



## Genetic variability in the peach palm genebank with RAPD markers

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**ABSTRACT** - Peach palm is a native Amazonian fruit species with broad genetic diversity in its wild and domesticated populations. This study completed the molecular characterization and genetic analysis of the accessions in the Peach Palm genebank, Brazil, using RAPD markers. Eight primers generated 132 markers, of which 128 were polymorphic. Average heterozygosity was 0.35, with 95.5% polymorphism. The genetic variability within the landraces ( $H_S$ ) was 0.32, while genetic divergence ( $G_{ST}$ ) was 0.09 among the four well-represented landraces. The average gene flow was 5.0, with high gene flow between the Solimões and Pampa Hermosa ( $N_m=10.07$ ), and between the Putumayo and Solimões ( $N_m=10.73$ ) landraces, all western landraces. The dendrograms of the Nei's and Rogers' genetic distances of the well-represented landraces presented similar groupings to previous analyses, with the Solimões, Putumayo and Pampa Hermosa landraces grouped together in western Amazonia, and distant from the Pará landrace in central and eastern Amazonia.

**Key words:** *Bactris gasipaes*, genetic distances, genetic relations, landraces, wild populations.

### INTRODUCTION

The peach palm (*Bactris gasipaes* Kunth, Palmae) is a native Amazonian fruit species with broad genetic diversity in its wild and domesticated populations, because of their different degrees of domestication and associated human preferences in terms of taste, use, processing and even color (Clement 1988, 1995, Mora Urpi et al. 1997). Peach palm populations are grouped in two botanical varieties (Henderson 2000): the var. *gasipaes* includes all the domesticated populations with large fruits (10 a 200 g) and the var. *chichagui* includes all the wild populations with small fruits (1 to 10 g). In the var. *gasipaes*, the populations are organized in landraces and modern hybrid populations (Mora Urpi et al. 1997).

During the last three decades, various Latin American institutions have created programs to transform peach palm from a minor fruit crop into an important source of income for farmers, with mixed success in terms of fruit (Clement et al. 2004), but considerable success in developing a viable agroindustry for hearts-of-palm (Clement 2008). All of these programs organized germplasm collections to support crop improvement efforts. The two largest collections are at the Universidad de Costa Rica, in association with the Ministry of Agriculture of Costa Rica, and at the National Research Institute for Amazonia (INPA), in association with the Embrapa network for genetic resources (Mora Urpi et al. 1997). INPA's Peach Palm genebank contains 390 accessions, down from 450 due to senescence of non-caespitose plants and natural accidents. The genebank

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contains good representation of four landraces and insufficient representation of most others (Clement et al. 2001), but with enough representation to allow analysis of peach palm genetic variability and how it is structured within and among landraces.

Previous studies with molecular markers evaluated the genetic variability of peach palm landraces, as well as gene flow among them, to determine the genetic validity of landraces defined with morphological criteria. Using RAPD markers, the first genetic analysis suggested that the Pará and Putumayo landraces are valid, while the Solimões landrace is part of the Putumayo landrace (Sousa et al. 2001). Another analysis validated the existence of four landraces (Pará, Pampa Hermosa, Putumayo and Utilis) of the seven examined, and suggested that the other three races (Solimões, Tuira and Guatuso) should be considered populations of two of the validated races (Rodrigues et al. 2004). A morphometric analysis suggested that the Solimões landrace should be maintained, but with a smaller geographic distribution than originally proposed (Martel et al. 2003). (CC Silva pers. com. 2004) confirmed the validations of Rodrigues et al. (2004) and the separation of the Pará landrace from the others. The valid Juruá landrace grouped with the three western landraces (Putumayo, Utilis and Pampa Hermosa), suggesting that their genetic similarity is due to the common origin of all the western landraces. The other landraces (Vaupés, Cauca and Inirida) were represented by very small numbers of individuals, not allowing a more precise analysis, but always showing a relationship with the western landraces (CC Silva pers. com. 2004). Recently, four hybrid populations (Belém, Manaus, Iquitos, Yurimaguas) were characterized; these hybrid populations do not have higher genetic variability than the landraces that surround them (RP Santos, pers. com. 2009), contrary to the hypothesis of Mora Urpí and Clement (1988).

(CC Silva pers. com. 2003) determined that three populations around Yurimaguas, Peru, are not different from the Pampa Hermosa landrace (also adjacent to Yurimaguas), contrary to the ideas of Mora-Urpí and Clement (1988), who had suggested the existence of other landraces based on the morphometric variability observed in the Yurimaguas hybrid population. Using AFLP, Adin et al. (2004) compared the genetic diversity between domesticated populations along the Parapapura and Cuiparillo Rivers (Yurimaguas, Peru), and observed little divergence and high gene flow, supporting the hypothesis that they are populations of the Pampa Hermosa landrace.

Although the majority of the landrace and hybrid population accessions in the BAG had been examined with molecular markers, several cultivated and wild populations had not yet been studied. The objective of this study was to complete the genetic characterization of the accessions present in the BAG, using RAPD markers. Although RAPD markers have reproducibility problems (Ferreira and Grattapaglia 1998), these markers do not adversely affect cluster analyses to identify major groups using genetic distances (Teixeira-Cabral et al. 2002), and continue to be used because most of the fragments are identical at the intra-specific level and the estimates of genetic diversity within and between populations are very similar when compared to other dominant markers (AFLP and ISSR) (Nybohm 2004). Thus, the continuity of their use allows the consistency of analysis needed to understand the genetic resources of peach palm maintained in *ex situ* collections.

## MATERIAL AND METHODS

### Material and DNA extraction

To finalize the characterization of the accessions of the Peach Palm Active Germplasm Bank, maintained by INPA at its Fruit Crop Experiment Station (BR 174, km 38, Manaus, Amazonas, Brazil), 314 plants of the var. *gasipaes* [Putumayo landrace (n = 68); Utilis (n = 2); Pará (n = 15); Pastaza (n = 1); Solimões (n = 62); Pampa Hermosa (n = 153); populations not yet designated to landraces - Pucallpa (n = 6), Contamana (n = 1) {analyzed jointly as Ucayali River}, Puerto Maldonado (n = 4), Plácido de Castro (n = 2) {analyzed jointly as Upper Madeira River}] and four plants of the var. *chichagui* type 3 from the Ucayali River, Peru, were sampled.

DNA was extracted using the CTAB method (Doyle and Doyle 1987), with modifications, from 100 mg of the apical meristem of a lateral offshoot or from 200 mg of leaves, which were macerated in liquid nitrogen (Weising et al. 1995).

### RAPD Technique

The RAPDs were obtained following Williams et al. (1990), with modifications of Rodrigues et al. (2004). The reaction amplifications contained 10 ng genomic DNA (2.5 ng  $\mu\text{L}^{-1}$ ), 250  $\mu\text{M}$  of dNTP (2.5 mM), 3 mM of  $\text{MgCl}_2$  (25 mM), 50 ng of primer (10 ng  $\mu\text{L}^{-1}$ ), 1.5 U of Taq

polimerase (CENBIOT/RS) and 3µL de Buffer 10X (CENBIOT/RS) and was completed to a final volume of 30 µl with distilled water. The primers were the same utilized by Rodrigues et al. (2004).

The samples were amplified in a thermocycler (Eppendorf) with two programs optimized for the primers. Program 1 (primer F919-3): 2 min at 94 °C, 40 cycles of 1 min at 92 °C, 1 min at 36 °C and 2 min at 72 °C, plus a final cycle of 3 min at 72 °C. Program 2 (other primers): 2 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C, and 33 cycles of 10 sec at 94 °C, 20 sec at 40 °C, 2 min at 72 °C, plus a final cycle of 5 min at 72 °C.

Polymorphic and monomorphic bands were classified as intense (1), moderate (2), weak (3) or absent (0), based on resolution and degree of amplification for visual interpretation, as described by Grattapaglia (1997). To evaluate the reliability of the interpretations, Dice similarities of the control plants were estimated for different combinations of intensity: 1 versus 2 = 0,3 = 0,0; 1,2 = 1 versus 3 = 0,0; 1,2 = 1,3 = 1 versus 0. The combination with greatest similarity among the control repetitions was used (Grattapaglia 1997, Rodrigues et al. 2004).

### Genetic and statistical analyses

In this study, we used principal components analysis (PCA) to identify a set of 30 plants that represented the central tendency of the over-represented landraces (Pampa Hermosa, Putumayo, Solimões) so as to avoid statistical bias by over-representation in the other analyses. PCA is designed to reduce the number of variables that need to be considered to a small number of indices (principal components) that are linear combinations of the original variables (Moreira et al. 1994). The 30 individuals grouped in the center of the two-dimensional PCA graph of each of these landraces were used in subsequent analyses.

The binary matrix was used to estimate each landrace's heterozygosity [assuming absence as recessive (Weir 1996)], using Nei (1972)'s criterion, and the percentage of polymorphism, using the TFPGA program (Miller 1997). Genetic diversity parameters ( $H_T$ ,  $H_S$  e  $G_{ST}$ ) were estimated using Hartl and Clark (1989)'s criterion, and gene flow was calculated from estimates of  $G_{ST}$  [ $Nm = 0.5(1-G_{ST})/G_{ST}$ ] (Slatkin and Barton 1989), using the POPGEN v. 1.31 program (Yeh et al. 1999). A Dice similarity matrix was generated among the plants, using the NTSYS-pc program (Rohlf 1990). The genetic relations among the populations were evaluated using Nei's (1972) distance and a UPGMA dendrogram was generated, using the

TFPGA program (Miller 1997). The Exact Test (Raymond and Rousset 1995) was used to determine significant differences in allele frequencies between landraces, using the TFPGA program (Miller 1997). A dissimilarity coefficient, Roger's distance (Rogers 1972), was used because it is appropriate for examining the relationships among populations that will contribute to a core collection (Reif et al. 2005), the final objective of this series of analyses in the peach palm genebank.

### RESULTS AND DISCUSSION

The eight primers amplified 132 useful markers, of which 124 were polymorphic, higher than the polymorphism obtained by Silva (pers. com., 2004) (101 polymorphic and 23 monomorphic), Rodrigues et al. (2004) (97 and 16, respectively) and Santos (pers. com., 2009) (88 and 11, respectively) with the same primers. The number of polymorphic bands ranged from 12 for primer F-919-3 to 20 for FC-13. Within of the landraces it ranged from 6 (F-919-3 in Pastaza, and OPA-5 in Utilis and Pastaza) to 20 (FC-13 in Putumayo), similar to the variability observed by Silva (pers. com., 2004) and Rodrigues et al. (2004).

The repeatability test found a Dice similarity of 0.86 among the control samples, using bands of all intensities (1=2=3 versus 0), a little less than the repeatabilities obtained by Rodrigues et al. (2004) and Silva (pers. com., 2004) (0.95 and 0.96, respectively), but reasonably trustworthy for this set of data.

The overall mean estimated heterozygosity was 0.35 and the polymorphism was 95.5% (Table 1), similar to the estimates of Silva (pers. com., 2004) (0.38 and 93% polymorphism) and Rodrigues et al. (2004) (0.31 and 89.4%). It is important to observe that this estimate of heterozygosity obtained with RAPD markers may be an overestimation due to estimates based on the frequency of the null homozygote (Weir 1996). In general, heterozygosity did not vary much among the well-represented landraces, although the Pará landrace was lower than the others, certainly due to low representation. The Pampa Hermosa landrace presented high polymorphism, although slightly less than the Putumayo landrace, contrary to Silva (pers. com., 2004) and Rodrigues et al. (2004), who observed higher polymorphism in the Pampa Hermosa landrace.

Using only the well-represented landraces (Pampa Hermosa, Putumayo, Solimões and Pará), overall diversity ( $H_T$ ) was 0.35, diversity within the landraces ( $H_S$ ) was 0.32 and diversity among the landraces ( $G_{ST}$ ) was 0.09,

**Table 1.** Genetic parameters of four peach palm (*Bactris gasipaes* var. *gasipaes*) landrace samples, of two synthetic non-designated populations and one wild population maintained in the peach palm genebank

Landraces <sup>1</sup>	N	Heterozygosity	Polymorphism	
			95%	99%
Putumayo	30	0.33	84.5	89.4
Pampa Hermosa	30	0.33	85.6	86.4
Solimões	30	0.30	81.8	86.4
Pará	15	0.27	70.5	75.0
<b>Non-designated populations</b>				
Ucayali River	7	0.25	66.0	66.0
Upper Madeira River	6	0.22	58.3	58.3
<b>Wild population</b>				
var. <i>chichagui</i> type 3	4	0.17	43.2	43.2
<b>Total</b>	<b>122</b>	<b>0.35</b>	<b>93.9</b>	<b>95.5</b>

<sup>1</sup> After the selection of 30 plants with PCA. The Utilis and Pastaza landraces are not included due to very small numbers.

suggesting close relationships. Slightly lower within and higher among landrace estimates ( $H_S = 0.25$  e  $G_{ST} = 0.16$ ) were found by Rodrigues et al. (2004) with the same landraces, although their analysis included the Utilis landrace and two other var. *chichagui* populations, the latter probably responsible for the higher  $G_{ST}$ .

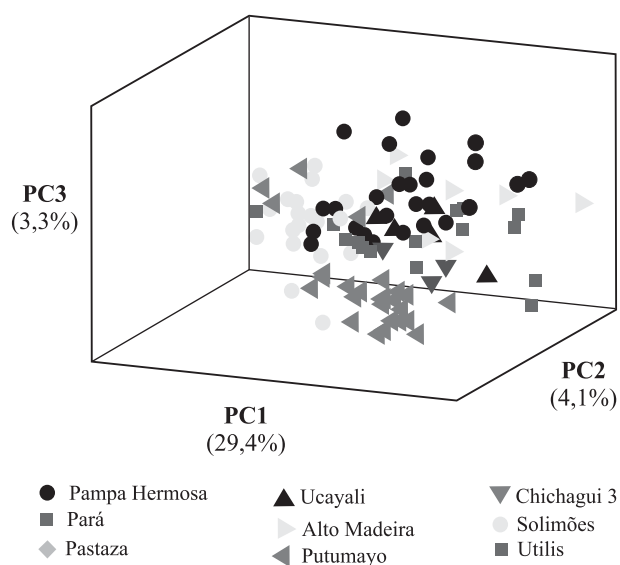
The dendrogram generated from the Dice similarities among individual plants contained 15 groups defined by similarity  $\geq 0.68$  (Table 2), with seven groups with predominance of one landrace and eight groups with no predominance of a landrace. Group two contained more than half the plants in the matrix, all from western Amazonian and the Central American landraces, confirming the affinities of these landraces. The landraces with small numbers of plants appeared in different groups. The Pastaza landrace grouped with most of the plants of the wild population. In contrast, the Utilis landrace grouped with the Putumayo landrace, as expected in other studies of this landrace (Rodrigues et al. 2004, Silva, pers. com., 2004).

The principal components analysis showed that molecular variation was not very widely dispersed in multivariate space, with the three principal components explaining 36.8% of the variation (Figure 1). As with the Dice similarities a large group occupied the center (mostly group 2 in Table 2), different from the PCA of Rodrigues et al. (2004) where four groups were clearly observed. Some of the Pará plants were dispersed much too widely, suggesting possible problems in accession identification, as observed by Sousa et al. (2001). The var. *chichagui* plants are dispersed within the cultivated cluster, rather than above the cultivated cluster (Rodrigues et al. 2004),

**Table 2.** Groups of the Dice similarity coefficients among plants based on a cut-off at 0.68, with the number of plants (n) in the group, the minimum Dice similarity (Sim), and the number of plants in the landraces<sup>1</sup>, non-designated populations<sup>2</sup> and the wild population<sup>3</sup> maintained in the peach palm genebank

Group	N	Sim	Put	PH	Pará	Sol	Past	Utilis	chi 3	Ucay	Mad
1	5	0.70	5	-	-	-	-	-	-	-	-
2	67	0.73	20	17	-	24	-	2	-	4	-
3	5	0.73	-	1	-	4	-	-	-	-	-
4	8	0.73	1	7	-	-	-	-	-	-	-
5	1	0.70	-	1	-	-	-	-	-	-	-
6	2	0.74	1	-	-	-	-	-	-	1	-
7	15	0.73	1	-	14	-	-	-	-	-	-
8	2	0.74	-	-	1	-	-	-	1	-	-
9	8	0.70	-	-	-	-	-	-	-	2	6
10	1	0.68	-	-	-	1	-	-	-	-	-
11	4	0.77	-	-	-	-	1	-	3	-	-
12	2	0.76	-	2	-	-	-	-	-	-	-
13	2	0.70	-	2	-	-	-	-	-	-	-
14	2	0.78	2	-	-	-	-	-	-	-	-
15	1	0.68	-	-	-	1	-	-	-	-	-
<b>Total</b>	<b>125</b>		<b>30</b>	<b>30</b>	<b>15</b>	<b>30</b>	<b>1</b>	<b>2</b>	<b>4</b>	<b>7</b>	<b>6</b>

<sup>1</sup> Put = Putumayo, PH = Pampa Hermosa, Sol = Solimões, Past = Pastaza, Pará and Utilis; <sup>2</sup> Ucay = Ucayali River and Mad = upper Madeira River; <sup>3</sup> chi 3 = *chichagui* type 3.



**Figure 1.** Principal components analysis (PCA) showing the three-dimensional distribution of plants of the cultivated and wild populations from the peach palm genebank.

suggesting introgression, as observed by Couvreur et al. (2006).

The Pará landrace was different from all the others ( $p = 0.00$ ) by the Exact Test (Raymond and Rousset 1995), even with the relatively small sample size. Putumayo and Solimões were also different ( $p = 0.00$ ), contrary to Rodrigues et al. (2004) ( $p = 0.09$ ). It is possible that this

**Table 3.** Gene flow (Nm) estimated by using RAPD markers among landraces<sup>1</sup>, non-designated populations<sup>2</sup> and the wild population<sup>3</sup> maintained in the peach palm genebank

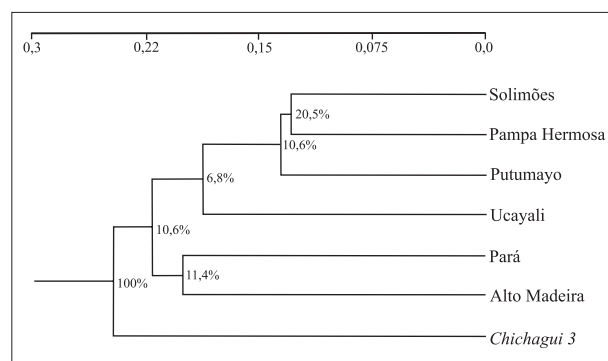
Landraces/ Populations	Put	Utilis	Pará	Chi. 3	Pastaza	Sol	PH	Ucayali
Utilis	1.66	-						
Pará	4.36	0.95	-					
<i>chichagui</i> 3	2.20	0.44	2.36	-				
Pastaza	0.92	0.10	0.78	0.59	-			
Solimões	10.73	1	4.48	2.16	0.84	-		
Pampa Hermosa	9.66	1.07	4.29	2.04	0.82	10.07	-	
Ucayali	4.40	0.75	2.53	1.47	0.5	4.12	5.21	-
Madeira	2.64	0.57	2.89	1.34	0.45	2.74	3.03	2.45

<sup>1</sup> Put = Putumayo, PH = Pampa Hermosa, Sol = Solimões, Past = Pastaza, Pará and Utilis; <sup>2</sup> Ucac = Ucayali River and Mad = upper Madeira River; <sup>3</sup> *chi* 3 = *chichagui* type 3.

difference is related to the localization of the samples of the Solimões landrace used in this study (chosen with PCA), which are geographically more distant from the Putumayo landrace (also chosen with PCA). This appears to support Martel et al. (2003)'s hypothesis of a smaller Solimões landrace in the lower-middle Solimões River. Pampa Hermosa was also different from all other landraces, as in previous studies (Rodrigues et al. 2004).

The average gene flow (Table 3) was 4.1 between the well-represented landraces (Putumayo, Pampa Hermosa, Solimões and Pará). The gene flow between the Pampa Hermosa and Solimões landraces, and between Putumayo and Solimões was high. The high gene flow between Putumayo and Solimões helps explain the dendrogram of Roger's distances (see below) and strengthens the hypothesis of Sousa et al. (2001) and Rodrigues et al. (2004) that only a single landrace exists along the Solimões River, contrary to Martel et al. (2003)'s hypothesis. Putumayo and Pampa Hermosa also presented high gene flow, as observed by Rodrigues et al. (2004) and Adin et al. (2004). The Pará landrace presented similar gene flow to the Solimões and Putumayo landrace, but reduced flow with other western landraces and the non-designated and wild populations.

The dendrogram of the landraces and populations based on Rogers' distance (1972) and that based on Nei's genetic distance (1972) were very similar, so only that based on Rogers' distance is presented (Figure 2). Rogers' distance (1972) is considered more appropriate to study relations among populations when the objective is the creation of a core collection (Reif et al. 2005), which is underway within the Peach Palm Active genebank (Clement et al. 2009). These dendrograms contain some consistent relations (when the number of plants was acceptable) and

**Figure 2.** UPMGA dendrogram of Rogers' (1972) distances among the landraces and cultivated non-designated populations and the var. *chichagui* type 3 wild population maintained in the peach palm genebank.

some inconsistencies (probably due to small numbers of plants). The general structure of the consistent part (Solimões, Pampa Hermosa, Putumayo, Pará) is similar to that observed by Silva (pers. com., 2004) and Rodrigues et al. (2004). As in other analyses, the relationship between the Pará landrace and the western Amazonian landraces is more distant. The Upper Madeira River synthetic population grouped with the Pará landrace, suggesting that it may be more primitive but closely related, and the Ucayali River population is associated with the set of Western Amazonia, as expected from their geographic location, although these relations need to be dealt with cautiously due to the reduced number individuals in these populations. Interestingly, the Rogers' distance dendrogram showed a closer relationship of the Pará landrace and the Upper Madeira River synthetic population, while showing a similar relationship of the Ucayali River population to the other western landraces (Figure 2). The position of the Ucayali River and Upper Madeira River populations is consistent with the proposal of a single origin of peach palm in southwestern Amazonia, as suggested by Rodrigues et al. (2004).

The two analyses of genetic distance were similar, and confirm the difference between the Pará landrace and the landraces of western Amazonia. Although some landraces and populations have small numbers of plants in the genebank and their genetic parameters were estimated with low precision, the information generated here will be useful to design the Core Collection within the peach palm genebank.



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# Variabilidade genética no germoplasma de pupunha analisada com marcadores RAPD

**RESUMO** - A pupunheira é uma fruteira nativa da Amazônia com ampla diversidade genética em suas populações silvestres e domesticadas. Este estudo finalizou a caracterização e análise genética dos acessos do germoplasma, usando marcadores moleculares RAPDs. Oito iniciadores geraram 132 marcadores, com 128 polimórficos. A heterozigosidade média foi 0,35 com 95,5% de polimorfismo, similares a outros estudos. A variabilidade dentro (Hs) das raças bem representadas foi 0,32, enquanto a divergência genética ( $G_{ST}$ ) foi 0,09 entre as quatro raças bem representadas. O fluxo gênico médio foi 5,0, com alto fluxo gênico entre as raças Solimões e Pampa Hermosa ( $N_m=10,07$ ), e as raças Putumayo e Solimões ( $N_m=10,73$ ), sendo todas ocidentais. Os dendrogramas das distâncias de Nei e Rogers mostraram que raças bem representadas apresentaram grupos similares às análises anteriores, com as raças Solimões, Putumayo e Pampa Hermosa agrupadas no oeste da Amazônia, e distantes da raça Pará no centro e leste da Amazônia.

**Palavras-chave:** *Bactris gasipaes*, distância genética, relações genéticas, raças primitivas, populações silvestres.

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