the same region. On the other hand, the smaller proportion of observed (Ho = 0.1091) and expected (He = 0.1668) heterozygotes for the lipases of locus *Lipase-5* in seeds from Picos is consistent with the low genetic diversity found in samples of a species of *C. jamacaru* plants by analyzing random DNA segments amplified by polymerases (PCR; Polymerase Chain Reaction) (Gutman et al. 2001). The main clonal form of plant propagation for these plants was used to justify the low genetic diversity in populations of *C. jamacaru*. The polymorphism analysis of lipases and/or α- and β-esterases of several loci in addition to locus *Est-5* in populations of mandacaru in the Northeast of Brazil may support or contradict these preliminary indications of the parameters of genetic diversity in plants cultivated in the South and Northeast of Brazil.

Low genetic identity (Nei 1978) between mandacaru from Maringá and Picos was evident in the *Lipase-5* locus analysis (I = 0.77). Plants showing low genetic identity, such as I < 0.85 are frequently related to geographically distant species in speciation process or to different species of genus. According to the present evidences, the mandacaru plants from Maringá and Picos may correspond to two species: *C. peruvianus* and *C. jamacaru*.

Our study showed that seeds of mandacaru from Maringá and Picos have demonstrated a similar germination pattern under laboratory conditions; the seeds require temperatures greater than 25°C for a faster germination process. On the other hand, the seeds from Maringá and Picos have different percentages of certain fatty acids and are genetically differentiated for lipases locus (*Lipase-5*). The analysis of lipases indicated that seeds from Maringá have high observed and expected mean heterozygosities and that seeds from Picos have a higher number of alleles per loci. Therefore, the procedure used in laboratory to germinate seeds of mandacaru of the South region may also be used to germinate seeds of the mandacaru plants of Northeast region. The seeds of mandacaru from semiarid region of the Northeast as well as the seeds from the South (the two contrasting regions of Brazil) are promises for use in the preservation of the biodiversity in the genome of mandacaru. These results are important in helping to increase the availability of these plants, which have characteristics that are of interest with respect to biotechnological applications and for the preservation of their biodiversity.

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RESUMO

A proposta deste estudo foi avaliar diferenças químicas, fisiológicas e genéticas em sementes de cactos do gênero *Cereus* (mandacaru) cultivadas nas regiões Nordeste (Picos, Estado do Piauí) e Sul (Maringá, Estado do Paraná) do Brasil. No período de oito dias, as temperaturas de 25°C e 30°C foram igualmente adequadas para a germinação de todas as sementes. O ácido oleico (C18:1) foi o ácido graxo mais comum encontrado nas sementes coletadas nas regiões Sul (41%) e Nordeste (45,5%). A análise de lipases indicou que as sementes de Maringá têm alta heterozigosidade média observada e esperada e que as sementes de Picos têm número mais alto de alelos por locos. Portanto, as sementes de mandacaru da região semiárida do Nordeste assim como as sementes do Sul (as duas regiões contrastantes do Brasil) são promissoras para a preservação da biodiversidade no genoma de mandacaru. A baixa identidade genética entre as sementes de Maringá e de Picos na análise do loco *Lipase-5* (I = 0,77) sugere que as plantas de mandacaru de Maringá e de Picos podem corresponder a duas espécies: *C. peruvianus* e *C. jamacaru*, respectivamente.

Palavras-chave: Cactos, *Cereus peruvianus*, *Cereus jamacaru*, germinação de sementes, ácidos graxos, lípases, diversidade genética.

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