

## Note

# Morphological variation and isozyme diversity in *Dioscorea alata* L. landraces from Vale do Ribeira, Brazil

Eduardo de Andrade Bressan<sup>1</sup>, Thiago Briner Neto<sup>2</sup>, Maria Imaculada Zucchi<sup>3</sup>, Ronaldo José Rabello<sup>4</sup>, Elizabeth Ann Veasey<sup>4\*</sup>

<sup>1</sup>USP/CENA – Programa de Pós-Graduação em Biologia na Agricultura e no Ambiente, Piracicaba, SP – Brasil.

<sup>2</sup>Rua Maestro Eufrozino de Azevedo, 2348 – 13633-016 – Pirassununga, SP – Brasil.

<sup>3</sup>APTA – Pólo Apta Centro Sul, C.P. 28 – 13400-970 – Piracicaba, SP – Brasil

<sup>4</sup>USP/ESALQ – Depto. de Genética, C.P. 83 – 13400-970 – Piracicaba, SP – Brasil.

\*Corresponding author <[eaveasey@esalq.usp.br](mailto:eaveasey@esalq.usp.br)>

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**ABSTRACT:** Traditional growers of the Vale do Ribeira, São Paulo State, grow and make use of several *D. alata* landraces. This study assessed the genetic diversity of 16 landraces, using isozymatic and morphological markers and comparing them with 19 commercial varieties of *D. alata*. Their distribution in different levels of organization such as households and communities of the Vale do Ribeira was evaluated. Isozymatic analyses were performed with polyacrylamide (six systems) and starch gels (one system), while the morphological analyses were carried out with 24 traits. Due to the polyploid nature of this species, the isozymatic bands were scored as binary data. Morphological traits were also scored as binary data. Principal coordinates and cluster analyses were conducted for both markers, using for the later the Jaccard's similarity coefficient and UPGMA method. The separation of the landraces from the commercial varieties, which showed lower genetic diversity, was reported for both markers. No correlation between genetic and geographical distances was found for both data, which suggests that the observed variability is not spatially structured. Also, no correlation was found between both markers. The analysis of molecular variance (AMOVA) indicated that genetic diversity was mainly found within households for both isozymatic (54%) and morphological (70%) markers. The results obtained for both markers revealed the importance of traditional agriculturists in the Vale do Ribeira in maintaining high diversity for *D. alata*, even higher than the varieties commercialized in São Paulo State, contributing for the *in situ/on farm* conservation of this crop.

Keywords: AMOVA, traditional agriculture, genetic diversity, water yam

## Introduction

Yam (*Dioscorea* spp.) is a horticulture crop with expressive world consumption, especially in developing countries. *Dioscorea alata* L. is probably the cultivated species with the largest world distribution and one of the oldest cultivated yam species (Lebot, 2009). *D. alata* is the most popular species for cultivation in Brazil, with varieties commonly known as: 'Mimoso', 'Florida' and 'Caipira'. In southern areas of the State of São Paulo (SP), Brazil, traditional farmers practice family farming using the slash and burn system and grow different *D. alata* landraces, used mostly for their own consumption (Bressan et al., 2005). Some households also grow landraces for local markets, following the same model of subsistence farming, which is characterized by low-energy and intense family labor.

Morphological characterization of *D. alata* accessions has been based on the list of descriptors recommended by IPGRI/IITA, in Nigeria (IPGRI/IITA, 1997), such as the studies of Cruz et al. (1999), Hasan et al. (2008), Mahalakshmi et al. (2007) and Rhodes and Martin (1972). The isozymatic genetic characterization in *D. alata* was performed by Lebot et al. (1998) and Twyford (1990). This species was also studied with molecular markers such as RAPD, for the evaluation of resistance to anthracnose (Mignouna et al., 2002a) and genetic diversity studies (Asemota et al.,

1996; Cheng and Liu, 1996; Zannou et al., 2009); AFLP, for the study of intraspecific genetic diversity (Egesi et al., 2006; Malapa et al., 2005) and development of a genetic map and analysis of QTL aimed at resistance to anthracnose (Mignouna et al., 2002b). Recently, Arnau et al. (2009) used six microsatellite primers to study the segregation patterns from four different progenies of *D. alata*.

This study assessed the genetic diversity, through isozyme markers and morphological traits, of commercial varieties and landraces of *D. alata* from the Vale do Ribeira, verifying how this diversity is distributed at different levels of organization: within households, between households within communities and between communities of the Vale do Ribeira, in the State São Paulo.

## Materials and Methods

We evaluated 16 *D. alata* landraces obtained from the communities that practice traditional farming in the Vale do Ribeira, SP, such as Aroeira, Porto Cubatão, Rio Branco, Mandira, Porto do Meio and Santa Maria in Cananéia (25°00'S; 47°55'W); Sapatu in Eldorado Paulista (24°36'S; 48°22'W); and Icapara, Momuna, Peropava and Arataca in Iguape (24°42'S; 47°33'W) (Table 1; Figure 1). In addition, we used two accessions from the germplasm collection of the Agronomic Institute (IAC), Campinas, SP, Brazil, (22°53'

Table 1 – List of *Dioscorea alata* accessions evaluated, with their identification, origin, common names and geographical coordinates.

No.	Municipality	Community	House-hold	Folk name	Geographic coordinates	
1	Iguape	Icapara	1	Cará guaçu	24°40' S	47°27' W
2	Iguape	Icapara	2	Cará aipim	24°41' S	47°27' W
3	Iguape	Momuna	3	Cará branco	24°42' S	47°40' W
4	Iguape	Momuna	4	Cará aipim	24°42' S	47°40' W
5	Iguape	Arataca	5	Cará guaçu	24°43' S	47°45' W
6	Iguape	Peropava	6	-	24°36' S	47°35' W
7	Iguape	- <sup>1</sup>	7	-	24°42' S	47°33' W
8	Iguape	-	7	-	24°42' S	47°33' W
9	Iguape	-	7	-	24°42' S	47°33' W
10	Iguape	-	7	-	24°42' S	47°33' W
11	Santa Rosa	-	8	-	21°28' S	47°21' W
12	Santa Rosa	-	8	-	21°28' S	47°21' W
13	Santa Rosa	-	8	-	21°28' S	47°21' W
14	Santa Rosa	-	8	-	21°28' S	47°21' W
15	Araras	-	9	-	22°21' S	47°23' W
16	Araras	-	9	-	22°21' S	47°23' W
17	Piracicaba	-	10	-	22°42' S	47°38' W
18	Piracicaba	-	10	-	22°42' S	47°38' W
19	Matão	-	11	-	21°36' S	48°22' W
20	Matão	-	11	-	21°36' S	48°22' W
21	Campinas (IAC)	-	12	-	22°53' S	47°04' W
22	Campinas (IAC)	-	12	-	22°53' S	47°04' W
23	Piracicaba	-	13	-	22°42' S	47°38' W
24	Piracicaba	-	13	-	22°42' S	47°38' W
25	Cruz das Almas (Embrapa)	-	14	Jibóia	12°40' S	39°06' W
26	Cananéia	Aroeira	15	São João	24°52' S	47°52' W
27	Cananéia	Aroeira	15	São João roxo	24°52' S	47°52' W
28	Cananéia	P. Cubatão	16	Cará	24°58' S	47°56' W
29	Cananéia	Rio Branco	17	São João branco	24°54' S	48°00' W
30	Cananéia	Rio Branco	17	São João roxo	24°54' S	48°00' W
31	Cananéia	Mandira	18	Bolo	24°59' S	48°51' W
32	Cananéia	Porto d Meio	19	Bolo	25°00' S	48°04' W
33	Cananéia	Santa Maria	20	Côco	22°04' S	48°08' W
34	Eldorado	Sapatu	21	Cará roxo	24°36' S	48°22' W
35	Eldorado	Sapatu	21	Cará branco	24°36' S	48°22' W

<sup>1</sup>Commercial varieties.

S; 47°04' W), and one from Embrapa Cassava and Tropical Fruits, Cruz das Almas, state of Bahia, Brazil, (12°40' S; 39°06' W). To compare with the diversity managed by traditional farmers in the Vale do Ribeira, 16 commercial varieties were also acquired in markets located in Iguape, Santa Rosa de Viterbo (21°28' S; 47°21' W), Piracicaba (22°42' S; 47°38' W) and Matão (21°36' S; 48°22' W), and at a farm in Araras (22°21' S; 47°23' W). From each farm visited in the Vale do Ribeira, a landrace represented by a tuber was collected, except for the households 15, 17 and 21 in the communities

of Aroeira, Rio Branco and Sapatu, respectively, which had more than one landrace identified as different varieties by the farmers. The average number of landraces per farm, therefore, was 1.2. These materials were planted in pots in a greenhouse in a first stage, and after two months, the seedlings were transplanted to an experimental field, in ridges, in Piracicaba, SP. The experiment was installed in a soil classified as Typic Haplustox, in a climate classified by the International Köppen as Cwa. The spacing between rows and between plants was 1.50 m and bamboo stakes were used.

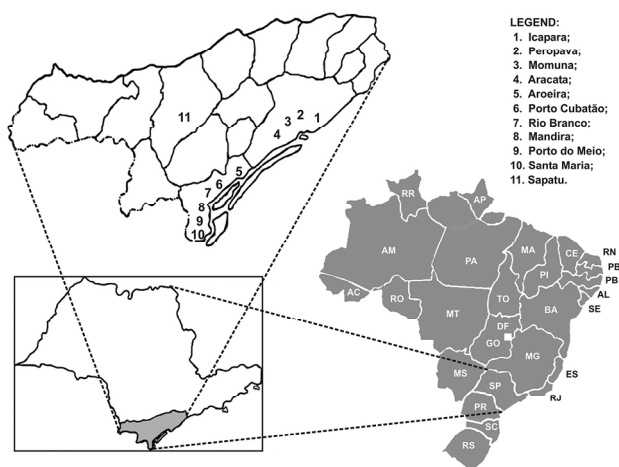


Figure 1 – Studied area in the Vale do Ribeira, São Paulo (SP), Brazil, showing the communities in the municipalities of Iguape (1-4), Cananéia (5-10) and Eldorado (11).

Morphological evaluations were carried out on individual plants of each variety in the experimental field. The morphological descriptors used in this study were recommended by the International Plant Genetic Resources Institute (IPGRI) and International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria (IPGRI/IITA, 1997). We have evaluated 24 traits, nine related to leaf characters, six to stem, seven related to the tubers and one related to flowering. These observations were based on phenotypic average of ten measurements for the quantitative descriptors for leaves, stems and tubers in each individual plant. We assessed a total of nine quantitative descriptors and 15 qualitative traits, which were transformed into different classes, attributing codes to each class (Table 2). The morphological evaluations were performed in two stages: the first assessment was carried out six months after transplanting the accessions on the field, as there was sufficient availability of tissues (young leaves, adult leaves and stems), and the second during harvest when the tubers were already formed.

Table 2 – Descriptors used for morphological assessment of *Dioscorea alata* accessions.

Traits	Classes (codes)
Stem descriptors	
1) Stem colour	1- Green; 2- Purplish green; 3- Brownish green; 4. Purple
2) Wings	1- Present; 2- Absent
3) Spines	1- Present; 2- Absent
4) Twining direction	1- Clockwise; 2- Anticlockwise
5) Stem diameter (at 15 cm from the base of the plant)	1- <0.4 cm; 2- 0.4-0.6 cm; 3- >0.6 cm
6) Stem cross-section shape at base	1- Octagonal; 2- Round
Leaves and flowering descriptors	
7) Position of leaves	1- Alternate; 2- Opposite
8) Leaf shape	1- Cordate; 2- Sagittate
9) Number of leaflets	1- One; 2- Three
10) Petiole length	1- Short (<5 cm); 2- Medium (5-10 cm); 3- Long (>10 cm)
11) Petiole colour	1- Green; 2- Brownish green; 3- Purple
12) [L3] Distance between the insertion of the petiole in the leaf to the upper tip of the leaf (adult leaves)	1- <2 cm; 2- 2-4 cm; 3- >2 cm
13) [L2] Distance between the insertion of the petiole in the leaf to the lower tip of the leaf (adult leaves)	1- <10 cm; 2- 10-15 cm; 3- >15 cm
14) [W1] Width of the leaf in its larger portion (adult leaves)	1- <10 cm; 2- 10-15 cm; 3- >15 cm
15) [W2] Width between the leaf lobes (adult leaves)	1- <6 cm; 2- 6-10 cm; 3- >10 cm
16) Flowering	1- Present; 2- Absent
Tuber descriptors	
17) Underground tubers	1- Present; 2- Absent
18) Number of tubers	1- One; 2- Few (2-5); 3- Several (>5)
19) Tuber shape	1- Elongate; 2- Irregular (not uniform)
20) Relationship of tubers	1- Totally fused; 2- Fused at neck
21) Tuber length	1- <20 cm; 2- 20-40 cm; 3- >40 cm
22) Tuber width (recorded at the widest part)	1- <7 cm; 2- 7-12 cm; 3- >12 cm
23) Tuber skin colour (beneath the bark)	1- Brown; 2- Yellow
24) Flesh colour	1- White; 2- Yellow; 3- Purple; 4- Purple with white; 5- White with purple

In a preliminary step we intended to adapt the protocols for polyacrylimide (discontinuous system) and starch gels (Alfenas et al., 1991; Mignouna et al., 2002c) for *D. alata*. After optimizing the methodology, we proceeded with the genetic evaluation based on isozymes.

The enzymes were extracted from recently expanded leaves (200 mg) from adult plants (6-8 months). The leaves were ground in liquid nitrogen in 1.5 mL microtubes using a power homogenizer, adding 1 mL of the extraction buffer as described by Mignouna et al. (2002c), and also adding 3%  $\alpha$ -mercaptoethanol to minimize damages caused by oxidation. The resulting raw extract was centrifuged at 18,000 g for 20 min at 4°C. After that, 130 mL of the supernatant were placed in 0.5 mL microtubes, diluted in 150 mL of a solution containing Tris-HCl pH 6.8 and Coomassie blue, the latter component indicating the protein migration in the gel. This amount of extract was enough for four gels, which in its turn allowed the assessment of 38 individuals each. The same control plant was added in each gel as a marker. The voltage was set at 50 V for three hours, and then adjusted for 100 V for the next 13 h, usually running overnight at 4°C.

A total of 18 enzymatic systems were initially tested and the main criteria used for the selection of the systems were high band resolution, presence of polymorphism and repeatability. Six systems were chosen to be used with polyacrylamide gels: glucose-6-phosphate isomerase (GPI; E.C. 5.3.1.9), aspartate aminotransferase (AAT; E.C. 2.6.1.1), phosphoglucosmutase (PGM; E.C. 2.7.5.1), shikimate dehydrogenase (SKD; E.C. 1.1.1.25), glucose-6-phosphate dehydrogenase ( $G_6$ PDH; E.C. 1.1.1.49) and superoxide dismutase (SOD; E.C. 1.15.1.1). The malate dehydrogenase system (MDH; E.C. 1.1.1.37) was also evaluated using starch gel. After electrophoresis, the enzymes were revealed according to Alfenas et al. (1991) and the gels were interpreted and photographed immediately after.

Due to the polyploid nature of *D. alata*, data was genotyped as binary data (presence = 1 and absence = 0), generating a binary matrix. The morphological data was also analyzed by transforming each character state into a code within each descriptor, and these codes were subsequently transformed into binary data. For the isozymic analyses, the number of bands per isozyme system, the total number of bands and the percentage of polymorphic bands were also registered.

For both markers Jaccard similarity indices were calculated between pairs of individuals based on binary matrices of allozyme and morphological data. Cluster analyses were performed using Jaccard's coefficient and the UPGMA (Unweighted pair group method with an arithmetic mean) clustering method, using the NTSYSpc software version 2.0 (Rohlf, 1992). The groups  $\times$  stability in the dendrograms were based on estimates of genetic dissimilarities by the procedure of resampling with 1,000 bootstraps using the program BOOD version 2.0 (Coelho, 2001). Principal coordinate analyses were also held for both data, utilizing the software PAST (Hammer et al., 2001).

Binary matrices of allozyme and morphological data were used for the analysis of molecular variance (AMOVA), in or-

der to be considered as a summary of molecular and morphological diversity, for a better understanding of the distribution of diversity, according to the hierarchical levels (households and communities). The AMOVA was used only for the materials collected in the fields of traditional farmers in the Vale do Ribeira. For this purpose, we used the squared distances, as described by Excoffier et al. (1992) with the program Arlequin (Schneider et al., 2004).

To evaluate the patterns of spatial variation, we attempted to correlate genetic diversity (isozymic and morphological) with spatial structure (geographic distances) in order to try to understand the evolutionary dynamics of this crop within traditional agricultural systems in the Vale do Ribeira. The coefficient used was the correlation coefficient ( $r$ ) through the matrix of genetic distances calculated by the similarity index of Jaccard and geographical distance between the collection sites. Geographical distances were estimated from the original coordinates of the home-gardens and swidden fields using the software TrackMaker version 12.3 (Ferreira Júnior, 2004). The Pearson correlation coefficient ( $r$ ) was also used to verify the association between the isozymic and morphological diversity. The significance levels of correlations were checked by the Mantel test ( $Z$ ), using 1000 random permutations (Mantel, 1967). To obtain the similarity matrixes and to apply the Mantel test the software NTSYSpc version 2.0 was used (Rohlf, 1992).

## Results and Discussion

The SOD system showed the highest number of bands (13) followed by PGI and MDH with 12, GOT with 10,  $G_6$ PDH with 9, SKDH with 8 and PGM with 7, in a total of 71 bands for all systems. Furthermore, we observed that 100% of the bands were polymorphic. The combination of different isozymic bands of all enzymes found in both types of gels (starch and polyacrylamide) discriminated the 16 landraces and 13 of the 19 commercial varieties analyzed.

The cluster analysis classified the 35 *D. alata* accessions into six groups, distributed as following: I (landraces 1 and 2 from Icapara and 3 from Momuna, Iguape); II (landrace 4 from Momuna, Iguape and landraces 31, 32, 33 from Mandira, Porto do Meio and Santa Maria, Cananéia respectively); III (landraces 5 from Arataca, Iguape; 34 from Sapatu, Eldorado; 27 from Aroeira, Cananéia; and variety 25 obtained from Embrapa, BA); IV (commercial varieties acquired in markets in the cities of Piracicaba, Iguape, Santa Rosa de Viterbo, Araras, Matão, IAC and landraces 6 from Peropava, Iguape and 28 from Porto Cubatão, Cananéia); V (landraces 26 from Aroeira, 29 and 30 from Rio Branco, Cananéia); and VI (landrace 35 from Sapatu, Eldorado) (Figure 2).

A clear distinction was observed between the landraces (groups I to III, V and VI) and the commercial varieties (group IV), including the accessions obtained from IAC and two landraces from Peropava and Porto Cubatão (Figure 2). The farmer in Peropava reported, during our visit, that he purchased his varieties at CEASA (market supply and distribution center) in the municipality of São Paulo, which explains their proximity to the group of commercial varieties.

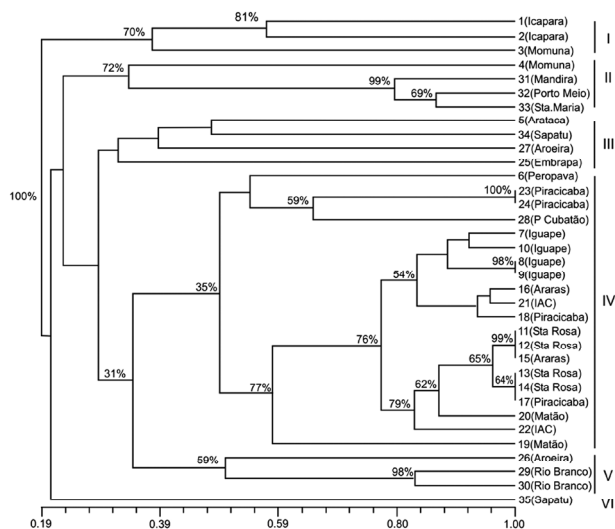


Figure 2 – Cluster analysis using the UPGMA method for the 35 *Dioscorea alata* accessions based on 71 isozymic bands, according to the similarity index of Jaccard.

Nevertheless, the variety from Porto Cubatão was acquired at a market in this community, which led us to the conclusion that it was also from CEASA. Another observation implied from the cluster analysis was that the commercial varieties showed less diversity (0.55 similarity range), while the landraces presented higher diversity (0.71). Similar results were reported by Raji et al. (2009) for cassava, where elite cultivars were clearly separated from African landraces evaluated by SSR markers, although less clearly with AFLP markers. Also, corroborating our results, the amount of within-population variation was higher for landraces for both markers, indicating a higher genetic diversity for the landraces. Total similarity ranged from 1.00 to 0.19 in our study indicating, thereby, high diversity among accessions of *D. alata*.

The presence of possible duplicates, showing the same isozyme phenotypes, was also observed in Figure 2. This occurred among the commercial varieties 23 and 24 from Piracicaba, 8 and 9 from Iguape, 11, 12 and 15 from Santa Rosa de Viterbo and Fazenda Pastre in Araras, and among individuals 13, 14 and 17 from the municipalities of Santa Rosa de Viterbo, Araras and Piracicaba (Table 1). These materials are commercialized in local markets or supermarkets in the State of São Paulo through CEASA, which explains its higher similarity and the possibility of being clones.

The scatter graph obtained from the principal coordinates analysis (Figure 3) showed the separation of landraces from the municipalities of Iguape, Cananéia and Sapatu, in the Vale do Ribeira, from the commercial varieties, which showed greater genetic similarity among each other and were grouped on the left, along with the main axis of the principal coordinate 1, which explained 30% of total variation. The variety originated from Embrapa, in Cruz das Almas, was closer to the landraces from Cananéia according to the dendrogram (Figure 2). The scatter graph also showed the highest variability observed among landraces, with the group II, which is composed of landraces from Cananéia and Iguape, being more distant from the other landraces (Figure 3).

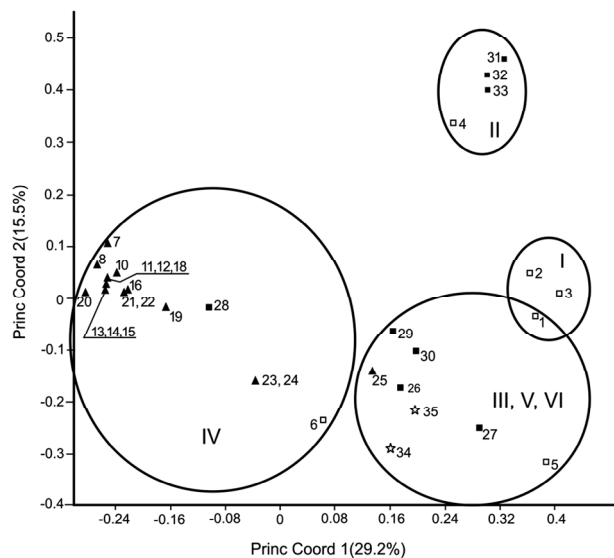


Figure 3 – Scatter graph from the principal coordinates analysis, based on 71 isozymic bands for 35 accessions of *Dioscorea alata*: # 1 to 6 - landraces from Iguape (empty squares), # 7 to 25 - commercial varieties (triangle), # 26 to 33 - landraces from Cananéia (filled squares), and # 34 to 35 - landraces from Eldorado (stars).

The data obtained from this study show the high isozymic diversity among landraces of the Vale do Ribeira, indicating that traditional farmers maintain and manage high levels of genetic diversity for this species in their home gardens or swidden fields. The communities that cultivate landraces with lesser levels of diversity among themselves are Porto do Meio and Santa Maria, which are geographically closer. A clear separation between the municipalities of the Vale do Ribeira was not observed in this study, as occurred for another studied species, *D. trifida* (Bressan et al., 2005).

We attempted to correlate the genetic distances and geographic distances of the landraces of the Vale do Ribeira, but they were nil ( $r = -0.11$ ), although statistically significant ( $p = 0.01$ ), suggesting that there is no correlation between these two parameters. The lack of correlation between genetic and geographical distances indicates that there must be a lot of exchange of landraces among farmers, which promotes gene flow among households and even among communities, and that the distribution of landraces is linked to anthropogenic factors, especially considering that this species is the most commercialized in the State of São Paulo.

Based on data from 71 polymorphic bands using molecular variance analysis (AMOVA) considering only the 16 landraces cultivated by traditional farmers in the Vale do Ribeira, we found that most of the isozymic variability is distributed within households (54%), thus emphasizing the importance of the basic evolutionary units (households) in the distribution of genetic diversity (Table 3). Similar results with isozyme markers were observed by Sambatti et al. (2000) for cassava (*Manihot esculenta*) cultivated by traditional farmers in the Vale do Ribeira, and Faraldo et al. (2000), who found higher genetic diversity in cassava within different re-

gions in Brazil. Veasey et al. (2008) also found higher diversity within households for sweet potato (*Ipomoea batatas*) landraces in the Vale do Ribeira using microsatellite markers.

In Brazil, this is the first report on isozyme diversity among landraces of *D. alata* grown by traditional farmers of the Vale do Ribeira. *D. alata* was studied using isozymes by Twyford (1990), identifying materials originated from tissue culture for the species *D. alata*, *D. cayenensis/rotundata*, *D. esculenta* and *D. bulbifera*. Another study using four polymorphic enzyme systems (MDH, PGI, SKDH, 6PGD [6-phosphogluconate dehydrogenase]) revealed 66 isozyme phenotypes in 269 cultivars of *D. alata* (Lebot et al., 1998), each uniquely characterized by the presence or absence of 27 electromorphs. The authors found that identical phenotypes were distributed in different geographical areas and vice versa, with different phenotypes within the same geographic zone.

From the 24 morphological characters evaluated, eight were monomorphic, ie, the 35 accessions showed opposite leaves with only one leaflet, absence of spines and presence of wings on the stem, anticlockwise twining direction, octagonal shape stem, presence of tubers in 100% of the accessions and brown tuber skin colour. Most of these characters define the species *D. alata*. For the other traits, most of the accessions showed green stems (91%), with 5.7% (two individuals) showing purplish green and 2.9% (one individual) brownish green stems. Stem diameters were, in the majority (69%) between 0.4-0.6 cm, followed by < 0.4 cm stems (31%). Most accessions (97%) had sagittate leaf shape and green coloured petioles (89%), followed by purple (8.6%), with only one individual (2.9%) showing brownish green petioles. The majority (80%) had petiole length measuring from 50 to 100 mm. Regarding the distance between the insertion of the leaf petiole and the upper tip of the leaf (L3), the majority (66%) had a < 20 mm distance, while the others (34%) ranged from 20-40 mm. Still, the majority (86%) had a distance of 100-150 mm between the insertion of the leaf petiole and the lower tip of the leaf (L2). With regard to the larger portion of width (W1), the majority (77%) showed a width of < 100 mm, while the width between the leaf lobes (W2) ranged from < 60 mm (46%) and 60-100 mm (54%). Concerning the flowering aspects, few accessions (8.6%) flowered under the experimental conditions in Piracicaba, SP. Seed production was not observed for the accessions that showed flowering.

Regarding the tubers, most accessions had several tubers (86%) of elongated shape (86%), with white coloured flesh (89%), followed by three individuals with purple flesh (8.6%) and only one with a yellow flesh (2.9%). The three accessions showing purple (27, 28 and 30) and yellow flesh (26) were landraces from Cananéia municipality. Most accessions (77%) had < 200 mm tuber length, while the width of the tubers ranged from 70-120 mm (49%), < 70 mm (37%) and > 400 mm (14%).

Rhodes and Martin (1972) used 100 characters to study the variation in 30 *D. alata* cultivars, concluding that only 28% of the characters used were useful for the classification of the cultivars. In our study, of the 24 traits used, 67% were discriminatory. Hasan et al. (2008) used 47 morpho-

logical traits to assess 70 *D. alata* accessions collected throughout Malaysia. The results obtained from the principal component analysis (PCA) indicated that the characters that most contributed to the species variability were those related to shape, size and flesh colour of underground tubers; shape and colour of aerial tubers; position, shape, size and vein colour of the leaves; petiole colour; shoot growth rate; and number of days for shoots to germinate. Mahalakshmi et al. (2007) reported the use of morphological traits to obtain a core collection for *D. alata*, evaluating a total of 772 accessions. The study identified 72 accessions to be part of the core collection using 77 qualitative and 11 quantitative traits.

A similarity range from 0.48 to 1.0 was observed in the cluster analysis of 35 *D. alata* accessions (16 landraces and 19 commercial varieties) (Figure 4), indicating that the set of landraces and commercial varieties evaluated present high variation in morphological characters, although the lesser magnitude than the variation observed for isozymes (Figure 2). This analysis classified the accessions in three groups (Figure 4), with group I containing most of the landraces from Iguape and the accession from Cruz das Almas, Embrapa (25), group II including all commercial varieties, and landraces from Cananéia (Rio Branco, Porto do Meio, Mandira, Santa Maria and Aroeira), Eldorado (Sapatu), and Iguape (Momuna and Peropava), while group III consisted of three landraces from Cananéia (Aroeira, Porto Cubatão and Rio Branco). Group III differed from the others mainly because of the purple colour of the petiole, purple flesh tuber colour, and the irregular shape of the tubers. Group I, comprised by landraces from Iguape and one accession from Cruz das Almas, differed from the others mainly due to the larger leaf sizes (W3, W2 and L2). Similarly to what was observed with isozymes, the commercial varieties obtained in markets, as well as all accessions from the IAC, were all clustered in group II, apart of the landraces from Cananéia and Eldorado, which stood in a differentiated sub-group. The

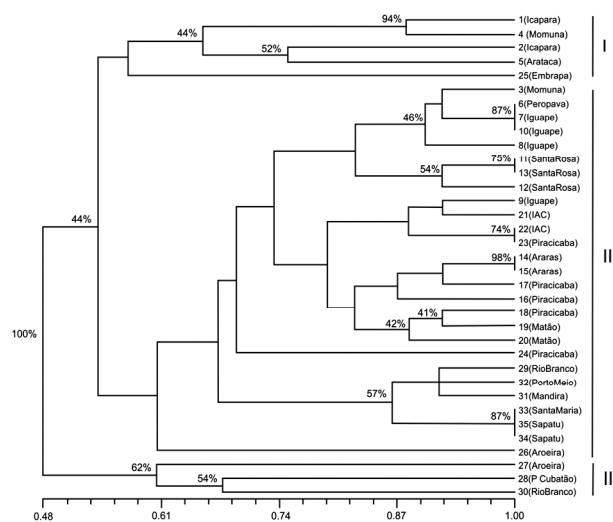


Figure 4 – Cluster analysis using the UPGMA method for the 35 *Dioscorea alata* accessions based on 24 morphological characters, according to the similarity index of Jaccard.

exception was observed for the landraces 3, from Momuna, and 6, from Peropava, both from Iguape, although the latter was acquired in CEASA, and therefore it was expected to show more similarity to the commercial varieties.

Duplicates were observed, with the similarity coefficient equal to 1, meaning that these individuals had the same morphological phenotype, such as landrace 6 from Peropava community and the commercial varieties 7 and 10, acquired in markets of Iguape municipality, again confirming the proximity of the Peropava accession with the commercial varieties. Duplicates were also found between the commercial varieties 11 and 13 from Santa Rosa de Viterbo, 22, from IAC, and 23, from Piracicaba, and between the landraces 33, from Santa Maria, in Cananéia, and 34 and 35, from Sapatu, Eldorado municipality. Unfortunately, the possible duplicates found using isozyme markers were not coincident with duplicates found using morphological characters.

On the scatter graph obtained from the principal coordinate analysis (Figure 5), with the first two coordinates accounting for 45% of total variability, the three groups ob-

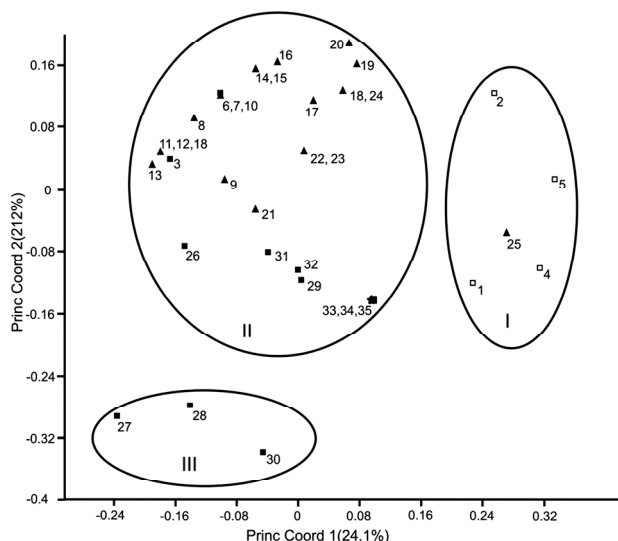


Figure 5 – Scatter graph from the principal coordinates analysis, based on 24 morphological characters for 35 *Dioscorea alata* accessions: # 1 to 6 - landraces from Iguape (empty squares), # 7 to 25 - commercial varieties (triangle), # 26 to 33 - landraces from Cananéia (filled squares), and # 34 to 35 - landraces from Eldorado (stars).

served in the dendrogram were clearly formed, with the first coordinate separating group I, on the left, from group II on the right of this parameter, and the second coordinate separating group III from the others.

The correlation between genetic distances and geographical distance was zero ( $r = 0.03$ ), suggesting that morphological diversity is not structured in space, probably due to the existence of an exchange of materials or mixture of varieties among farmers. Lebot et al. (1998), studying 269 cultivars of *D. alata* to determine genetic relationships among *D. alata* originating from the South Pacific, Asia, Africa and the Caribbean with four polymorphic enzyme systems (MDH, PGI, SKDH, 6PGD) and 25 morpho-agronomic descriptors, did not find an agreement between patterns of morphological variation, geographic origins and isozyme polymorphism either.

The molecular variance analysis (AMOVA) based on morphological markers (24 characters), showed that 70% of the morphological diversity were found within households (Table 3), indicating the management of traditional farmers in the selection and maintenance of different materials within the evolutionary units (households). This finding confirms the results obtained by Sambatti et al. (2000) with cassava and Veasey et al. (2007) with sweet potato cultivated by traditional farmers in the Vale do Ribeira. Sambatti et al. (2000) demonstrated that most of the variability, both morphological and isozymic, for the cassava crop was distributed within households. According to Peroni and Martins (2000), the maintenance of diversity and its amplification at the garden level are consequences of the interaction between farmer management and life history components of the crops. In this study we found a large number of local names (“cará guaçu” [guaçu yam], “cará aipim” [cassava yam], “cará branco” [white yam], “cará são João” [Saint John’s yam], “cará São João branco” [white Saint John’s yam], “cará São João roxo” [purple Saint John’s yam], “cará bolo” [cake yam], “cará coco” [coconut yam] and “cará roxo” [purple yam]), that are used by farmers, sometimes hiding levels of diversity not recognized by them. However, the name “cará guaçu” was attributed to landraces 1 and 5 (both from group I), and the name “cará aipim” was attributed to landraces 2 and 4 (both also from Group I). The name “cará branco” was assigned to accessions 3 and 35 (both from Group II) (Figure 4), along with all commercial varieties of white flesh.

The correlation coefficient between morphological and isozymic genetic distances was positive ( $r = 0.27$ ) but not

Table 3 – Analysis of molecular variance (AMOVA) for allozyme and morphological data assessed on 16 *D. alata* landraces from the Vale do Ribeira.

Source of variation	Isozyme markers			Morphological markers		
	DF	MS	% of total variation	DF	MS	% of total variation
Between communities	10	118.87	26.38 <sup>ns</sup>	10	70.31	0.00 <sup>ns</sup>
Between households within communities	2	15.00	19.63*	2	15.00	29.89*
Within households	3	16.50	53.99*	3	14.00	70.11 <sup>ns</sup>
Total	15	150.37		15	99.31	0.00

\*Significant at  $p < 0.01$ ; <sup>ns</sup>Non-significant.

significant ( $p = 0.99$ ), suggesting that there is no correlation between these two parameters. Therefore, traditional farmers are faced with some morphological changes that do not reflect the isozymic differences in *D. alata*. The lack of correlation between molecular and morphological genetic distances is due to poor genomic coverage achieved with the isozyme loci. However, Telles et al. (2001) showed in their research on natural populations of a Brazilian fruit tree known as “cagaiteira” (*Eugenia dysenterica*), a positive ( $r = 0.289$ ) and significant ( $p < 0.05$ ) correlation between the isozymic and morphological matrices of genetic distances.

### Conclusions

Both isozymatic and morphological markers revealed the existence of large genetic diversity among the landraces cultivated by traditional farmers in the Vale do Ribeira, with an even larger diversity for the isozymes. Moreover, both markers separated the commercial varieties, which showed less genetic diversity when compared to the landraces. Most of the diversity in *D. alata*, which was not spatially structured, was found to be within households of traditional farmers in the Vale do Ribeira. This is due to the management practices and material exchange among farmers, thus emphasizing the important role they have played in the *in situ/on farm* conservation of *D. alata*.

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### References

- Alfnas, A.C.; Peters, I.; Brune, W.; Passador, G.C. 1991. Electrophoresis of Proteins and Isozymes of Fungi and Forest Plants. UFV, Viçosa, MG, Brazil. (in Portuguese).
- Arnau, G.; Nemorin, A.; Maledon, E.; Abraham, K. 2009. Revision of ploidy status of *Dioscorea alata* L. (Dioscoreaceae) by cytogenetic and microsatellite segregation analysis. *Theoretical and Applied Genetics* 118: 1239-1249.
- Asemota, H.N.; Ramser, J.; López-Peralta, C.; Weising, K.; Kahl, G. 1996. Genetic variation and cultivar identification of Jamaican yam germplasm by random amplified polymorphic DNA analysis. *Euphytica* 92: 341-351.
- Bressan, E.A.; Veasey, E.A.; Peroni, N.; Felipim, A.P.; Santos, M.P. 2005. Collecting yam (*Dioscorea* spp.) and sweet potato (*Ipomoea batatas*) germplasm in traditional agriculture small-holdings in the Vale do Ribeira, São Paulo, Brazil. *Plant Genetic Resources Newsletter* 144: 8-13.
- Cheng, K.T.; Liu, S.Y. 1996. Genetic relationships in *Dioscorea alata* L. revealed by RAPD analysis. *Chinese Pharmaceutical Journal* 48: 197-205.
- Coelho, A.S.G. 2001. BOOD: Dendrogram Assessment Based on Distances/Genetic Similarities Estimates through the Bootstrap Procedure. Laboratório de Genética Vegetal/Instituto de Ciências Biológicas/Universidade Federal de Goiás, Goiânia, GO, Brazil. (in Portuguese).
- Cruz, V.M.V.; Altoveros, N.C.; Mendiolo, M.S.; Ramirez, D.A. 1999. Geographical patterns of diversity in the Philippine edible yam collection. *Plant Genetic Resources Newsletter* 119: 7-11.
- Egesi, C.N.; Asiedu, R.; Ude, G.; Ogunyemi, S.; Egunjobi, J.K. 2006. AFLP marker diversity in water yam (*Dioscorea alata* L.). *Plant Genetic Resources* 4: 181-187.
- Excoffier, L.; Smouses, P.E.; Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.
- Faraldo, M.I.F.; Silva, R.M.; Martins, P.S. 2000. Genetic variability of landraces of cassava in geographical regions of Brazil. *Scientia Agricola* 57: 499-505 (in Portuguese, with abstract in English).
- Ferreira Júnior, O. TrackMaker. Available at: <http://www.gpstm.com/port> [Accessed Nov. 12, 2004].
- Hammer, O.; Harper, D.A.T.; Ryan, P.D. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, 4: 9. Available at: [http://palaeo-electronica.org/2001\\_1/past/issue1\\_01.htm](http://palaeo-electronica.org/2001_1/past/issue1_01.htm) [Accessed Oct. 10, 2009].
- Hasan, S.M.Z.; Ngadin, A.A.; Shah, R.M.; Mohamad, N. 2008. Morphological variability of greater yam (*Dioscorea alata* L.) in Malaysia. *Plant Genetic Resources* 6: 52-61.
- International Plant Genetic Resources Institute/International Institute of Tropical Agriculture [IPGRI/IITA]. 1997. Descriptors for Yam (*Dioscorea* spp.). Rome, Italy; Ibadan, Nigeria.
- Lebot, V. 2009. Tropical Root and Tuber Crops: Cassava, Sweet Potato, Yams and Aroids. CABI, Wallingford, UK.
- Lebot, V.; Trilles, B.; Noyer, J.L.; Modesto, J. 1998. Genetic relationships between *Dioscorea alata* L. cultivars. *Genetic Resources and Crop Evolution* 45: 499-509.
- Mahalakshmi, V.; Atalobhor, N.N.G.J.; Ogunsoola, D.; Lawson, M.; Ortiz, R. 2007. Development of a West African yam *Dioscorea* spp. core collection. *Genetic Resources and Crop Evolution* 54: 1817-1825.
- Malapa, R.; Arnau, G.; Noyer, J.L.; Lebot, V. 2005. Genetic diversity of the greater yam (*Dioscorea alata* L.) and relatedness to *D. nummularia* Lam. and *D. transversa* Br. as revealed with AFLP markers. *Genetic Resources and Crop Evolution* 52: 919-929.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209-220.
- Mignouna, H.D.; Abang, M.M.; Onosanya, A.; Asiedu, R. 2002a. Identification and application of RAPD markers for anthracnose resistance in water yam (*Dioscorea alata*). *Annals of Applied Biology* 141: 61-66.
- Mignouna, H.D.; Mank, R.A.; Ellis, T.H.N.; Bosch, N.V.D.; Asiedu, R.; Abang, M.M.; Peleman, J. 2002b. A genetic linkage map of water yam (*Dioscorea alata* L.) based on AFLP markers and QTL analysis for anthracnose resistance. *Theoretical and Applied Genetics* 105: 726-735.
- Mignouna, H.D.; Dansi, A.; Zok, S. 2002c. Morphological and isozymic diversity of the cultivated yams (*Dioscorea cayenensis*/*Dioscorea rotundata* complex) of Cameroon. *Genetic Resources and Crop Evolution* 49: 21-29.
- Peroni, N.; Martins, P.S. 2000. Influence of itinerant agriculture dynamics in the generation of diversity of landraces grown vegetatively. *Interciência* 25: 22-29. (in Portuguese, with abstract in English).
- Rhodes, A.M.; Martin, F.W. 1972. Multivariate studies of variation in yams (*Dioscorea alata* L.). *Journal of the American Society for Horticultural Science* 97: 685-688.
- Rohlf, J. 1992. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 1.70 (software). Stony Brook, New York, NY, USA.
- Sambatti, J.B.M.; Ando, A.; Martins, P.S. 2000. Distribution of isozyme and morphological diversity of cassava in the autochthonous agriculture of Ubatuba. *Scientia Agricola* 57: 75-80. (in Portuguese, with abstract in English).
- Schneider, S.; Roessli, D.; Excoffier, L. 2004. Arlequin v. 2000: a software for population data analysis (software). Genetic and Biometry Laboratory/University of Geneva, Geneva, Switzerland. Available at: <http://anthropologie.unige.ch/arlequin> [Accessed Oct. 14, 2004].



- Telles, M.P.C.; Silva, R.S.M.; Chaves, L.J.; Coelho, A.S.G.; Diniz Filho, J.A.F. 2001. Divergence between subpopulations of cagaiteira (*Eugenia dysenterica*) in response to edaphic patterns and spatial distribution. *Pesquisa Agropecuária Brasileira* 36: 1387-1394. (in Portuguese, with abstract in English).
- Twyford, C.T.; Viana, A.M.; James, A.C.; Mantell, S.H. 1990. Characterization of species and vegetative clones of *Dioscorea* food yams using isoelectric focusing of peroxidase and acid phosphatase isoenzymes. *Tropical Agriculture* 67: 337-341.
- Van Raji, A.J.; Fawole, I.; Gedil, M.; Dixon, A.G.O. 2009. Genetic differentiation analysis of African cassava (*Manihot esculenta*) landraces and elite germplasm using amplified fragment length polymorphism and simple sequence repeat markers. *Annals of Applied Biology* 155: 187-199.
- Veasey, E.A.; Borges, A.; Rosa, M.S.; Queiroz-Silva, J.R.; Bressan, E.A.; Peroni, N. 2008. Genetic diversity in Brazilian sweet potato (*Ipomoea batatas* (L.) Lam., Solanales, Convolvulaceae) landraces assessed with microsatellite markers. *Genetics and Molecular Biology* 31: 725-733.
- Veasey, E.A.; Queiroz-Silva, J.R.; Rosa, M.S.; Borges, A.; Bressan, E.A.; Peroni, N. 2007. Phenology and morphological diversity of sweet potato (*Ipomoea batatas*) landraces of the Vale do Ribeira. *Scientia Agricola* 64: 416-427.
- Zannou, A.; Agbicodo, E.; Zoundjihékpon, J.; Struik, P.C.; Ahanchédé, A.; Kossou, D.K.; Sanni, A. 2009. Genetic variability in yam cultivars from the Guinea-Sudan zone of Benin assessed by random amplified polymorphic DNA. *African Journal of Biotechnology* 8: 26-36.

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