

USE OF AFLPS TO DISTINGUISH LANDRACES OF PEJIBAYE (*Bactris gasipaes*) IN BRAZILIAN AMAZONIA

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ABSTRACT: Although the first inhabitants of western Amazonia domesticated pejibaye (*Bactris gasipaes* Kunth, Palmae) or peach palm for its fruits, today it is widely planted for its heart-of-palm. Like other domesticates, pejibaye presents a complex hierarchy of landraces developed before the conquest of the Americas. The existence of three landraces (Pará, Solimões, Putumayo) was proposed along the Amazonas and Solimões Rivers, Brazil, based on morphological characteristics. There are some questions remaining about the intermediate landrace being an artifact of the morphometric analysis. AFLPs were used to evaluate the relationships among samples of these putative landraces. DNA was extracted from 99 plants representing 13 populations maintained in the Pejibaye Germplasm Bank, Manaus, AM; six primer combinations generated 245 markers via PCR, which were scored in an ABI Prism 310 sequencer and analyzed with GeneScan Software; Jaccard similarities were estimated and a dendrogram was generated with UPGMA. Two groups of plants were observed in the dendrogram instead of three, and were similar at 0.795. Each group contained two subgroups, similar at 0.815. One group (n=41) contained 73% Pará landrace plants, with one subgroup (n=22) containing 91% Pará, and the other (n=19) containing 53% Pará. The other group (n=58) contained 53% Solimões and 40% Putumayo landrace plants, with one subgroup (n=21) containing 52% Solimões and 43% Putumayo, and the other (n=35) containing 57% Solimões and 37% Putumayo. The first group confirmed the Pará landrace. The second group suggested that the Solimões landrace does not exist, but that the Putumayo landrace extends along the Solimões River to Central Amazonia.

Key words: molecular markers, population differentiation, genetic resources, genetic analysis

USO DE AFLPS PARA DISCRIMINAR RAÇAS PRIMITIVAS DE PUPUNHA (*Bactris gasipaes*) NA AMAZÔNIA BRASILEIRA

RESUMO: Os primeiros povos da Amazônia ocidental domesticaram a pupunha (*Bactris gasipaes* Kunth, Palmae) por seu fruto, embora hoje seja muito plantada por seu palmito. Como outros cultivos domesticados, a pupunha apresenta uma hierarquia complexa de raças primitivas criadas antes da conquista das Américas. A existência de três raças (Pará, Solimões, Putumayo) foi proposta ao longo dos rios Amazonas e Solimões, Brasil, com base em características morfológicas. Algumas dúvidas existem sobre a raça intermediária, pois podia ser um artefato da análise morfométrica. AFLPs foram usados para avaliar as relações entre amostras destas raças hipotéticas. DNA foi extraído de 99 plantas representando 13 populações mantidas no Banco de Germoplasma de Pupunha, Manaus, AM; seis combinações de 'primers' geraram 245 marcadores via PCR, que foram codificados num sequenciador ABI Prism 310 e analisados com o programa GeneScan; similaridades de Jaccard foram estimadas e um dendrograma foi criado com UPGMA. Dois grupos de plantas foram observados no dendrograma, em lugar de três, com similaridade de 0,795. Cada grupo continha dois subgrupos, similares a 0,815. Um grupo (n=41) continha 73% de plantas da raça Pará, com um subgrupo (n=22) contendo 91% Pará e o outro (n=19) contendo 53% Pará. O outro grupo (n=58) continha 53% de plantas da raça Solimões e 40% da Putumayo, com um subgrupo (n=21) contendo 52% Solimões e 43% Putumayo, e o outro (n=35) contendo 57% Solimões e 37% Putumayo. O primeiro grupo confirmou a raça Pará, mas o segundo grupo sugeriu que a raça Solimões não existe; em lugar desta raça, a raça Putumayo se estende ao longo do rio Solimões até a Amazônia Central.

Palavras-chave: diferenciação populacional, recursos genéticos, marcadores moleculares, análise genética

INTRODUCTION

Pejibaye (*Bactris gasipaes* Kunth, Palmae) is widely distributed in the lowland humid Neotropics (Mora Urpí et al., 1997), and its cultivated populations

contain ample genetic diversity due to their different stages of domestication in different environments, as well as to pejibaye's mainly allogamous reproductive system. There is a complex hierarchy of landraces, that has been partially characterized morphologically and

mapped (Mora Urpí & Clement, 1988). These landraces were created by the first inhabitants of Amazonia over a period of thousands of years, starting from different genetic bases, resulting in each landrace being morphologically and chemically different, and having different yield potential (Mora Urpí et al., 1997). Each landrace is also expected to be genetically different.

Morphometric characterization, using a descriptor list designed for both *in situ* and *ex situ* use (Clement, 1986), allowed the classification of several Amazonian landraces of pejibaye (Mora Urpí & Clement, 1988). The landraces were grouped by fruit size into 'microcarpa', 'mesocarpa' and 'macrocarpa,' because fruit size is thought to reflect their degree of domestication. The Pará landrace (microcarpa) occurs along the Amazonas River, Brazil, and has small, oily and fibrous fruits, with numerous fruits/bunch (Clement, 1987). The Solimões landrace (mesocarpa) occurs along the lower and middle Solimões River, Brazil, has intermediate size fruits and bunches, and is one of the best for direct consumption because of its texture and moderate levels of carotene and oil, which appear to contribute to its agreeable flavor (*ibid.*). The Putumayo landrace (macrocarpa) occurs along the upper Solimões River, Brasil, and adjacent areas of Colombia and Peru, has large, starchy fruits and few fruits/bunch (*ibid.*), and is starting to supply raw material to the new agribusiness producing flour for human consumption, both in western Amazonia and in Acre state.

Based on this morphometric characterization, Clement (1986) evaluated the phenetic relationships among eight Amazonian landraces with discriminant analysis. Only the three landraces along the Solimões and Amazonas Rivers were not well discriminated, as demonstrated by the overlap of the standard error volumes around their centroids. This overlap suggested that there was significant introgression between the landraces (a logical assumption due to their linear distribution along these rivers) or that the Solimões landrace was an artifact of the morphometric analysis (since the discriminant analysis was based on the visual impressions of a group of researchers (Mora Urpí & Clement, 1988), rather than being based on an exploratory analysis, e.g., principal components). These doubts about the Solimões landrace can probably be resolved by molecular analysis.

Currently, molecular markers are important complementary tools for germplasm characterization (Ferreira & Grattapaglia, 1996) and, consequently, for identification of populations and landraces. Due to lack of environmental effects, molecular markers are more reliable across environments than morphological traits. The AFLP technique is based on PCR amplification of restriction fragments from digested total DNA. PCR primers partially complementary to the adapters and the restriction site are used to create multiple marker bands (Vos et al., 1995). AFLP markers have been found to be suitable for studies on genetic diversity (Arens et al., 1998; Rahman et al., 1998).

Sousa et al. (2001) used RAPDs to evaluate the status of the Solimões landrace. They found that it is probably an extension of the Putumayo landrace, rather than a distinct landrace, but their results suggested the necessity of more sophisticated analysis, especially due to the lack of grouping of plants of the same progeny and distances more typical of species than of populations. The objective of this study was to evaluate the three landrace hypothesis (Pará, Solimões and Putumayo) along the Solimões and Amazonas Rivers using AFLPs to characterize the pejibayes present there and also to expand the preliminary RAPD analysis of Sousa et al. (2001).

MATERIAL AND METHODS

In Brazilian Amazonia, the National Research Institute for Amazonia (INPA) maintains the Pejibaye Active Germplasm Bank (BR 174, km 38, Manaus, AM, Brazil; 2° 30' S; 60° 15' W), in collaboration with Embrapa Recursos Genéticos and Embrapa Amazônia Ocidental. The bank contains 450 accessions collected from cultivated and wild populations of pejibaye and 5 from related species. This study used 34 plants from the Pará, 32 from the Solimões and 33 from the Putumayo landraces, from a total of 13 populations (Figure 1). Eighty one of these plants were the same as those used by Sousa et al. (2001), allowing validation of their study. The plants were selected to represent the Brazilian distribution of the Putumayo landrace, the majority of the distribution of the Solimões landrace (the eastern part is poorly represented in the bank), and the western and eastern parts of the Pará landrace (the central part is poorly represented in the bank).

DNA was extracted with the DNAsy Plant Mini-kit (Quiagen) from 100 mg of apical meristem of a lateral shoot, as explained by Clement et al. (1997) for extraction of enzymes, yielding an average (\pm standard error) of 14.4 \pm 6.0 μ g of DNA (minimum 3.2; maximum 28 μ g). The DNA was analysed at Madrid's National Agricultural Research Institute (INIA).

AFLP markers were developed following the protocol supplied by Perkin Elmer (1995), based on Vos et al. (1995). DNA digestion was carried out using the restriction enzymes *EcoRI* and *MseI*. Forty-two combinations of primers (Table 1) were tested for number of fragments, ample fragment size range and sufficient fluorescent emission, and six pairs were chosen and grouped in two sets. Set A included the following sequences: ACA/CAC, AAG/CTG and AAC/CTG. Set B comprised the sequences ACT/CAG, AAG/CAG and AGC/CAG.

Aliquots (2 μ L) of PCR products were mixed with 12 μ L of formamide and 0.5 μ L of a red DNA size standard (GENESCAN-500 ROX). Samples were denatured at 94°C for 3 min prior to separation by capillary electrophoresis at 15 kV for 25 min in an ABI Prism 310 DNA Sequencer

Table1 - Number of AFLP fragments of peijibaye DNA detected with 42 primer combinations.

Primer combination	No. of fragments	Primer combination	No. of fragments
E-CAA/M-ACA	58	E-CAT/M-ACT	68
E-CAA/M-AAG	103	E-CAT/M-AGG	0
E-CAA/M-AAC	102	E-CAT/M-AGC	82
E-CAA/M-ACT	136	E-CTA/M-ACA	27
E-CAA/M-AGG	0	E-CTA/M-AAG	29
E-CAA/M-AGC	97	E-CTA/M-AAC	40
E-CAC/M-ACA	62*	E-CTA/M-ACT	51
E-CAC/M-AAG	94	E-CTA/M-AGG	0
E-CAC/M-AAC	78	E-CTA/M-AGC	81
E-CAC/M-ACT	83	E-CTC/M-ACA	77
E-CAC/M-AGG	0	E-CTC/M-AAG	86
E-CAC/M-AGC	60	E-CTC/M-AAC	62
E-CAG/M-ACA	63	E-CTC/M-ACT	70
E-CAG/M-AAG	59*	E-CTC/M-AGG	0
E-CAG/M-AAC	63	E-CTC/M-AGC	81
E-CAG/M-ACT	47*	E-CTG/M-ACA	0
E-CAG/M-AGG	0	E-CTG/M-AAG	71*
E-CAG/M-AGC	48*	E-CTG/M-AAC	53*
E-CAT/M-ACA	48	E-CTG/M-ACT	65
E-CAT/M-AAG	102	E-CTG/M-AGG	0
E-CAT/M-AAC	83	E-CTG/M-AGC	48

**selected primer combinations.

E=*EcoRI* and M=*MseI* in the primer descriptions.

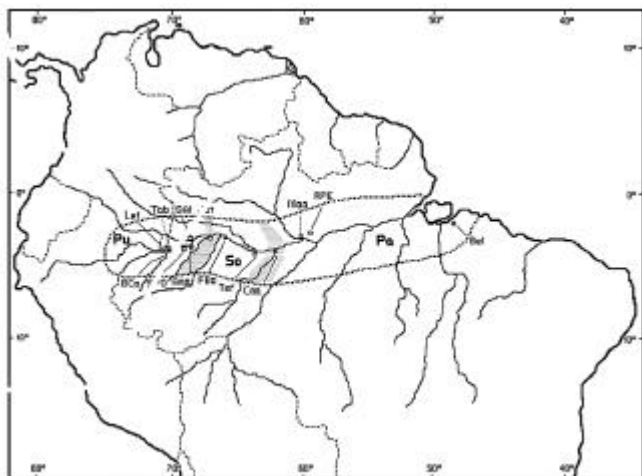


Figure 1 - Geographic distribution of the Pará, Solimões and Putumayo landraces of peijibaye (*Bactris gasipaes*) in Brazilian Amazonia. The sampled populations were: Pará - Belém (Bel: 5 accessions; 13 plants), Manaus (Mao: 4 ac; 8 pl), Rio Preto de Eva (RPE: 6 ac; 13 pl); Solimões - Coarí (Coa: 1 ac; 1 pl), Fonte Boa (FBo: 8 ac; 17 pL), Jutáí (Jut: 2 ac; 5 pl), Tefé (Tef: 4 ac; 9 pl); Putumayo - Amaturá (Ama: 2 ac; 5 pl), Benjamin Constant (BCo: 3 ac; 8 pl), Leticia, Colombia (Let: 2 ac; 4 pl), Santo Antonio do Içá (SAI: 1 ac; 2 pl), São Paulo de Olivença (SPO: 1 ac; 2 pl), Tabatinga (Tab: 6 ac; 12 pl). Hatched bands represent areas of probable introgression between landraces.

(PE Biosystems) and analyzed by using Genescan Analysis software 3.1 (PE Biosystems).

The number of fragments generated by each pair of primers was obtained directly from the Genescan Analysis software, using the Local Southern method to size the fragments. On the basis of presence and absence of amplification products, data matrices were built. Genetic distances were estimated using Jaccard's algorithm. This coefficient was used for clustering data with the UPGMA method (Rolf, 1990).

RESULTS AND DISCUSSION

Eight of the 42 combinations of primers failed to amplify (Table 1). The 34 combinations that amplified yielded 2377 fragments (70 bands per primer pair), in the size range of 50 bp to 500 bp. Six combinations that generated an intermediate number of fragments, with ample size range and with enough fluorescent emission to be detected were chosen. After discarding the peaks that were difficult to score, a total of 245 fragments for analysis were selected, of which 135 (55.10%) were polymorphic between two or more samples.

The dendrogram contained two large groups that joined at a similarity of 0.795 (Figure 2), rather than three groups expected if the three landrace hypothesis was valid, as also found by Sousa et al. (2001). The smaller group contained 41 plants, of which 30 were from the

Pará, 10 from the Putumayo and one from the Solimões landraces, and corresponds approximately to the Pará landrace, since 73% of the plants are from that landrace. The larger group contained 58 plants, of which 31 were from the Solimões, 23 from the Putumayo and 4 from the Pará landraces, and will henceforth be called the Solimões River group because the Solimões (53%) and the Putumayo (40%) were roughly similar in importance.

Each large group was composed of two subgroups and, in the case of the Solimões River group, two outliers, one from the Pará and one from the Putumayo landraces (Figure 2). In both groups, the subgroups were joined at similarities close to 0.815.

The first subgroup of the Pará landrace contained 22 plants (91% Pará), of which 13 (of 13 sampled) from Belém (eastern Pará landrace), five from Rio Preto de Eva (western Pará landrace), two from Manaus (western Pará), one from Tabatinga (Putumayo) and one from Coarí (Solimões). This was a very homogeneous subgroup and appeared to represent the Pará landrace well. The second subgroup contained 19 plants (53% Pará), of which six from Manaus (western Pará landrace), four from Rio Preto da Eva (western Pará), five from Amaturá, two from Tabatinga and two from São Paulo de Olivença (all eastern Putumayo). This sub-group was very heterogenous and only marginally dominated by Pará landrace plants. Mora Urpí & Clement (1988) proposed that the principal urban centers of Amazonia, such as Manaus, are centers of hybridization, which may help to explain the heterogeneity of the second subgroup, although possible errors (see below) can not be ignored.

The first subgroup of the Solimões River group contained 21 plants (52% Solimões; 43% Putumayo), of which seven from Fonte Boa (western Solimões), three from Tefé (central Solimões), one from Jutáí (western Solimões), three from Letícia, three from Benjamin Constant, two from Tabatinga, one from Santo Antônio do Iça (all Putumayo), and one from Rio Preto de Eva (western Pará). The second subgroup contained 35 plants (57% Solimões; 37% Putumayo), of which 10 from Fonte Boa, six from Tefé, four from Jutáí (all Solimões), seven from Tabatinga, five from Benjamin Constant, one from Letícia (all Putumayo) and two from Rio Preto de Eva (western Pará). The two subgroups had similar mixtures of Solimões and Putumayo, with no apparent organization within either group, similar to the situation reported by Sousa et al. (2001). This apparently random mixture of the two landraces suggests that the Solimões landrace does not exist at the level of DNA, as visualized with AFLPs, as also reported by Sousa et al. (2001).

However, there were some unexpected results that need clarification, as also presented by Sousa et al. (2001). Plants of the same accession (members of a progeny that originated from the same open-pollinated female plant) rarely clustered together, although they almost always clustered in the correct group: three pairs

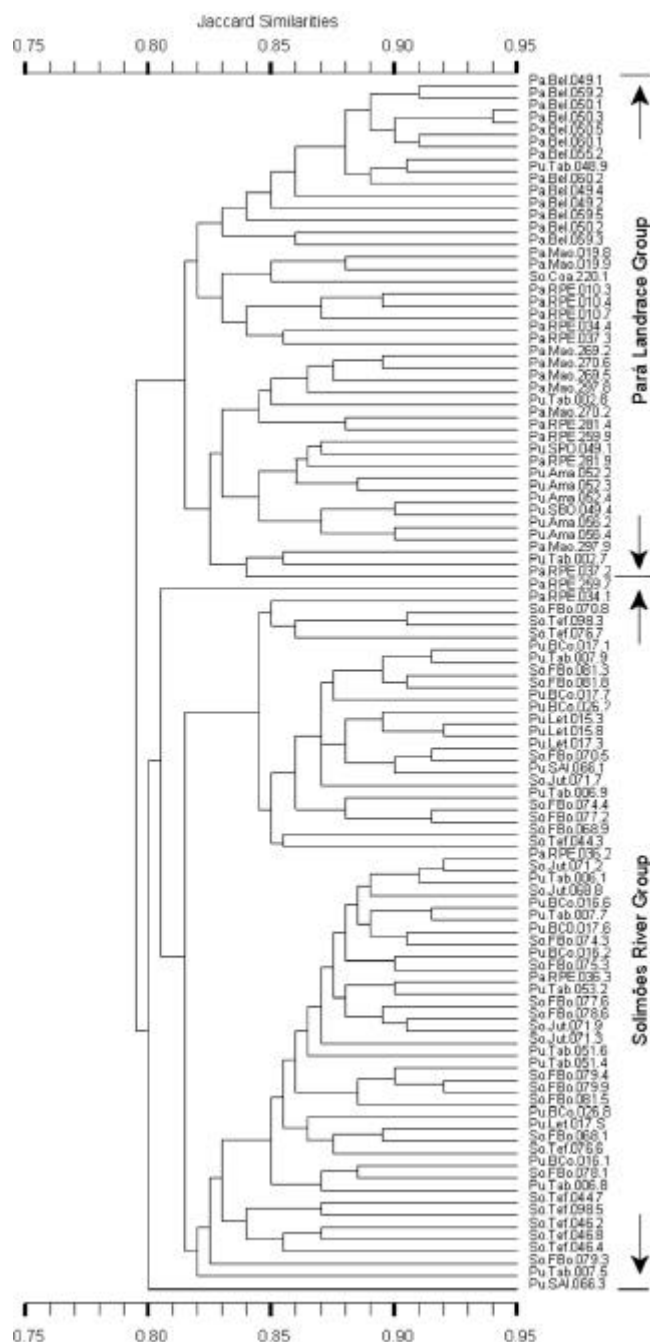


Figure 2 - Dendrogram of the relationships among plants of peijibaye (*Bactris gasipaes*) based on Jaccard's similarities (245 AFLP markers). Key: rr.ppp.nnn.n, where rr are the first letters of the landrace name, ppp are the abbreviations of the populations listed in Figure 1, and nnn.n are accession and plant numbers in the Pejibaye Germplasm Bank, INPA, Manaus, AM.

of Pará plants and one pair of Putumayo in the Pará group; three pairs of Solimões plants and one pair of Putumayo in the Solimões River group. Although the lack of expected clustering was also observed in the American oil palm (*Elaeis oleifera* Cortes), it was an exception rather than the norm in that species (Edson Barcelos, Embrapa, pers. com.). Also, in each group there were intrusive plants: in the Solimões River group there were

four Pará plants, while in the Pará group there were 10 Putumayo plants and one Solimões plant. Both of these unexpected results suggest that there may be errors of identification in the Pejibaye germplasm bank, or sampling and handling errors occurred during collection and laboratory work, or they represent plants introduced from the original landrace into the current. These plants all need to be reanalyzed to clarify their anomalous position in this study.

Sousa et al. (2001) found that the similarities among plants and groups using RAPDs were less than expected. Using AFLPs, the similarities were much closer to expectation, ranging from 0.96 in one Pará progeny (versus 0.85 for the same progeny in Sousa et al. (2001) to 0.82 for the extreme outlier of the Solimões River group (versus 0.54 for its extreme outlier). The major groups joined at nearly 0.80 with AFLPs, while they joined at 0.53 with RAPDs. These differences confirm the greater reliability of AFLPs (Ferreira & Grattapaglia, 1996).

CONCLUSIONS

Using AFLP markers, only two groups were found instead of three, suggesting that the original hypothesis is not valid. The composition and mixture of the Solimões River group suggests that the Putumayo landrace extends along the Solimões River at least to Tefé and possibly to Coarí, instead of being restricted to the upper Solimões River, as originally proposed by Mora Urpí & Clement (1988). Nonetheless, this genetic analysis does not clarify the entire question; for example, the location of the eastern limit of the Putumayo and the western limit of the Pará landraces remains uncertain. A more complete sample of these landraces, with better representation of unclarified areas, will be necessary, and may be enriched with morphometric data as well.

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