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 (HT = 0.82). The low levels of divergence [FST (0.023), GST' (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 Urpi 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 açai (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 Urpi 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 off-shots 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 Urpi 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 Yurimaguas, 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 Urpi 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 Yurimaguas, as well as from traditionally cultivated populations in nearby communities along the Cuiparillo, Parapura 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 lead 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 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 (Martinez 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; Hernandez 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 (Hallaer 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 accesses (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 Yurimaguas 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 (Martinez 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 (Ap) for each locus, with CONVERT (Glaubitz, 2004). The inbreeding coefficient (f), RST and GST were calculated with FSTAT v. 2.9.3.2 (Goudet, 2002). The populations’ pairwise degrees of divergence (FST) and estimates of gene flow (Nm) [assuming Nm = (1/FST - 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

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

*Martinez et al. 2002; Billotte et al. 2004; Rodrigues 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 (considering the three populations and the market, the total (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 Yurimagus.

Table 2 - Estimates of genetic parameters found for three peach palm (Bactris gasipaes var. gasipaes) populations and the Yurimagus 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* |

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, estimates 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 Yurimagus 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.
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 latter 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 trial, 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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