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Genetic diversity of endangered populations of *Butia capitata*: Implications for conservation

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The flora and fauna of the Cerrado biome in central Brazil both show great diversity and high levels of endemism. *Butia capitata* is a palm native to this biome that has significant economic, social, and environmental value. We sought to identify and quantify the genetic diversity of four fragmented populations of *B. capitata* growing in northern Minas Gerais State, Brazil, as well as one located at the Institute of Agricultural Sciences (ICA) at UFMG, assessing 93 genotypes using 11 ISSR markers. The relationships among populations were evaluated by constructing dendrograms, principal coordinate analysis, genetic distances, as well as Bayesian inference, including and excluding the ICA population. High genetic diversity was found in the populations studied, with most of that diversity occurring within populations. Bayesian inference regrouped the original populations into four populations, redistributing the ICA individuals to the Abóboras and Cristália populations. The analysis that excluded the ICA population arranged the original populations into two groups - with the Abóboras and Cristália populations together in the same group. The ICA population was found to be a repository for future reintroductions into the Abóboras and Cristália regions, as they genetically resemble those populations. It should be noted that other management measures outlined in this study should be adopted before these palm populations enter critical decline phases, such as: stimulus to plants seedlings derived from seeds originated in each area (principally the Abóboras site); quantification of the genetic diversity of neighboring populations to the Mirabela site for future reintroductions as this population showed low intrapopulation diversity.

Key words: Arecaceae, inter simple sequence repeats (ISSR) molecular markers, Bayesian analysis, management measures.

INTRODUCTION

The Cerrado (Brazilian savannas) is the second largest biome in Brazil, but extensive areas of this natural vegetation have been removed for grain production and pasture formation (Klink and Machado, 2005). The Ministry of the

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Environment (Ministério do Meio Ambiente, 2014) has estimated that almost 8,000 km² of this biome were converted between 2008 and 2009 and many species have become endangered due to habitat loss and the fragmentation of natural populations (Silva and Bates, 2002; Klink and Machado, 2005; Carvalho et al., 2009). *Butia capitata* Mart. Becc., is a palm tree native to the Cerrado biome that is considered as an endangered species (Lopes et al., 2011). Its fruits are widely consumed either fresh or processed to produce pulp, juices, jams and popsicles (Faria et al., 2008), constituting one of the main sources of income for small farmers and harvesters that supply local markets and schools. *B. capitata* also has ornamental value and is used in gardening and landscape projects. This species is usually found bordering rivers in northern Minas Gerais State, contributing to the formation of riparian forests and providing food for local wildlife (Mercadante-Simões et al., 2006). This species experiences intensive extractive exploitation of its fruits as there are no commercial orchards (Magalhães et al., 2012). As demand is always greater than natural supplies, collectors will harvest virtually everything that is produced. The fruits of *B. capitata* show great heterogeneity, coming in many different shapes, epicarp colors, scents, and flavors (Moura et al., 2008; Faria et al., 2008), indicating a high genetic diversity that is now threatened by over-exploitation. While the genetic diversity within populations of *B. capitata* is probably high, it is now threatened by habitat loss and fragmentation. Fragmentation is known to be a significant threat to the maintenance of biodiversity in palms (Bouzat, 2010; Shapcot et al., 2012; Avalos et al., 2013; Federman et al., 2013) as it reduces population sizes and increases the spatial distances between them (Young et al., 1996) – with serious implications for genetic drift, inbreeding, and gene flow (Biebach and Keller, 2010; Silva et al., 2011). The loss of heterozygosity can reduce the viability of the remaining population, and have long-term effects on the ability of a species to respond to environmental changes (Young et al., 1996; Bouzat, 2010). Intensive extraction of fruits/seeds will compromise the natural regeneration of a species (Byg and Balslev, 2001), and deplete its genetic reserves (Homma, 2012).

Genetic data can contribute to the quantification of genetic diversity and aid the management of conservation and recovery programs of endangered palm populations (Shapcott et al., 2009; Nazareno et al., 2013). Molecular information can complement ecological information (Nazareno et al., 2013) and morphological studies, increase the efficiency of collection processes and genetic enrichment, and aid in species classifications (Gaiero et al., 2011). There have been no studies yet for the genetic diversity of *B. capitata* populations growing in northern Minas Gerais State, and it is hoped that this study will be a useful starting point for the pro-active conservation and management programs of *B. capitata* palm trees (and other species) in the Cerrado biome. One way to assess this genetic diversity is to examine (Inter Simple Sequence Repeats (ISSR) markers – which do not require prior knowledge of genome sequences (Kumar et al., 2009) and is relatively simple, quick, and inexpensive technique, with high reproducibility (Karim et al., 2010). ISSR include many polymorphic loci and generate a great number of informative bands per reaction that can be used to differentiate between even closely related individuals (Gaiero et al., 2011). These markers have been successfully employed in examining the genetic diversity and taxonomy of *Phoenix dactylifera* L. (Hamza et al.; 2012; Srivashtav et al.; 2013) and of species of *Butia* (Rossato et al. 2007 and Gaiero et al.; 2011).

Ecological restoration is a relatively young science, however, numerous criteria must be verified (Hufford and Mazer, 2003) regarding the genetic composition of plants before the use in restoration projects (Jones, 2003; Hufford and Mazer, 2003; McKay et al., 2005) and researchers have noted that molecular markers may provide guidelines for this process (McKay et al., 2005). That is why we also examined the genetic diversity of individuals of *B. capitata* growing on the campus of the Institute of Agricultural Sciences (ICA), UFMG, to evaluate potential as a genetic repository for the re-introduction of individuals into threatened populations of this palm. We therefore sought to evaluate the levels of genetic diversity in four fragmented natural populations of *B. capitata* in northern Minas Gerais State, Brazil, as well as specimens growing on the campus of the Institute of Agricultural Sciences (ICA) UFMG, using ISSR markers.

MATERIALS AND METHODS

Characterization of populations

The *B. capitata* populations studied here were located in the central region of northern Minas Gerais State, Brazil. Ninety-three individuals were sampled from four wild populations growing in four municipalities (Cristália, Bonito de Minas, Mirabela, and Montes Claros) as well as from a nine-year-old orchard at the Institute of Agricultural Sciences (ICA) of the Federal University of Minas Gerais State (which were grown from seeds derived from the Abóboras population) (Figure 1).

The Abóboras population

This population consists of 60 plants economically exploited. The landscape is dominated by a mosaic of small savannah fragments surrounded by croplands (sugar cane and pineapples) and pastures. The plants there were nearly all adults that were more than 27 years old. No juvenile plants were encountered (Figure 2A and 2B).

The Bonito de Minas population

The Bonito de Minas population is composed of approximately 100 plants (generally 25-year-old adults in their reproductive phase),

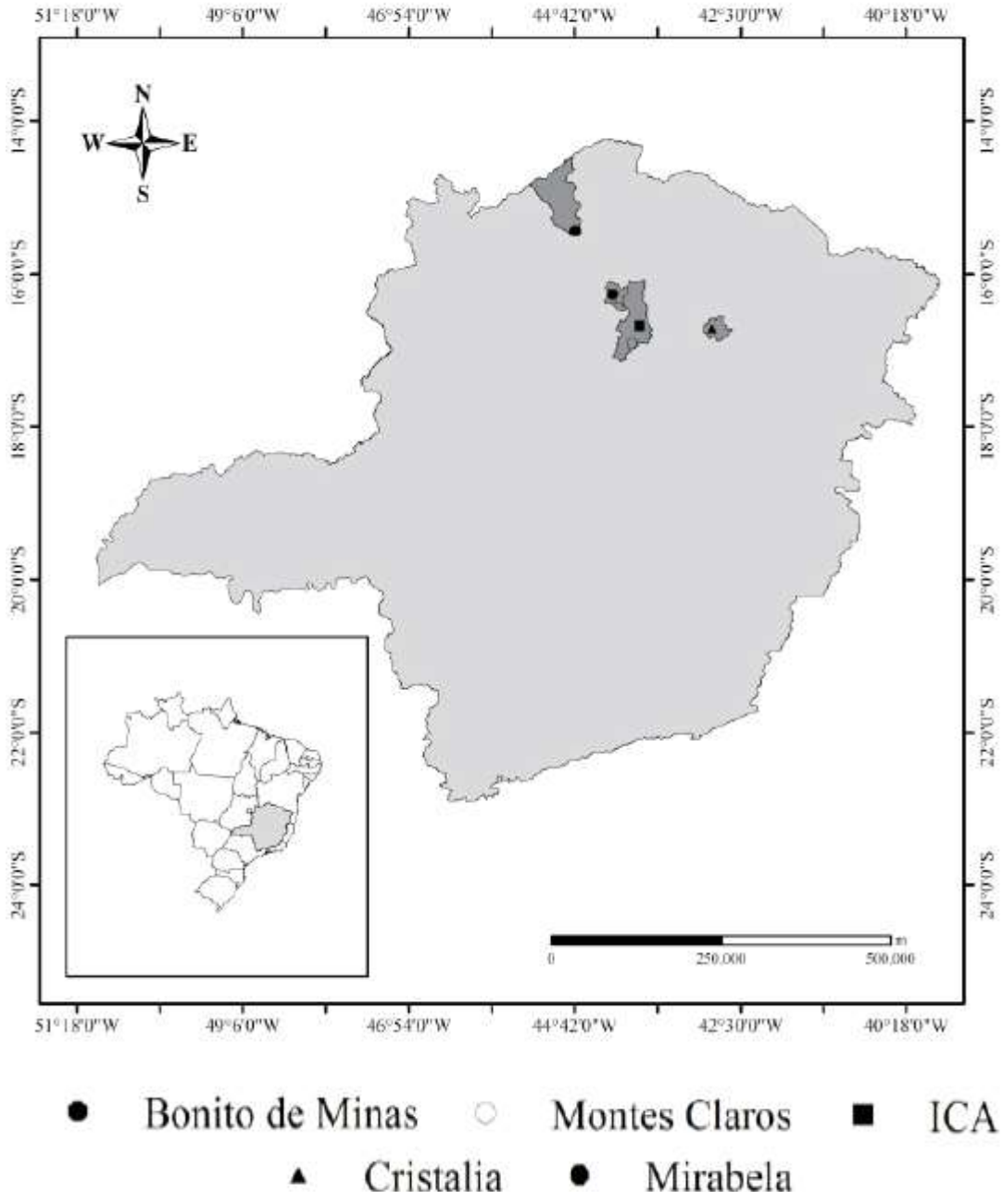

Figure 1. Geographical distributions of *Butia capitata* populations in the municipalities of Bonito de Minas (B), Mirabela (M), Abóboras (A), and Cristália (C), and at ICA (I) in northern Minas Gerais State, Brazil. The dark gray areas indicate the municipal boundaries.

although juveniles were observed in their initial seedling stages. The area is also used as pasture for cattle. The fruits of this population are not commercially harvested for human consumption but used to feed pigs, chickens and cattle (Figure 2D).

The Mirabela population

This population comprises approximately 150 adult plants of reproductive age; seedlings were also observed. The area is used for cattle grazing and the palm fruits are collected to be sold for human consumption. The mean age of this population is approximately 20 years old (Figure 2E).

The Cristália population

This population is composed of approximately 80 plants in their reproductive phase (average of 20 years old). The area is used for grazing, and the palm fruits are harvested to feed pigs, chickens, and cattle (Figure 2F).

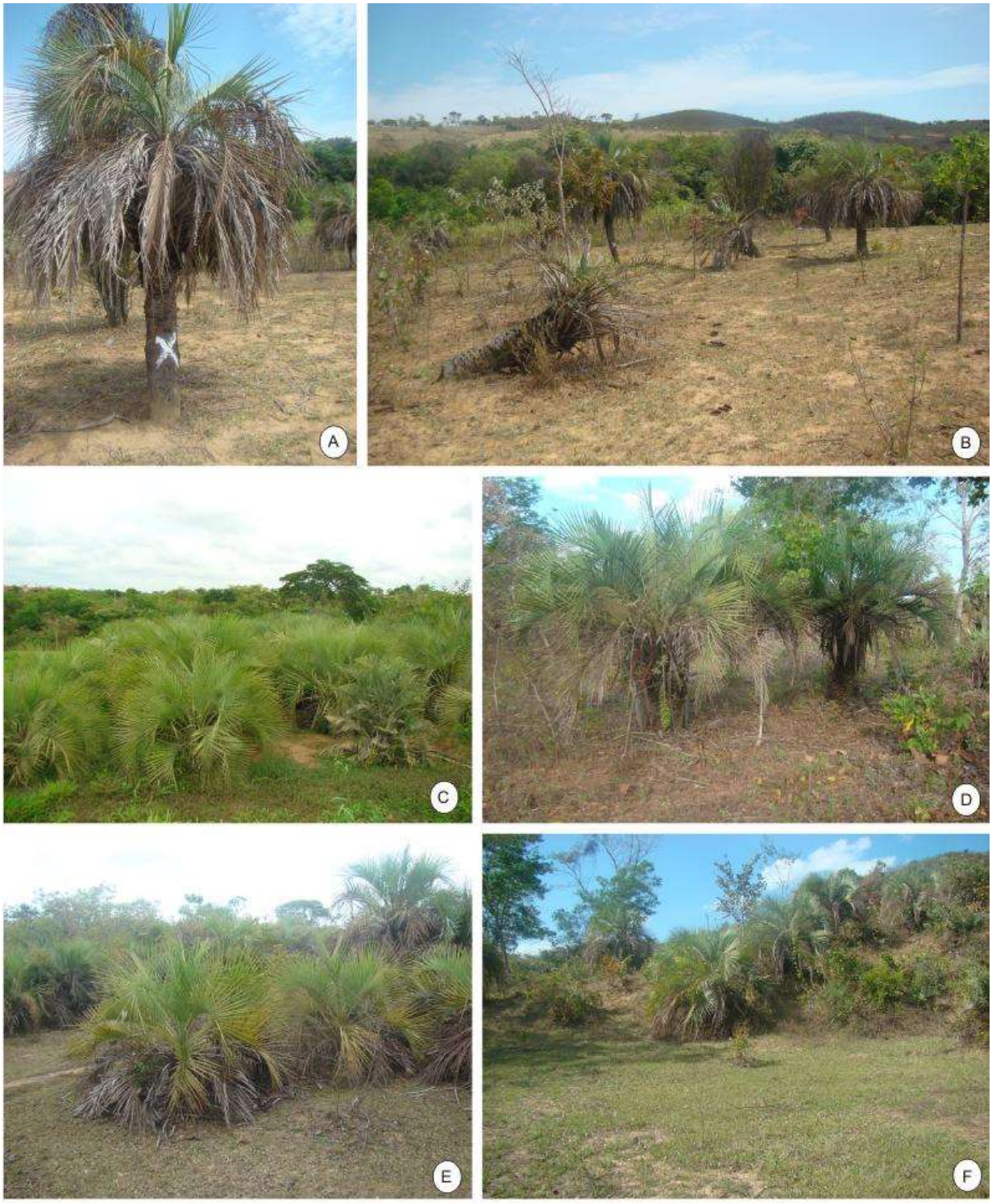

Figure 2. Populations of *Butia capitata* studied in the municipalities of Abóboras (A,B), Bonito de Minas (D), Mirabela (E), and Cristália (F), and ICA (C) in northern Minas Gerais State, Brazil.

The ICA population

The ICA orchard was planted in 2006 by researchers at the Institute of Agricultural Sciences, UFMG, in Montes Claros. The seeds used were collected at the Abóboras site, and 40 seedlings were planted with 2.5 m x 3.0 m spacing. This population is now in its reproductive phase after approximately 8 years (Figure 2C). All plants sampled in these populations were geo-referenced using a GPS (Garmin III Plus). Their geographical coordinates and the numbers of plants sampled are shown in Table 1. The distances (in

Table 1. Geographical coordinates of the populations of *Butia capitata* and the numbers of plants sampled in five locations in northern Minas Gerais State, Brazil.

Population	Latitude	Longitude	Samples	Approximate population sizes
Bonito de Minas (B)	15.434.037	44.691.877	21	~ 100
Abóboras (A) - Montes Claros	16.915.687	43.937.155	17	~150
ICA (I) - Montes Claros	16.682.370	43.839.108	20	~80
Cristália (C)	16.723.888	42.879.871	21	40
Mirabela (M)	16.266.282	44.691.977	14	60

Table 2. Sequences of the ISSR primers used and the annealing temperatures of the PCR reactions.

Marker code	ISSR Sequence (5' → 3')	Annealing temperature (°C)	Nab	Npb
1	$(AC)_8T$	50	10	9
1a	$(GACA_3)RG$	48	14	12
7	$GAG(CAA_4)$	55	15	14
817	$(CA_8)A$	55	14	11
849	$(GT_8)YA$	55	12	10
851	$(GT_8)YG$	55	15	15
853	$(TC_8)RT$	48	23	20
855	$(AC_8)YT$	50	14	10
857	$(AC_8)YG$	50	13	10
864	$(ATG)_6$	48	14	10
876	$(GATA_2)$ $(GACA_2)$	48	22	20

Nab = Number of amplified bands; Npb = number of polymorphic bands.

Table 3. Estimates of genetic (below) and geographic distances (km) (above) of different populations of *Butia capitata* from the municipalities of Bonito de Minas, Mirabela, Abóboras, ICA, and Cristália in northern Minas Gerais State, Brazil.

Population	Bonito de Minas (B)	Abóboras (A)	ICA (I)	Cristália (C)	Mirabela (M)
Bonito de Minas (B)	0.000	263.0	218.0	377.0	149.0
Abóboras (A)	0.078	0.000	45.0	210.0	114.0
ICA (I)	0.082	0.060	0.000	165.0	69.0
Cristália (C)	0.121	0.094	0.065	0.000	277.0
Mirabela(M)	0.162	0.177	0.137	0.109	0.000

km) between the populations are listed in Table 3.

DNA extraction and amplification using ISSR

DNA was extracted from the youngest leaves (still contained within the leaf sheaths and characterized by their yellow color). Leaf samples were ground in a porcelain mortar in the presence of liquid nitrogen (N2) and 0.1 g polyvinylpyrrolidone (PVP) to prevent oxidation, and then stored at -80°C in an Ultrafreezer in 1.5 ml tubes. DNA extraction was performed according to the methodology described by Ferreira and Grattapaglia (1995). A NanoDrop ND-1000 spectrophotometer was used to quantify the DNA samples, and they were subsequently diluted in TE (TRIS - 10 mM HCl; 1 mM EDTA; pH 8.0) to a final concentration of 10 ng/µL. After standardization of the DNA concentrations for each PCR reaction, 10 ng of DNA, 100 µM of each dNTP, 1U of Taq DNA polymerase buffer composed of 50 mM TRIS, pH 8.3, 20 mM KCl, 2 mM $MgCl_2$, 10 mg BSA, 0.25% Ficoll 400, 10 mM tartrazine and pure water was added to a final reaction volume of 12.01 /µL. The amplification reactions were performed in a Master Cycler Gradient thermocycler model 5331. Sixty-one primers (© Life Technologies) were tested to examine polymorphism, and 11 were selected based on producing high numbers of fragments and good quality bands. Their respective base sequences and quantities of bases are shown in Table 2. The amplification programme consisted of an initial denaturation of DNA at 95°C for 5 min followed by 40 cycles of 20 s at 94°C (denaturation), 20 s annealing at varying temperatures (depending on the primer – Table 2), and 20 s at 72°C (polymerization), followed by one cycle of 4 min at 72°C, with final stabilization at 10°C. The amplified fragments were separated on 1.5% agarose by gel electrophoresis in 1X TBE buffer with 10 µL of GelRed™ (Uniscience®) fluorescent dye, at 90 V from 2.5 h to 4 h (depending on the primer used). Comparisons of fragment sizes were based on a standard 100 bp DNA ladder. The fragments were visualized under ultraviolet light (Fotodyne) and the images were

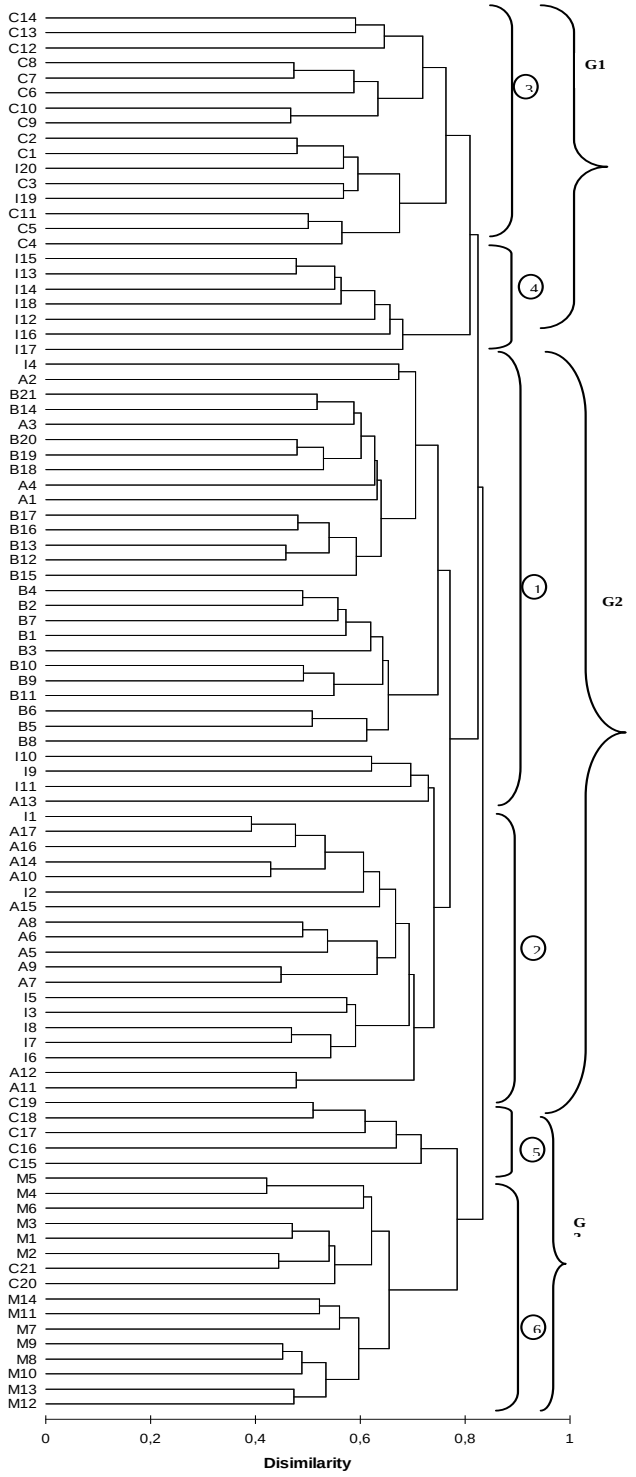

Figure 3. UPGMA grouping and the Jaccard (1908) genetic similarity coefficient of different genotypes of *Butia capitata* in the municipalities of Bonito de Minas (B), Mirabela (M), Abóboras (A), Cristália (C), and at the ICA experimental orchard (I) in northern Minas Gerais State, Brazil. G1= group 1; G2 = Group 2; G3 = Group 3.

captured and documented using an EDAS 290 system (Kodak ®).

Statistical analyses of the data

The electrophoretic profiles of each gel were transformed into a binary matrix, with the presence of a fragment being represented by (1) and its absence by (0). Binary data was used to evaluate all subsequent analyzes. The genetic dissimilarity between each pair of individuals was determined using the Jaccard coefficient (Jaccard, 1908). The Unweighted Pair Group Method Arithmetic Average (UPGMA) was used to group the genotypes according to their genetic similarity, using XLSTAT software (Addinsoft ® version 2011.2.04, 2009)). The genetic similarities between populations were calculated by the Nei index using Genalex v.6.3 software (Peakall and Smouse, 2006). Population groupings using principal coordinates analysis (PCoA), with the help of XLSTAT software (Addinsolft, 2009) was also performed.

The Shannon index (I) (Brown and Weir, 1983) and expected heterozygosity (He) were calculated as described by Lynch and Milligan (1994) and Maguire et al. (2002), using Genalex v.6.3 software (http://www.anu.edu.au/BoZo/GenAlEx/). The genetic variation within and among populations was estimated by AMOVA using the GenAlEx program, version 6.43 (Peakall and Smouse, 2006) like the Mantel test was performed based on the genetic and geographic distances of individuals. The significance of variance components and of the Φ statistics and the Mantel test was estimated using permutation procedures (5000 permutations). Inferences about genetic structures were performed using Structure version 2.2 software (Pritchard et al., 2000; Falush et al., 2007). We estimated the most likely cluster (K) number, and the number of reconstructed panmictic populations (RPP) using values ranging from 1 to 10 and assuming that the sampled genotypes were of unknown origin. The burn-in was 20,000, and a Markov chain Monte Carlo (MCMC) clustering was performed with 20,000 iterations, with five replicates. This program estimates the most likely number of clusters (K) by calculating the log likelihood of the data for each value of (K). We evaluated the best (K) value using the method proposed by Evanno et al. (2005).

Genetic distance analysis of molecular variance (AMOVA) and Bayesian inference analyzes were performed in two ways: excluding and including the ICA samples. The same programs described above were used in both analyses.

RESULTS

Eleven primers were selected for this study that generated 141 polymorphic bands. The numbers of polymorphic bands ranged from 9 to 20 per primer, with a mean of 12.81. The ISSR 876 and 853 primers produced most fragments (20 each) (Table 2). The UPGMA analyses revealed high genetic diversity in the *B. capitata* populations, distributing them into three groups. The largest group comprised 49 genotypes, with 100% of the individuals sampled in Bonito de Minas, 100% of the Abóboras individuals, and 55% of the ICA specimens (Figure 3). The second largest group consisted of 23 plants from the populations at Cristália (71.42%) and from ICA (45%). Group 3 was the smallest group, with 21 individuals, comprising 33% of the population of genotypes sampled at Cristália and 100% of those from Mirabela (Figure 3). The Mantel test revealed a significant positive correlation between genetic and geographic distances among populations of *B. capitata* ($r = 0.472$; $P < 0.005$). The genetic distances varied when the five populations were analyzed together and, in this

Table 4. Analysis of molecular variance (AMOVA) of populations of *Butia capitata* in the municipalities of Bonito de Minas, Mirabela, Abóboras, ICA, and Cristália in northern Minas Gerais State, Brazil.

Origin	Degrees of freedom	Estimate of variation	Percentage of variation
between populations	4	5.561	19
within populations	88	23.415	81
Total	92	28.975	100

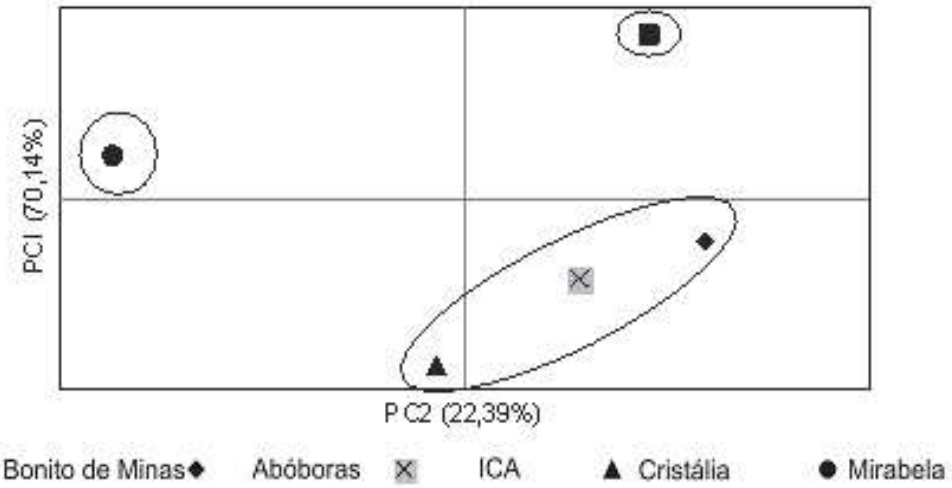

Figure 4. Principal Coordinates Analysis (PCoA of different populations of *Butia capitata* in the municipalities of Bonito de Minas, Mirabela, Abóboras, and Cristália, and at ICA (I) in northern Minas Gerais State, Brazil.

case, the population of Mirabela was the farthest from the others (Table 3). The largest genetic distance was found between the Mirabela and Abóboras populations (0.177), followed by Bonito de Minas, ICA, and Cristália. The smallest distances were observed between the ICA and Abóboras population (0.060) and between ICA and Cristália (0.065) (Table 3). When the ICA population was excluded, the results were similar to the first analysis. The Mirabela population showed the greatest genetic distance in relation to others. The largest genetic distance was found between the Mirabela (M) and Abóboras (B) populations (0.084), and the smallest distance was observed between the Abóboras and Cristália populations (0.041). Diversity between populations was lower than within populations. This result was confirmed by AMOVA, which showed variations of 19% between populations and 81% within populations (Table 4) when excluding the ICA individuals, the diversity variations were 22% between populations and 72% within populations.

The graph generated by principal coordinate analysis (PCA) explained 92.53% of the observed diversity (Figure 4). Populations of *B. capitata* from different municipalities formed three distinct groups. The first was composed only of the Mirabela population, the second of the Bonito de Minas population, and the third comprised the Abóboras, Cristália, and ICA populations together (Figure 4). The Shannon index (I) identifies genetic diversity, and can range between 0 and 1. The closer its value is to 1, the greater diversity among the genotypes (Perry and McIntosh, 1991). The Shannon index (I) values of the *B. capitata* populations ranged from 0.374 to 0.432, with a mean of 0.41. The lowest value was seen with the Mirabela population (0.374), which also showed a lower percentage of polymorphic loci (67.38%) (Table 5). The PCA and UPGMA analyses indicated that this population was the most divergent from the others, although it had a lower internal diversity. The highest Shannon index (I), genetic diversity (He), and percentage of polymorphic loci values were observed with the Cristália and ICA populations (Table 5).

Bayesian inference was performed to evaluate the genetic structure of the genotypes of *B. capitata*. The best (K) to represent the number of reconstructed panmictic populations including the ICA individuals was K = 4 (RPP1 to RPP4), and K = 2 (RPP1 to RPP2) when excluding the ICA population. The results were mostly in agreement with the UPGMA method described above, although they provide some interesting insights into these populations. Twenty-seven individuals were grouped in

Table 5. Shannon index (I), genetic diversity (He), and percentage of polymorphic bands (P%) of different populations of *Butia capitata* in the municipalities of Bonito de Minas, Mirabela, Abóboras, ICA, and Cristália in northern Minas Gerais State, Brazil.

Population	I	Ĥe	P%
Bonito de Minas	0.409	0.275	76,60
Abóboras	0.414	0.277	78.01
ICA	0.432	0.287	83.69
Cristália	0.420	0.276	85.82
Mirabela	0.374	0.254	67.38
Mean	0.410	0.274	78.30
Standard deviation	0.010	0.007	3.22

RPP1 (15 from Cristália and 12 from ICA). Of the genotypes found in the Cristália population, 14 had adhesion probabilities of over 90% for RPP1. Of the 11 genotypes identified in the ICA population, seven had at probabilities of over 90% for RPP1 (Figure 5a). These results were generally consistent with the UPGMA analysis (Figure 3), which grouped the Cristália genotypes and part of the ICA genotypes together (1) (Figure 3). RPP2 was reconstructed with 21 individuals – 12 from the Abóboras population and 9 from ICA. There was more than 95% adherence of Abóboras genotypes to RPP2 and more than 80% adherence of ICA genotypes (Figure 5a). The results of this analysis show that these two populations are connected only through the ICA population (Figure 5a). RPP3 was composed of all of the genotypes from Bonito de Minas and four from Abóboras, totaling 24 individuals; more than 85% of the Abóboras individuals showed adherence to this reconstructed population (Figure 5a). Finally, RPP4 comprises all 14 individuals from Mirabela and 6 from Cristália (total of 20 genotypes), this being the smallest reconstructed population compared with the others.

The best (K) number of panmictic populations (excluding the ICA specimens) was K = 2 (RPP1 and RPP2). RPP1 included all individuals of the Abóboras population, Bonito de Minas, and the vast majority of Cristália specimens with more than 90% adherence, as RPP2 included all of the Mirabela individuals and four from Cristália (C18, C19, C20, and C21) (Figure 5b).

DISCUSSION

In general, the number of polymorphisms found in *B. capitata* was comparable to other studies of diversity in the family Arecaceae, and in some cases superior. A number of populations of the genus *Butia* were characterized in terms of their diversity in the study undertaken by Nunes et al. (2008), with 77 polymorphic bands being observed in *Butia odorata* using 21 RAPD primers. Rossato et al. (2007) and Gaiero et al. (2011) obtained 150 and 74 polymorphic loci, respectively using ISSR primers in various species of *Butia*. In both studies the primer sequence (AC)8T generated the highest number of polymorphic fragments (26 and 17, respectively); this primer was also efficient in generating polymorphic fragments in the present study. The mantel test indicated positive correlations between geographical and genetic distances, with spatial patterns influencing genetic variability between populations, so that closer populations tend to be more genetically similar - with genetic differences tending to increase with geographic distance. Similar results were reported by Gaiero et al. (2011) for species of *Butia* (r = 0.652) in Uruguay. Shapcott et al. (2012) and Rossetto et al. (2004) did not, however, find significant correlations using the mantel test with the palm species *Lemurophoenix halleuxii* and *Elaeocarpus grandis* in fragmented environments. Estimates of genetic distance in the analyses (including or excluding the ICA population) showed that the Mirabela population was the most divergent, although the genetic distance values decreased when the ICA population was excluded. Among all of the populations sampled, Mirabela was the only one that showed fruits with different colors (ranging from purple through different shades of red to yellow), reinforcing the expectation that this population was actually more divergent than the others, although only minor intrapopulational variations were, in fact, encountered. The results of genetic distance analyses were similar to those of other species of the genus *Butia* (Gaiero et al., 2011) and *Euterpe edulis* (Cardoso et al., 2000). Principal coordinates analysis (Figure 4) reinforced the positioning of both nearby populations as well as the more distant populations identified by UPGMA (Figure 3). The group formed by the Abóboras, ICA, and Cristália populations in the dendrogram showed that the ICA individuals were partly grouped with the Cristália population while a larger number and grouped with the Abóboras population, a fact that is probably related to the source of this plant population, since the ICA orchard was planted with seeds collected in Abóboras. This can be seen in Figure 5a, as the Abóboras and Cristália populations are linked

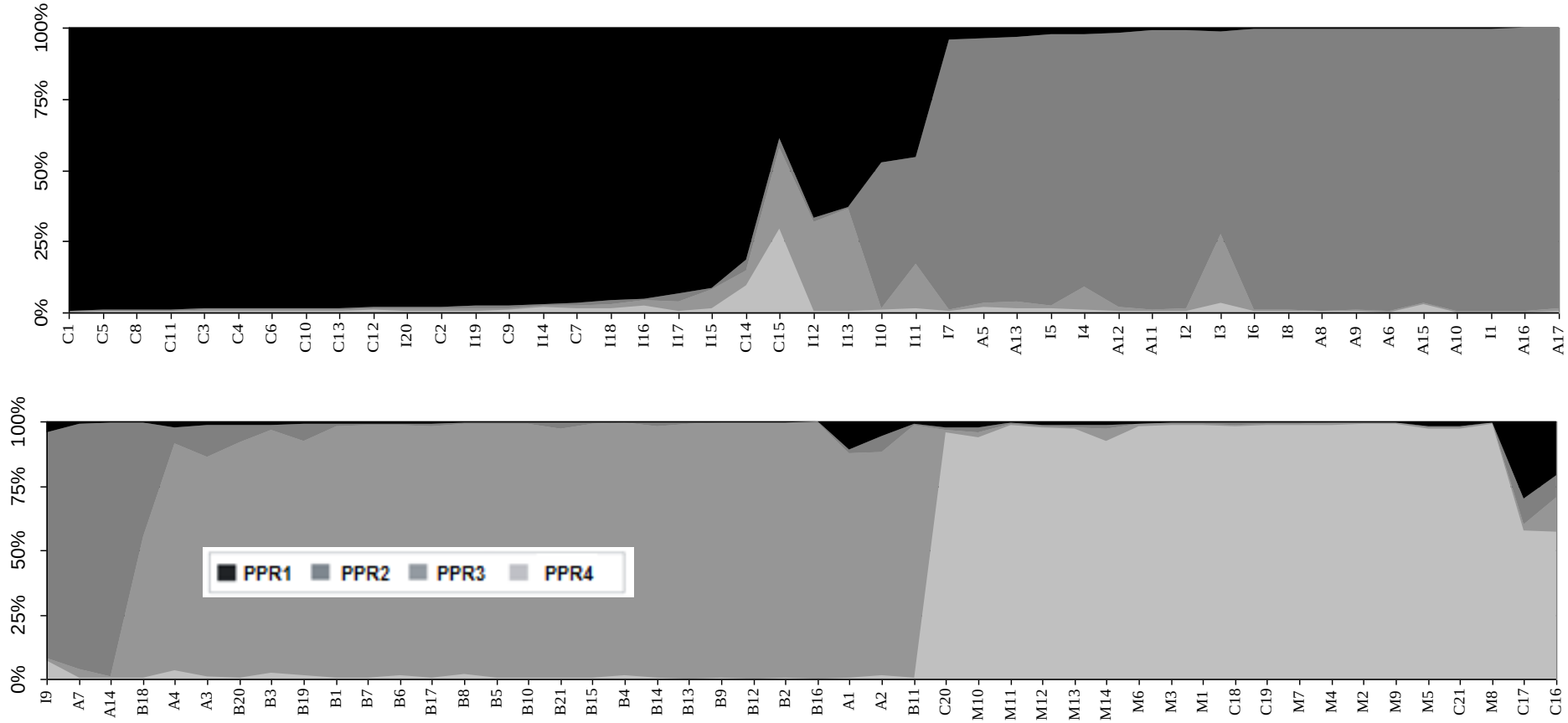

Figure 5a. Bayesian Inference for populations of *Butia capitata* from the municipalities of Bonito de Minas (B), Mirabela (M), Abóboras (A), and Cristália (C), and at ICA (I) in northern Minas Gerais State, Brazil (using K = 4).

by the ICA population and, when it is removed, appear grouped into a single population (Figure 5b).

Genetic diversity in a population is determined by several well-known factors, including gene flow (Lenormand, 2002), genetic drift and habitat fragmentation (Lienert 2004), reproductive isolation (Charlesworth and Charlesworth, 2000), biotic and abiotic factors, topographic relief (Escudero et al., 2003), human interference, mutation (Lande, 1995) and reproductive biology. Most of the genetic diversity of *B. capitata* was found to be distributed within populations (Table 4), in agreement with the work of Gaiero et al. (2011) who reported that 94% of the diversity within the genus *Butia* was at the population level, with only 6% between populations. Similar results were reported by Nunes et al. (2008) for *B. odorata*, and with palm trees of other species, such as *Calamus thwaitesii* Becc. and *Acrocomia aculeata* (with 79.79 and 82.8% intra-population genetic diversity, respectively) (Sreekumar and Renuka, 2006; Oliveira et al., 2012.). These results can be explained by hypothesizing that populations of *B. capitata* had a common origin, but geographical

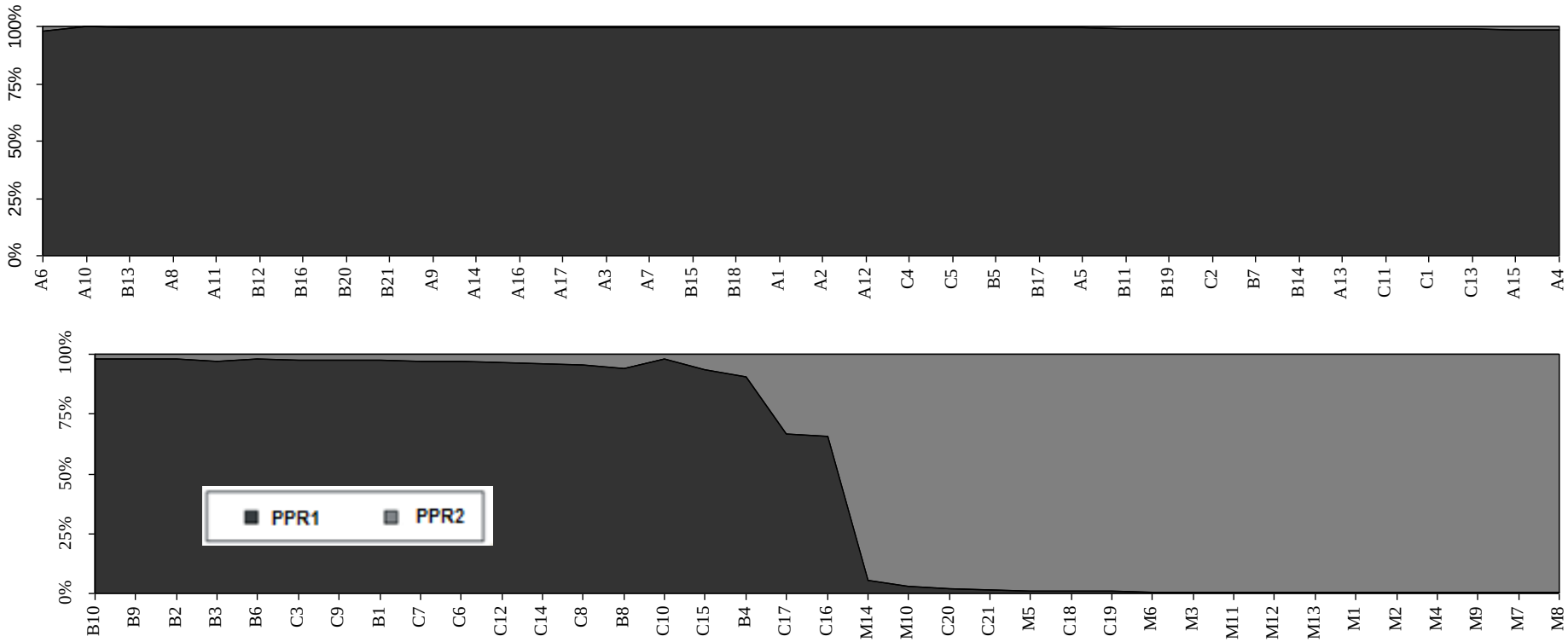

Figure 5b. Bayesian Inference for populations of *Butia capitata* in the municipalities of Bonito de Minas (B), Mirabela (M), Abóboras (A), and Cris tália (C), in northern Minas Gerais State, Brazil (using K = 2).

isolation processes, including selection, drift, and historical process of landscape and human activities across the savanna region caused the observed differences between them, structuring them into subpopulations (Buttow et al., 2010). Studies have indicated that the vegetation of this biome originally consisted of a mosaic of large areas of different vegetation types (from rocky, open fields to dense forests) (Klink and Machado, 2005). In the 60s and 70s stimulus for the production of grain and pasture in the central areas of the country caused large areas of natural vegetation to be removed or modified (Klink and Machado, 2005; Sano and Brito, 2010).

Another hypothesis is that the reproductive biology of *B. capitata* may have contributed to the high diversity seen within populations (Zehdi et al., 2004), as female flowers are found only on the basal portion of the rachillae while male flowers are formed only on the distal portion. Dichogamy of the protandry type was also observed, with anthesis of male flowers occurring before maturation of the female flowers. The low occurrence of synchrony between the male and female phenolphases on the same plant thus favors xenogamic pollination in this species (Mercadante-Simões et al., 2006).

Aguilar et al. (2008) and Shao et al. (2009) suggest that high genetic diversity within populations may also reflect the effects of time, in the sense that more recent habitat fragmentation may not yet be reflected in the current genetic diversity of long-lived species. Thus, habitat fragmentation may be a relatively recent event compared to the lifespans of these species, perhaps involving less than a few generations (Collevatti et al., 2010). Cluster analysis, ordination, and other indexes revealed a large genetic diversity in *B. capitata*, although we initially thought that its diversity might already have

been compromised due to anthropogenic impacts on the cerrado biome. The vast majority of fragmentation studies have reported negative effects on vegetation (Lowe et al., 2005; Aguilar et al., 2006), including decreasing areas of occupation and increasing isolation – leading to genetic drift and increased inbreeding (Young and Brown, 1996) and impairment of seed dispersal (as dispersers encounter difficulties in gaining access to other areas) (Ghazoul, 2005). Fragmentation was found to be detrimental to *Dipteryx alata* in the cerrado, as it showed very low levels of diversity and high levels of inbreeding (Collevatti et al., 2010; Collevatti et al., 2013). *Mauritia flexuosa* (Federman et al., 2012), *Acrocomia aculeata,* and other native Cerrado palm tree populations growing in Mirabela showed lower heterozygosity and lower percentages of polymorphic loci (Oliveira et al., 2012). Other studies, however, have shown that fragmentation may promote more gene flow between populations (Fore et al., 1992; Born et al., 2008) than seen when they are growing fairly close to each other. The effects of fragmentation may not be detectable or not initially compromise the genetic diversity in some species of palms, as noted by Nazareno et al. (2013) in *Butia eriospatha* and Shapcot et al. (2012) in *Voanioala gerardii*. These species showed increased coefficients of inbreeding, however, and reduced numbers of rare alleles (Nazareno et al., 2013; Shapcott et al., 2012). Generally, adults individuals are used to access genetic diversity, while the anthropogenic disturbances and fragmentation were recent events in evolutionary time, so this does not, however, rule out the possibility that these populations in fact have been damaged by fragmentation even though it is not yet detectable by the analyses used (Collevatti et al., 2010; Collevatti et al., 2014). It was not always possible to evaluate inbreeding or the presence of rare alleles in our studies with *B. capitata*, so that future studies should consider these analyzes due to the danger of these populations becoming extinct (Collevatti et al., 2010). While the results of the present study are encouraging in terms of the high genetic diversity observed in their populations, conservation programs of species conservation that must be initiated, as deforestation in the Cerrado biome (MMA 2014) and the extraction of *B. capitata* continues, and this species may soon cease to exist outside of protected reserves (Homma, 2012). Therefore, one should not wait for further population declines before taking action. Conservation and management measures that can be adopted for the preservation and maintenance of genetic diversity of *B*. *capitata* are discussed here based on the information generated in this study.

The Abóboras population exhibited high heterozygosity and Shannon diversity compared to the other areas, although it is one of the populations that most suffers from harvesting, fragmentation, and farmland conversion; steps should be taken to encourage awareness among local communities that some palm seeds should be planted (Gaiero et al., 2011; Nazareno et al., 2013), especially in forest areas near crop fields. Cattle also feed on the inflorescences of this palm, and it is suggested that these areas be fenced off, at least until fruit set. The formation of small palm orchards and the restoration of native forests by planting seeds or seedlings originating from local palm populations will help reduce extraction pressures on native populations (Lopes et al., 2011; Gaiero et al., 2011). Overcoming dormancy and promoting seedling formation, however, is currently not a serious obstacle (Lopes et al., 2011; Magalhães et al., 2012; Oliveira et al., 2013; Dias et al., 2013). The ICA Orchard proved to be a viable repository for future conservation activities in the Abóboras and Cristália sites as our analyses showed high similarities between those three populations. Furthermore, our results showed that the ICA individuals demonstrated high heterozygosity and Shannon diversity. The advantage of this collection is that it is irrigated and produces fruits throughout the year (which does not occur with natural populations). However, the use of this material for restoring other populations (principally Mirabela) should be undertaken with great caution – as Hufford and Mazer (2003) and McKay et al. (2005) warn of the dangers of introducing external genes that can affect plants specifically adapted to local conditions.

The Mirabela population had the lowest Shannon index, heterozygosity, and numbers of polymorphic loci, and was the most divergent from the others, even though it showed genotypes with varying colors of fruits (from purple through red in different shades and, most commonly, yellow). In the future, new populations of *B. capitata* in the region Mirabela should be evaluated for their genetic diversity, and if they were found to be similar, genetic material could be exchanged between these populations – as has been suggested for the ICA, Abóboras, and Cristália populations – thus avoiding the introduction of extraneous genes that could jeopardize their populations Hufford and Mazer (2003) and McKay et al. (2005). These steps were suggested for *D. alata* by Diniz-Filho et al. (2012) for *Annona crassiflora* (Collevatti et al., 2013), and for different species of the genus *Butia* in Uruguay (Gaiero et al., 2011). The formation of genebanks by institutions may also be assisted by this study, as it may serve as a guide for the collection of different genotypes and protecting the greatest possible diversity and representivity of native populations. In the future, new populations should be visited to collect unique genotypes from each region to be used to strengthen their local populations (Collevatti et al., 2010), similar to how the ICA collection can be used. These measures have already been adopted for other species of the Cerrado Biome, such as *D. alata*, *Calophyllum brasiliense* and *Tibouchina papyrus* (Collevatti et al., 2010; Collevatti et al., 2013).

Our results indicate the urgent need for action and long-term studies to preserve the remaining genetic diversity of *B. capitata*. There are still several issues that

raise concerns about the future of this species and about collecting information about other populations across the range of threatened taxa. Our results showed that populations of *B. capitata* show high genetic diversity in spite of the high degree of fragmentation and disturbance of the Cerrado biome in northern Minas Gerais State. Intensive deforestation and extraction should serve as a warning of population declines – and other factors should be investigated, including complementary studies of demography, the genetic diversity of young individuals, gene flow, and inbreeding. The ICA collection has excellent potential for restoring populations at Cristália and Abóboras as they show high genetic diversity similarities; however, their use for restoring other populations should be avoided, and additional populations investigated with a view to designing more effective restoration strategies.

REFERENCES

Addinsoft (2009). XLSTAT Statistical analysis software, versão 2009. IOP Publishing PhysicsWeb www.xlstat.com Accessed 10 june 2014.

Aguilar R, Ashworth L, Galetto L, Aizen MA (2006). Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecol. Lett. 9: 968-980.

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008). Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol. Ecol. 17: 5177-5188.

Avalos G, Otárola MF, Engeln JT (2013). Successional stage, fragmentation and exposure to extraction influence the population structure of *Euterpe precatoria* (Arecaeae). Rev. Bio. Trop. 61(3): 1415-1424.

Biebach I, Keller L (2010). Inbreeding in reintroduced populations: the effects of early reintroduction history and contemporaryn processes. Cons. Gen. 11: 527-538.

Born C, Hardy OJ, Chevallier MH, Ossari S, Attéké C, Wickings EJ, Hossaert-McKey M (2008). Small-scale spatial genetic structure in the Central African rainforest tree species *Aucoumea klaineana*: a stepwise approach to infer the impact of limited gene dispersal, population history and habitat fragmentation. Mol. Ecol. 17: 2041-2050.

Bouzat J (2010). Conservation genetics of population bottlenecks: the role of chance, selection, and history. Cons. Gen. 11: 463-478.

Brown AHD, Weir BS (1983). Measuring genetic variability in plant populations. In: Tanksley SD, Orton, T J (Eds.). Isozymes in plant genetics and breeding. Part A. Amsterdan: Elsevier Science Publishers, pp. 73-86.

Buttow MV, Castro CM, Schwartz E, Tonietto A, Barbieri RL (2010). Caracterização molecular de populações de *Butia capitata* (Arecaceae) do sul do Brasil através de marcadores AFLP. Rev. Bras. Frut. 32:(1): 230-239.

Byg A, Balslev H (2001) Diversity and use of palms in Zahamena, eastern Madagascar. Biodiv. Cons. 10: 951-970.

Cardoso SRS, Eloy NB, Provan J, Cardoso MA, Ferreira PCG (2000). Genetic differentiation of *Euterpe edulis* Mart. populations estimated by AFLP analysis. Mol. Ecol. 9: 1753-1760.

Carvalho FMV, Júnior PM, Ferreira LG (2009). The Cerrado into-pieces: Habitat fragmentation as a function of landscape use in the savannas of central Brazil. Biol. Cons. 142: 1392-1403.

Charlesworth B, Charlesworth D (2000). Reproductive isolation: Natural selection at work. Curr. Biol. 10(2): 68-70.

Collevatti RG, Lima JS, Soares TN, Telles MPC (2010). Spatialgenetic structure and life history traits in Cerrado tree species:inferences for conservation. Nat. Conserv. 8: 54-59.

Collevatti RG, Telles MPC, Lima JS, Gouveia FO, Soares TN (2014). Contrasting spatial genetic structure in *Annona crassiflora* populations from fragmented and pristine savannas. Plant. Syst. Evol. DOI 10.1007/s00606-014-0997-9

Collevatti RG, Telles MPC, Nabout JC, Chaves LJ, Soares TN (2013). Demographic history and the low genetic diversity in *Dipteryx alata* (Fabaceae) from Brazilian Neotropical savannas. Heredity 1: 1-9.

Dias DS, Lopes PSN, Ribeiro LM, Oliveira LAA, Mendes EV, Carvalho VS (2013). Effects of seed structures, sucrose and gibberellic acid on the germination of *Butia capitata* (Arecaceae). Seed. Sci. Technol. 41: 371-382.

Diniz-Filho JAF, Melo DB, Oliveira G, Collevatti RG, Soares TN, Nabout JC, Lima JS, Dobrovolski R, Chaves LJ, Naves RV, Loyola RD, Telles MPC (2012). Planning for optimal conservation of geographical genetic variability within species. Conserv. Genet. 13: 1085-1093.

Escudero A, Iriondo JM, Torres ME (2003). Spatial analysis of genetic diversity as a tool for plant conservation. Biol. Cons. 113: 351-365.

Evanno G, Regnaut S, Goudet J (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. Mol. Ecol. 14: 2611-2620.

Falush D, Stephens M, Pritchard JK (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. 7(4): 574-578.

Faria JP, Almeida F, Silva LCR, Vieira RF, Agostini-Costa TS (2008). Caracterização da polpa do coquinho-azedo (*Butia capitata* var *capitata*). Rev. Bras. Frut. 30(3): 827-829.

Federman S, Hyseni C, Clement W, Oatham MP, Caccone A (2013). Habitat fragmentation and the genetic structure of the Amazonian palm *Mauritia flexuosa* L.f. (Arecaceae) on the island of Trinidad. Conserv. Genet. DOI 10.1007/s10592-013-0543-2

Ferreira ME, Grattapaglia D (1995). Introdução ao uso de marcadores RAPD e RFLP em análise genética. EMBRAPA-CENARGEN, Documento 20, p 220.

Fore SA, Hickey JR, Vankat JL, Guttman SI, Schaefer RL (1992). Genetic structure after forest fragmentation: a landscape ecology perspective on *Acer saccharum.* Can. J. Bot. 70: 1659-1668.

Gaiero P, Mazzella C, Agostini G, Bertolazzi S, Rossato M (2011). Genetic diversity among endangered Uruguayan populations of *Butia* Becc. species based on ISSR. Plant. Syst. Evol. 292: 105-116.

Ghazoul J (2005). Pollen and seed dispersal among dispersed plants. Biol. Rev. 80: 413-443.

Hamza H, Benabderrahim MA, Elbekkay M, Ferdaous G, Triki T, Ferchichi A (2012). Investigation of genetic variation in Tunisian date palm (*Phoenix dactylifera* L.) cultivars using ISSR marker systems and their relation with fruit characteristics. Turk. J. Biol. 36: 449-458.

Homma AKO (2012). Extrativismo vegetal ou plantio: qual a opção para a Amazônia? Estudos avançados 26(74):167-186.

Hufford KM, Mazer SJ (2003). Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends. Ecol. Evol. 18(3): 147-155.

Jaccard P (1908). Nouvelles recherches sur la distribution florale. Bulletin Société Vaudoise des Sci Naturelles 44(2): 223-270.

Jones, TA (2003). The restoration gene pool concept: beyond the native versus non-native debate. Restoration Ecol. 7: 42-50. Karim, k. Chokri B, Amel S, Wafa H, Richid H, Nouredine D (2010). Genetic diversity of Tunisian date pal germplasm using ISSR markers. Inter. J. Bot. 6(2): 182-186.

Klink C, Machado RB (2005). Conservation of the Brazilian Cerrado. Conserv. Biol. 19(3): 707-713.

Kumar P, Gupta VK, Misra AK, Modi DR, Pandey BK (2009). Potential of Molecular Markers in Plant Biotec. Plant. Omics. J. 2(2):141-162.

Lande R (1995). Conservation Biol. 9: 782-791.

Lenormand T (2002) Gene flow and the limits to natural selection. Trends Ecol. Evol. 17(4):183-189.

Lienert J (2004). Habitat fragmentation effects on fitness of plant populations – a review. J. Nat. Conser 12: 53-72.

Lopes PSN, Aquino CF, Magalhães HM, Brandão Júnior DS (2011). Tratamentos físicos e químicos para superação de dormência em sementes de *Butia capitata* (Martius) Beccari. Pesq. Agrop. Trop. 41: 120-125.

Lowe AJ, Boshier D, Ward M, Bacles CFE, Navarro C (2005). Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. Heredity 95: 255-273.

Lynch M, Milligan BG (1994). Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3: 91-99.

Magalhães HM, Lopes PSN, Ribeiro LM, Sant'anna-Santos BF, Oliveira DMT (2012). Structure of the zygotic embryos and seedlings of *Butia capitata* (Arecaceae). Trees v. 27: 273-283.

Maguire TL, Pearkall R, Saenger P (2002). Comparative analysis of diversity in the mangrove species *Avicennia marina* (Fosk.) Vierth. (*Avicenniaceae)* detected by AFLPs and SSRs. Theo. Appl. Genet. 1004: 388-398.

McKay JK, Christian CE, Harrison S, Rice KJ (2005) ''How Local Is Local?''- A Review of Practical and Conceptual Issues in the Genetics of Restoration. Restoration Ecol. 13(3):432-440.

Mercadante-Simões MO, Fonseca RS, Ribeiro LM, Nunes YRF (2006). Biologia reprodutiva de *Butia capitata* (Mart.) Beccari (*Arecaceae*) em uma área de cerrado no norte de Minas Gerais. Unim. Cient. 8(2): 311-316.

Ministerio Meio Ambiente (MMA) (2014). http://www.mma.gov.br/ Accessed 28 june 2014.

Moura RC, Lopes PSN, Brandao Junior DS, Gomes JG, PEREIRA MB (2008). Biometria de frutos e sementes de *Butia capitata* (Mart.) Beccari (Arecaceae), em vegetação natural no Norte de Minas Gerais, Brasil. Biota Neotro. 10: 415-419.

Nazareno AG, Reis MS (2013). At Risk of Population Decline? An Ecological and Genetic Approach to the Threatened Palm Species *Butia eriospatha* (Arecaceae) of Southern Brazil. J. Heredity 28: 1-10.

Nunes AM, Bianchi VJ, Fachinello JC, Carvalho AZ, Cardoso G (2008). Caracterização molecular de butiazeiro por marcadores RAPD. Ver. Bras. Frut.30(3): 702-707.

Oliveira DA, Melo Júnior AF, Brandão MM, Rodrigues LA, Menezes EV, Ferreira PRB (2012) Genetic diversity in populations of *Acrocomia aculeata* (*Arecaceae*) in the northern region of Minas Gerais, Brazil. Genet. Mol. Res. 11(1): 531-538.

Oliveira NCC, Lopes PSN, Ribeiro LM, Mercadante-Simões MO. Oliveira LAA, Silverio FO (2013). Seed structure, germination, and reserve mobilization in *Butia capitata* (Arecaceae). Trees 27:1633-1645, 2013.

Peakall R, Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537-2539.

Perry MC, Mcintosh MS (1991). Geographical patterns of variation in the USDA soybean germplasm collection: I. Morphological traits. Crop. Sci. 31(5):1350-1355.

Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Rossato M, Barbieri R L, Aloiss Chäfer A, Zacaria J (2007). Caracterização molecular de populações de palmeiras do gênero *Butia* do rio grande do sul através de marcadores ISSR Magistra 19(4):311-318.

Rossetto M, Jones R, Hunter J (2004). Genetic effects of rainforest fragmentation in an early successional tree (*Elaeocarpus grandis*). Heredity 93: 610-618.

Sano EE, Rosa R, Brito JLS, Ferreira LG (2010). Land cover mappingof the tropical savanna region in Brazil. Environ. Monit. Assess 166: 113-124.

Shao JW, Chen W L, Peng YQ, Zhu GP, Zhang X (2009). Genetic diversity within and among populations of the endangered and endemic species *Primula merrilliana* in China. Bioch. Syst. Ecol 37: 699-706.

Shapcott A, Dowe JL, Ford H (2009). Low genetic diversity and recovery implications of the vulnerable Bankoualé Palm *Livistona carinensis* (Arecaceae), from North-eastern Africa and Southern Arabian Peninsula. Conserv Genet 10: 317-327.

Shapcott A, Quinn J, Rakotoarinivo M, Dransfield J (2012). Contrasting patterns of genetic diversity between two endangered palms with overlapping distributions, *Voanioala gerardii* (Arecoideae) and *Lemurophoenix halleuxii* (Arecoideae), from North-east Madagascar. Conserv. Genet. 13:1393-1408.

Silva JMC, Bates JM (2002). Biogeographic patterns and conservation in the South American Cerrado: a tropical savanna hotspot. BioSci. 52:225-233.

Silva MS, Vieira FA, Carvalho D (2011). Diversity and genetic in natural populations of *Geonoma schottiana* Mart (Arecaceae): implications for conservation. Cerne 17(2): 195-201.

Sreekumar VB, Renuka C (2006). Assessment of genetic diversity in *Calamus thwaitesii* BECC. (Arecaceae) using RAPD markers. Bioch. Syst. Ecol. 34: 397-405.

Srivashtav VS, Kapadia CV, Mahatma MK, Jha SK, Ahmad T (2013). Genetic diversity analysis of date palm (*Phoenix dactylifera* L.) in the Kutch region of India using RAPD and ISSR markers. Emir. J. Food Agric. 25(11):907-915.

Young A, Boyle T, Brown T (1996). The population genetic consequences of habitat fragmentation for plants. Trends Ecol. Evol. 11:413-418.

Zehdi S, Trifi M, Billotte N, Marrakchi M, Pintaud JC (2004). Genetic diversity of Tunisian date palms (*Phoenix dactylifera* L.) revealed by nuclear microsatellite polymorphism. Hereditas 141:278-287.