



Genetic divergence among populations and accessions of the spineless peach palm from Pampa Hermosa landrace used in the heart-of-palm agribusiness in Brazil

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Abstract

Although originally domesticated for its fruit, exploitation of the peach palm (*Bactris gasipaes* Kunth) in the production of gourmet heart-of-palm has also become an important activity, hence the need for improved material for large-scale production, on employing the Pampa Hermosa landrace as the seed source. In this study 11 microsatellite markers were used to evaluate genetic divergence among 96 elite plants representing four populations of spineless peach palm from the above cited source. Genetic variability was high ($H_t = 0.82$). The low levels of divergence [F_{ST} (0.023), G_{ST}' (0.005)] and the high number of migrants (Nm - 3.8 to 52.2) indicated significant interpopulation gene flow. Some of the plants presented high levels of genetic divergence, but the plants were grouped independently of their geographic origins. When combined with morpho-agronomic evaluation, the results found could substantially contribute towards mounting an efficient tool for obtaining superior genotypes with wide genetic variability for improvement programs.

Key words: *Bactris gasipaes*, heart-of-palm, microsatellites, genetic divergence, genetic improvement.

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The cultivated peach palm (*Bactris gasipaes* Kunth var. *gasipaes*) is the only domesticated palm in the Neotropics (Clement, 1988; Mora Urpí *et al.*, 1997). Although initially domesticated for its fruit, it has currently attained greater importance in the production of gourmet quality heart-of-palm, of considerable economic impact in Brazil, Costa Rica and Ecuador (Clement, 2008). The peach palm has advantages when compared to other sources of heart-of-palm, such as *açaí* (*Euterpe oleracea*) and *juçara* (*Euterpe edulis*), notably faster growth and higher yields per hectare (Bovi, 1997), as well as better adaptability to modern agricultural systems (Mora Urpí *et al.*, 1997).

Given the outstanding expansion of the market, attention is now directed to selecting vigorous plants presenting higher yields (Kalil Filho *et al.*, 2010). Currently, the characters aimed for in improvement programs are spineless stems and leaf petioles, quick growth, more than four offshoots and high frequency of hearts-of-palm over 45 cm long (Yuyama *et al.*, 2002). An intra-population recurrent selection strategy has been proposed as the most efficient for

long-term gain (Clement *et al.*, 2009; Kalil Filho *et al.*, 2010), as against the inter-population recurrent selection strategy used for African oil palms (Cunha *et al.*, 2009). This is justified by best genetic resources coming from a single landrace, the Pampa Hermosa (Mora Urpí *et al.*, 1999).

Most of the seeds used in the expansion of the Brazilian agribusiness and improvement programs are from the Pampa Hermosa landrace, which occurs in the outskirts of the town of Yurimáguas, Loreto, Peru. This peach palm landrace is ideal for the production of heart-of-palm, through presenting high frequencies of spineless palms (60% to 80%) and higher yields than other landraces (Mora Urpí *et al.*, 1999).

The Instituto Nacional de Pesquisas da Amazônia (INPA) has a collection of peach palms from the Pampa Hermosa landrace compiling a progeny trial in support of an ongoing improvement program (Yuyama *et al.*, 2002). The germplasm was collected from the local market at the Yurimáguas, as well as from traditionally cultivated populations in nearby communities along the Cuiparillo, Parana-pura and Shanusi Rivers. Preliminary morphometric evaluation indicated differences in production components among the populations, with greater vigor in plants from the Shanusi River, and longer heart-of-palms in plants from

the Paranapura River (Yuyama *et al.*, 2002). Heart-of-palm length, as a basis, has led to the selection of three categories of plants, viz., 45-54 cm; 54-63 cm; and >63 cm. However, as heritability in most of the pertinent production components is extremely low, response to selection is reduced (Farias Neto *et al.*, 2002) (Clement CR, 1995, PhD thesis University of Hawaii at Manoa, Honolulu, USA). Thus, the characterization of genetic variability and selection of elite plants through molecular markers constitute potential implements for improvement (Dawson and Jaenicke, 2006; Jamnadass *et al.*, 2009).

Microsatellites (SSR) are currently considered the most appropriate markers for population genetic studies, since they are randomly distributed in the eukaryote genome, highly polymorphic and co-dominant (Powell *et al.*, 1996). Currently, 46 SSR loci of peach palm are available (Martínez *et al.*, 2002; Billotte *et al.*, 2004; Rodrigues *et al.*, 2004), some of them have already been used in studies of genetic variability, and structure of wild and cultivated populations (Couvreur *et al.*, 2006; Cole *et al.*, 2007; Hernández Ugalde *et al.*, 2008). These SSR can generate estimates of genetic divergence among groups of germplasm accessions, which, combined with field evaluation, can provide a solid basis for the detection of heterotic groups, *i.e.*, elite progenies possibly presenting heterosis, higher vigor and fertility than inbred relatives (Simmonds and Smart, 1999; Reif *et al.*, 2003). Recognition of heterotic patterns among genetically divergent groups of accessions is fundamental in hybrid breeding for the maximum exploitation of this characteristic (Hallauer *et al.*, 1988).

The aim of this study was to evaluate genetic variability and the genetic structure of populations of spineless peach palms from the Pampa Hermosa landrace, as well as

genetic divergence among elite plants from this heart-of-palm progeny trial, by using microsatellite markers.

Meristems of lateral off-shots of selected plants were collected from 96 accessions (1 plant/accession) in the peach palm progeny trial located at the INPA Tropical Fruit Experimental Station (BR-174, km 40, Manaus, AM, Brazil). Their geographic origins were the Yurimáguas local market (n = 43), and the Paranapura (n = 25); Cuiparillo (n = 21) and Shanusi (n = 7) Rivers. Heart-of-palm lengths were >45 cm but <54 cm (n = 47), >54 cm but <63 cm (n = 45), and >63 cm (n = 4).

DNA extraction was according to the CTAB protocol (Doyle and Doyle, 1987). DNA was quantified by comparison with known concentrations of a standard DNA (Lambda DNA) in 0.9% agarose gel stained with ethidium bromide. Eleven SSR primer pairs developed for *B. gasipaes* (Martínez *et al.*, 2002; Billotte *et al.*, 2004; Rodrigues *et al.*, 2004) were chosen for this study (Table 1). The amplification and detection of polymorphisms were as described by Rodrigues *et al.* (2004).

The number of alleles (A), as well as expected (H_E) and observed (H_O) heterozygosities were calculated using ARLEQUIN v.3.11 (Excoffier *et al.*, 2005), and the number of private alleles (A_p) for each locus, with CONVERT (Glaubitz, 2004). The inbreeding coefficient (f), R_{ST} and G_{ST} were calculated with FSTAT v. 2.9.3.2 (Goudet, 2002). The populations' pairwise degrees of divergence (F_{ST}) and estimates of gene flow (Nm) [assuming $Nm = (1/F_{ST} - 1)/4$ and $M = 2Nm$], as well as hierarchical Analysis of Molecular Variance (AMOVA – Michalakis and Excoffier, 1996) were obtained with ARLEQUIN v. 3.11 (Excoffier *et al.*, 2005). Further estimates of genetic divergence among plants and among the populations and market samples were obtained by calculating the distance

Table 1 - Characteristics and estimates of genetic parameters of microsatellite loci for *Bactris gasipaes* var. *gasipaes* used in this study of 96 plants from the Pampa Hermosa progeny trial at INPA, Manaus, AM, Brazil. (A) number of alleles, (A_p) number of private alleles, observed (H_O) and expected (H_E) heterozygosities, (f) inbreeding coefficient. Ta = annealing temperature; bp = base pairs. Significant values ($p < 0.05$) are indicated with asterisks.

Locus	Ta	Allele size range (bp)	A	A_p	H_O	H_E	f
Bg6 ^a	50 °C	185-209	11	2	0.69	0.82	0.157*
Bg17 ^a	52 °C	228-252	13	1	0.79	0.85	0.080*
mBg10 ^b	52 °C	138-168	10	2	0.52	0.71	0.272*
mBg41 ^b	52 °C	140-178	14	3	0.47	0.85	0.432*
mBg57 ^b	52 °C	242-268	12	3	0.69	0.74	0.065
mBg62 ^b	52 °C	174-224	20	3	0.64	0.90	0.276*
mBg87 ^b	52 °C	153-179	13	0	0.47	0.87	0.454*
Bg02-4 ^c	64 °C	144-178	16	3	0.73	0.88	0.169*
Bg02-5 ^c	64 °C	183-225	14	4	0.76	0.80	0.055
Bg02-10 ^c	64 °C	150-164	8	0	0.79	0.78	0.025
Bg02-12 ^c	58 °C	152-180	13	1	0.79	0.82	0.026
All loci			144	22	0.67	0.82	0.182*

^aMartínez *et al.* 2002; ^bBillotte *et al.* 2004; ^cRodrigues *et al.* 2004.

of shared alleles (D_{AS} - Chakraborty and Jin, 1993) using POPULATIONS v. 1.2.31 (Langella, 1999). These were grouped by applying the Neighbor-Joining (NJ) method with MEGA v. 4.0 (Kumar *et al.*, 2004). To counteract effects of unbalanced sample sizes during the analysis, Factorial Analysis of Correspondence (FAC) was carried out with GENETIX v. 4.05 (Belkhir *et al.*, 2004), to thus select the seven most typical individuals from each population (except for Shanusi), whereat population sample sizes were equalized.

The number of alleles varied from 8 (Bg02-10) to 20 (mBg62), with an average of 13.1 alleles per locus. Considering the three populations and the market, the total number of alleles found was 144 (Table 1). Observed (H_O) and expected (H_E) heterozygosities were high for most of the loci (Table 1), with H_E higher than H_O in all but one locus (Bg02-10). The Hardy-Weinberg Equilibrium was not tested for two reasons: (1) the plants were not free from selective pressures, since they were selected for heart-of-palm length; (2) the plants were not sampled randomly, since sampling was directed towards selected spineless plants. Genetic diversity can be considered high, and was similar to that from other studies with cultivated peach palm (Adin *et al.*, 2004; Couvreur *et al.*, 2006; Cole *et al.*, 2007).

Nine of the 11 loci presented private alleles, the larger the sample, the more numerous (Table 2). In particular, the high number of private alleles found in the market samples was due to the ample geographic area sampled, viz., the Huallaga River and other regions surrounding Yurimáguas.

Table 2 - Estimates of genetic parameters found for three peach palm (*Bactris gasipaes* var. *gasipaes*) populations and the Yurimáguas Market in the Pampa Hermosa landrace using 11 microsatellite loci. (A) number of alleles, (Ap) number of private alleles, observed (H_O) and expected (H_E) heterozygosities, (f) inbreeding coefficient. n = number of individuals genotyped. Significant values ($p < 0.05$) are indicated with asterisks.

Population	A	Ap	H_O	H_E	f
Cuiparillo (n = 21)	109	4	0.69	0.82	0.156*
Paranapura (n = 25)	115	7	0.70	0.83	0.160*
Shanusi (n = 7)	62	1	0.64	0.79	0.210*
Market (n = 43)	121	10	0.64	0.81	0.204*
Pampa Hermosa (n = 96)	144		0.67	0.82	0.182*

Table 3 - Genetic divergence (F_{ST} - below diagonal) and gene flow (Nm - above diagonal) among 96 peach palm (*Bactris gasipaes* var. *gasipaes*) samples from three populations and the market of Yurimáguas, estimated with 11 microsatellite loci. The values to the left of the dash represent the estimates for the full data set, those to the right the FAC-selected balanced data set. Significant values ($p < 0.05$) are indicated with asterisks.

	Cuiparillo	Paranapura	Shanusi	Market
Cuiparillo	-	Infinite / Infinite	11.4 / 11.8	38.6 / 52.2
Paranapura	-0.0003 / -0.006	-	7.1 / 3.8	52.2 / 12.8
Shanusi	0.021 / 0.020	0.034* / 0.061*	-	8.2 / 6.1
Market	0.006 / 0.004	0.004 / 0.019	0.029* / 0.039	-

Inbreeding coefficients were significantly different from zero ($p < 0.05$) for seven loci (Table 1), and for the set of populations (Table 2). This was to be expected, since the progenies displayed significant biparental inbreeding (Picanço-Rodrigues R, 2007, Doctoral thesis, Universidade Federal do Amazonas, Manaus, AM), as a result of smallholders' practice of growing half or full sibs (Clement, 1988; Cole *et al.*, 2007). Selection undertaken by Amerindians during the domestication process, and later by the breeding program, gives to understand that heterozygosity has decreased over time, notable by the inbreeding coefficient.

AMOVA revealed 99.1% of total genetic variance among plants within the populations and the market, and only 0.9% among the populations and the market, thus in accordance with the low indices of genetic structure [F_{ST} (0.009), G_{ST} ' (0.011) and R_{ST} (0,001)]. However, due to possible over- or underestimation through unbalanced sample numbers, plants that were the most typical in each population were identified by FAC. This was followed by further AMOVA, whereby 97.7% of total intrapopulation genetic variance was found, and 2.3% interpopulation, thus still in agreement with the low indices of genetic structure [F_{ST} (0.023), G_{ST} ' (0.005) and R_{ST} (-0.002)].

Although interpopulation pairwise genetic divergences (F_{ST}) were low, they were significantly different from zero ($p < 0.05$) between Shanusi and Paranapura, and between Shanusi and the market (Table 3). Since unbalanced sample sizes could have affected the analysis, estimations of F_{ST} were recalculated with the balanced database. Again, estimates of F_{ST} were low, although this time only significant between Shanusi and Paranapura (Table 3). The high number of migrants (Nm) may explain the very low genetic structuring in both analyses. This is probably so because most of the gene flow detected among populations is the result of human action (Adin *et al.*, 2004). Smallholders in the Yurimáguas region grow numerous selections, besides commonly exchanging seeds with other local smallholders, and acquiring new seeds from local markets (Adin *et al.*, 2004; Cole *et al.*, 2007). This, besides helping to maintain variability within the properties and contributing to gene flow, even between distant localities (up to 600 km; Cole *et al.*, 2007), counteracts the reduction of variability caused by human selection and genetic drift.

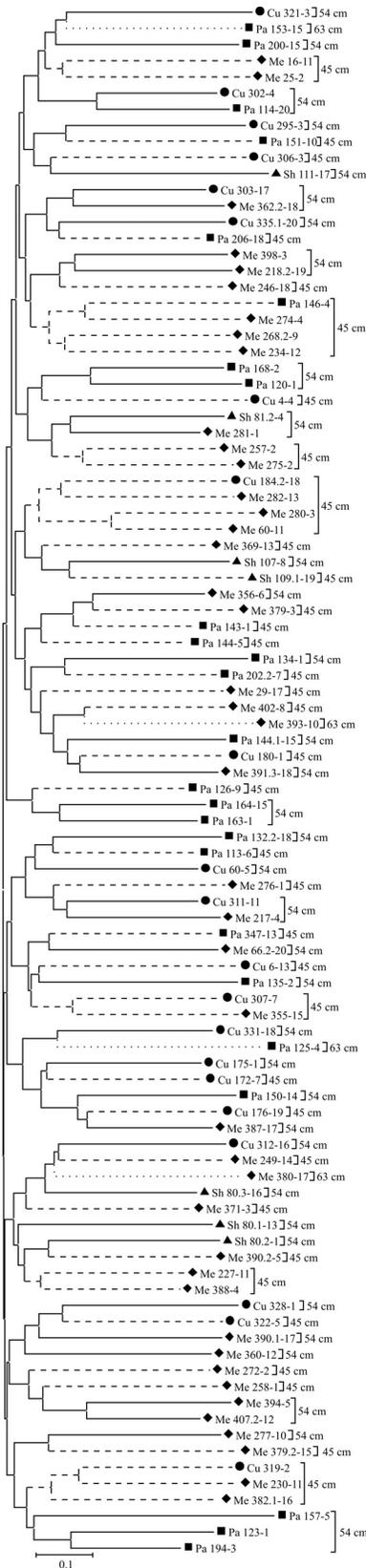


Figure 1 - Neighbor-Joining dendrogram based on genetic distances (D_{AS}) showing the genetic relationships among 96 selected peach palm plants (*Bactris gasipaes* var. *gasipaes*) from the Pampa Hermosa landrace (Yurimáguas, Peru), maintained in the INPA progeny trial. The type of line corresponds to plants with different heart-of-palm lengths: 45-54 cm (dashed); 54-63 cm (solid); and >63 cm (dotted). Symbols correspond to the different populations: Paranapura (squares); Cuiparillo (circles); Shanusi (triangles) and the market of Yurimáguas (diamonds).

The distance of shared alleles (D_{AS}), considered appropriate for recently diverged populations (Goldstein and Pollock, 1997), as those studied here, ranged from 0.44 to 1.0. Some pairwise D_{AS} were extremely high (1.0), as were around 41% of the distances (0.71-0.96), thereby confirming the high genetic variability and genetic divergence among many of the plants in this progeny trial.

Based on D_{AS} , the NJ dendrogram revealed more similarity between Cuiparillo and Paranapura on the one hand, and Shanusi and the market on the other, thereby implying that the four, though weakly structured, present a certain degree of divergence, notable through the former two clustering separately from the later two (data not shown). Neighbor-Joining analysis of the set of plants based on D_{AS} showed most of the individuals to be clustered independent of geographical origin and heart-of-palm length (Figure 1). This pattern corroborates both the low levels of genetic structure and the high divergence ultimately encountered.

The microsatellites used in this study revealed that, despite the form of selection, the plants still maintained high genetic variability. Low levels of genetic differentiation among populations and market were found, probably due to human action in the exchange of germplasm among different localities (seed flow). This suggests that the first steps in an intrapopulation recurrent selection improvement program of the peach palm must be the creation of heterotic groups within the Pampa Hermosa landrace. Although there was no grouping by geographic origin within the landrace, geography can still be considered as a basis for creating heterotic groups, since through morphometric evaluation it was possible to identify specific population tendencies as to vigor and heart-of-palm length (Yuyama *et al.*, 2002). On the other hand, by grouping plants with long hearts versus plants with rapid growth rates it may be possible to create the desired heterotic groups. Further studies with a larger number of SSR loci, covering the entire progeny trail, will improve genetic information on creating either of the two grouping strategies. In association with information on the components of heart-of-palm production and growth analysis, these data will certainly be useful for planning crossing within and among groups, as a mean of reaching the main goals of the peach palm improvement program, viz., genetic gains and the maintenance of genetic variability.

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