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DNA fingerprinting of water yam (*Dioscorea alata*) cultivars in Brazil based on microsatellite markers

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ABSTRACT

This study aimed to fingerprint 36 water yam (*Dioscorea alata*) accessions using microsatellite markers. Ten accessions were collected in local markets from several municipalities in Brazil, eight were obtained from the 'Instituto Agronômico de Campinas' (IAC) germplasm collection and eighteen were collected directly from growers from São Paulo state. A total of nine microsatellite loci were used in the analysis. Loci revealed high polymorphism verified by elevated PIC values (0.57-0.77), and by high gene diversity and Shannon-Wiener indices (0.69 and 1.29 on average, respectively). The accessions were classified into two groups based on clustering analysis. One group contained mostly accessions from the IAC collection, including a commercial cultivar acquired in a market in the city of Cuiabá, Mato Grosso state. The second group was composed of most accessions, including those collected directly from growers and markets in São Paulo, a few accessions from the IAC collection, and an accession from Puerto Rico, named 'Florida', which is the most cultivated in Brazil. Several duplicates were identified in this study, including accessions obtained from two farmers in Mogi Guaçu and Mogi Mirim, São Paulo state. However, some of these accessions were allocated in different sub-groups, within this second group. Results suggested the hypothesis of different origins for accessions currently cultivated in Brazil. Similar accessions obtained from different municipalities revealed the commercialization of the same accessions at different locations.

Keywords: *Dioscorea alata*, commercial varieties, genetic diversity, germplasm collection, SSR.

RESUMO

Diversidade genética de cultivares de inhame (*Dioscorea alata*) no Brasil utilizando microssatélites

Este estudo teve como objetivo a análise genética de 36 acessos de inhame (*Dioscorea alata*) utilizando marcadores microssatélites. Dez acessos foram coletados em mercados locais de vários municípios no Brasil, oito foram obtidos no banco de germoplasma do Instituto Agronômico de Campinas (IAC), e dezoito foram coletados diretamente com os agricultores no estado de São Paulo. Um total de nove locos de microssatélites foram utilizados para amplificação. Alto polimorfismo foi encontrado entre os locos, verificado pelos valores de PIC elevados (0,57-0,77) e altos índices de heterogosidade esperada e Shannon-Wiener (0,69 e 1,29 em média, respectivamente). Os acessos foram classificados em dois grupos pela análise de agrupamento. O primeiro grupo consiste principalmente de acessos obtidos da coleção do IAC, incluindo um acesso comercial obtido num mercado na cidade de Cuiabá, estado de Mato Grosso. O segundo grupo classificou os acessos coletados diretamente dos agricultores, incluindo um importante acesso proveniente de Porto Rico, denominado 'Florida', a cultivar mais plantada no Brasil. Este grupo incluiu também os acessos obtidos em mercados de vários municípios do estado de São Paulo, além de outros acessos da coleção do IAC. Várias duplicatas foram identificadas neste estudo, incluindo acessos obtidos junto aos dois agricultores de Mogi Guaçu e Mogi Mirim, em São Paulo. Entretanto, parte desses acessos foi alocada em diferentes sub-grupos, dentro do segundo grupo. Os resultados sugerem a hipótese de diferentes origens para os acessos atualmente comercializados e cultivados no Brasil. Acessos similares obtidos de diferentes municípios mostrou a comercialização dos mesmos em locais diferentes.

Palavras-chave: *Dioscorea alata*, banco de germoplasma, diversidade genética, SSR, variedades comerciais.

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Water yam (*Dioscorea alata*), also known as "greater yam", was never found in the wild and its hybridization with other *Dioscorea* species is unknown, although two Asian species (*D. hamiltonii* and *D. persimilis*) could be part of its origins (Burkill, 1960). Water yam is believed to be a true cultigen derived from wild forms through human selection, although there is no concrete evidence to support this claim (Hahn, 1995). Tubers from this species are known for their high nutritional content, with crude protein content of 7.4%, starch content of 75-84%, and vitamin C content ranging from 13.0 to 24.7 mg/100 g. Due to high starch content of the tubers, *D. alata* provides a good source of dietary carbohydrates in tropical and subtropical regions (Osagie, 1992).

World annual production of yam, in 2010, was estimated to be 48.3 million tons (FAOSTAT, 2012). About 94% of this production was from Western and Middle Africa, with Nigeria being the world largest producer, the remaining was produced mainly in Central and South America, Oceania and Asia (FAOSTAT, 2012). Brazilian yam production, including *D. alata* and *D. cayenensis/D. rotundata* species, was estimated to be 232,100 tons in 2010 in a cultivated area of 25,300 ha (FAOSTAT, 2012), concentrated in the Northeastern and Southeastern regions, grown mainly

by small-scale farmers and in reduced planting areas, in the states of Paraíba, Pernambuco, Bahia, Alagoas, Minas Gerais, São Paulo and Espírito Santo, accounting for roughly 55% of total national yield (Mesquita, 2001). Small-scale farmers produce *D. alata* in most Brazilian states (Veasey *et al*., 2010).

Dioscorea alata is a polyploid, with several ploidy levels, revealing a predominance of tetraploidy (Arnau *et al.*, 2009; Obidiegwu *et al*., 2009a). Effective breeding programs, genetic diversity analyses and elucidation of the phylogeny and the species origin are urgently necessary (Lebot, 2009).

Despite a growing interest in water yam, published data on molecular characterization and genetic diversity of this crop are scanty (Siqueira, 2011). Few studies on genetic diversity of water yam have been reported using isozymes (Lebot *et al*., 1998; Bressan *et al*., 2011), RAPDs (Random amplified polymorphic DNA) (Asemota *et al.*, 1996; Mignouna *et al*., 2002; Zannou *et al*., 2009), AFLPs (Amplified fragments length polymorphism) (Malapa *et al*., 2005; Egesi *et al.*, 2006; Tamiru *et al*., 2007) and Expressed Sequence Tags (Narina *et al.*, 2011), with each method differing in terms of principle, application, type and amount of polymorphism detected and time requirements (Agarwal *et al*., 2008). Microsatellites or SSR (Simple sequence repeats) markers are, in general, more abundant in most genomes, possessing highly informative content. Microsatellite primers have been developed for a few *Dioscorea* species (Mizuki *et al.*, 2005; Hochu *et al*., 2006), including *D. alata* (Tostain *et al*., 2006; Siqueira *et al*., 2011), and have been used on segregation studies, and genetic characterization of *Dioscorea* species (Mignouna *et al*., 2003; Scarcelli *et al*., 2005; Bousalem et al., 2006; Tostain *et al.*, 2007; Arnau *et al.*, 2009; Obidiegwu *et al.*, 2009b,c).

Little is known about the genetic variability of Brazilian accessions. Knowledge of the genetic variation within and among accessions is necessary for efficient breeding and management programs. This study aimed to assess the genetic diversity of 36 *D. alata* commercial cultivars obtained from markets in various municipalities in Brazil, and accessions introduced from Puerto Rico and the Democratic Republic of Congo, using SSR markers, and to infer about possible origins of accessions commercialized in Brazil.

MATERIAL AND METHODS

Thirty six accessions from the germplasm collections of the 'Instituto Agronômico de Campinas' (IAC) and from the Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo (ESALQ/USP), Piracicaba, São Paulo state were studied. Ten accessions were collected from markets in distinct municipalities in Brazil; eight were obtained from the IAC collection, with part being introduced from Puerto Rico and the Democratic Republic of Congo, and 18 were collected directly in the field from farmers. From these 18 accessions, eight tubers were obtained from a farmer field in Mogi Guaçu (DGC 309.1 – 309.8), and ten tubers from a farmer field in Mogi Mirim (DGC 311.1-311.10), representing possible clones (Table 1). IAC accessions were identified as SRT, followed by a number, and the ESALQ/USP accessions, as DGC followed by a number. Tubers from each accession were planted in pots in a greenhouse and further, the plantlets were transferred to the field.

Recently expanded young leaves were collected and dried in an oven at 45ºC for 48 h. A macerated sample was submitted to a CTAB (Cetyl Trimethyl Ammonium Bromide) extraction buffer (3% CTAB; 1.2 M NaCl; 0.1 M TRIS HCl, pH 8.0; 30 mM EDTA, pH 8.0; 1% β-Mercapthoethanol added just before use), following the procedure described in Sharma *et al*. (2008), with modifications. During each wash solution with ethanol 70% we used two extra centrifugations (5900 g for 300 s), and an extended time (2 h) with RNAse incubation. DNA quality was visually assessed on 1% agarose gels, stained with Blue Green Loading Dye (LGC Biotechnology, Brazil) following electrophoresis at 2.7 V/cm. DNA concentration was determined with known concentrations of undigested λ DNA (Invitrogen, CA, USA) varying from 5 to 100 ng/uL. The gel was visualized by UV and photo documented (Canon Utilities Remote Capture DC).

Nine microsatellite loci based on Tostain *et al*. (2006) and Siqueira *et al*. (2011) were used (Table 2). The polymerase chain reactions (PCR) were conducted in a final volume of 16 µL, consisting of 1 U of *Taq* DNA polymerase (LGC, Brazil); 1X Amplification Buffer (Mg^+ free); 1.5 mM of $MgCl_2$; 0.3 µM of each primer; 0.2 mM of each dNTPs and 20 ng of DNA template. Reactions were conducted in the thermocycler *Bioer Lifepro* (Brazil) model, under the following amplification conditions: initial denaturing at 94ºC for 5 min, followed by 35 cycles of denaturing at 94ºC for 30 s, 1 min at the defined annealing temperature for each primer (Table 2), and 1 min at 72ºC, with a final extension at 72ºC for 8 min (Tostain *et al*., 2006). Amplification products were submitted to electrophoresis in 6% denaturing polyacrylamide gels under an initial voltage of 60 V for 30 min and 120 V for 3 h. Gels were stained using a silver staining procedure (Creste *et al*., 2001) and photo-documented with a digital camera.

The number of alleles (bands) per loci was recorded and the Polymorphism Information Content (PIC) was calculated according to Botstein *et al*. (1980). The expected heterozygosity (H_E) was also determined. PIC and H_E were calculated according to Botstein *et al.* (1980). To estimate the Shannon-Wiener Diversity Index for each locus we used POPGENE v. 1.32 (Yeh *et al.*, 1997).

A similarity matrix was obtained for the 36 yam accessions using binary data and the Jaccard similarity coefficient method. With this coefficient and the UPGMA (Unweighted Pair Group Method with an Arithmetic Mean) method (Sneath & Sokal, 1973), a cluster analysis was conducted using the NTSYS-pc software (Rohlf, 1992). The precision of the generated groupings was estimated from sampling simulations, considering 10,000 bootstraps, using BOOD, version 2.0 software (Coelho, 2001). Analysis on principal coordinates and the scatter graph using the two

first principal coordinates was also accomplished with the NTSYS-pc software (Rohlf, 1992).

RESULTS AND DISCUSSION

Despite the fact that 12 microsatellite markers were used, only nine showed polymorphism across the analyzed accessions, producing well-defined and reproducible fragments. A total of 45 alleles (fragments) was recorded with an average of 5 alleles per loci (Table 2). The highest number of alleles (7) was recorded for loci *A7*, and the lowest number (4) was found for loci *A4*, *F1*, *H12* and *9C*.

Gene diversity or the expected heterozygosity (H_E) was high in this study, 0.69 on average, varying from 0.52 (locus *B5*) to 0.79 (locus *A7*), as well as the Shannon-Wiener diversity index, 1.29 on average, varying from 0.82 (locus *B5*) to 1.65 (locus *A7*). This

Table 1. List of 36 *Dioscorea alata* accessions used in this study, including their origin and common names (lista dos 36 acessos de *Dioscorea alata* usados neste estudo, incluindo suas origens e nomes comuns). Piracicaba, ESALQ, 2011.

Nº	Germplasm Nº [1]	Municipalities/state/country	Introduction year	Origin	Common name
1	SRT 24.0	Sorocaba – SP – Brazil	1947	IAC	Sorocaba
2	SRT 71.0	Congo Belga	1949	IAC	Bira
3	SRT 80.0	Minas Gerais – Brazil	1949	IAC	Branco Viçosa
4	SRT 3.0	Campinas – SP – Brazil	1936	IAC	Mimoso
5	SRT 84.0	Campinas – SP – Brazil	1951	IAC	Cova Campinas
6	SRT 112.0	Mato Grosso do Sul – Brazil	2000	IAC	Cará do Mato
7	SRT 29.0	Puerto Rico	1947	IAC	Florida
8	SRT 89.0	Araraquara – SP – Brazil	1959	IAC	Araraquara I
9	DGC 45	Campinas – SP – Brazil	2002	Market	---
10	DGC 40	Piracicaba – SP – Brazil	2002	Market	---
11	DGC 43	Matão – SP – Brazil	2002	Market	---
12	DGC 46	Piracicaba – SP – Brazil	2002	Market	---
13	DGC 97.0	Cuiabá – MT – Brazil	2006	Market	---
14	DGC 107.0	Botucatu – SP – Brazil	2006	Market	---
15	DGC 36	CEASA – SP – Brazil	2002	Market	---
16	DGC 132.0	Fernandópolis – SP – Brazil	2007	Market	---
17	DGC 123.0	Mogi-Guaçu – SP – Brazil	2006	Market	---
18	DGC 127.0	Santa Mercedes – SP – Brazil	2007	Market	---
19.1	DGC 309.1	Mogi Guaçú – SP - Brazil	2009	Farmer	Florida
19.2	DGC 309.2	Mogi Guaçú – SP	2009	Farmer	Florida
19.3	DGC 309.3	Mogi Guaçú – SP	2009	Farmer	Florida
19.4	DGC 309.4	Mogi Guaçú – SP	2009	Farmer	Florida
19.5	DGC 309.5	Mogi Guaçú – SP	2009	Farmer	Florida
19.6	DGC 309.6	Mogi Guaçú – SP	2009	Farmer	Florida
19.7	DGC 309.7	Mogi Guaçú – SP	2009	Farmer	Florida
19.8	DGC 309.8	Mogi Guaçú – SP	2009	Farmer	Florida
20.1	DGC 311.1	Mogi Mirim – SP - Brazil	2009	Farmer	cará
20.2	DGC 311.2	Mogi Mirim – SP – Brazil	2009	Farmer	cará
20.3	DGC 311.3	Mogi Mirim – SP – Brazil	2009	Farmer	cará
20.4	DGC 311.4	Mogi Mirim – SP – Brazil	2009	Farmer	cará
20.5	DGC 311.5	Mogi Mirim – SP – Brazil	2009	Farmer	cará
20.6	DGC 311.6	Mogi Mirim – SP – Brazil	2009	Farmer	cará
20.7	DGC 311.7	Mogi Mirim – SP – Brazil	2009	Farmer	cará
20.8	DGC 311.8	Mogi Mirim – SP – Brazil	2009	Farmer	cará
20.9	DGC 311.9	Mogi Mirim – SP – Brazil	2009	Farmer	cará
20.10	DGC 311.10	Mogi Mirim – SP - Brazil	2009	Farmer	cará

[1]SRT: germplasm from the Agronomic Institute (IAC); DGC: germplasm from USP-ESALQ; unknown variety name (SRT: germoplasma do IAC; DGC: germoplasma da USP-ESALQ; nome da variedade desconhecido).

Table 2. Polymorphism detected based on SSR primers, including annealing temperature (Temp), fragment size, allele (band) number (A), expected heterozigosity (H_E), Shannon-Wiener diversity index and polymorphism information content (PIC) when assessing 36 *Dioscorea alata* accessions (polimorfismo detectado, baseado em primers SSR, incluindo temperatura de anelamento (Temp), tamanho do fragmento, número (A) de alelos (bandas), heterozigosidade esperada (H_E), índice de diversidade de Shannon-Wiener e informação do conteúdo de polimorfismo (PIC) na avaliação de 36 acessos de *Dioscorea alata*). Piracicaba, ESALQ, 2011.

Primers	Temp (ºC)	Fragment size (bp)	*A*	H_E	Shannon-Wiener	PIC
A4[1]	53	140–180	4	0.69	1.25	0.65
B5[1]	60	100–145	5	0.52	0.82	0.61
C5[1]	58	210–270	5	0.78	1.56	0.77
E11[1]	55	165–190	6	0.78	1.59	0.74
F1[1]	58	145–205	4	0.71	1.31	0.66
A7[*]	52	215–250	7	0.79	1.65	0.75
H12[1]	53	310–325	4	0.68	1.18	0.60
E10[1]	58	190–250	6	0.68	1.18	0.62
9C[2]	58	140–180	4	0.62	1.08	0.57
Mean			5	0.69	1.29	0.66

[1]Siqueira *et al.* (2011) [2]Tostain *et al*. (2006); [*] Unpublished primer (F: GCCCCACCTTA-ATTTCAT; R: GGAATGAGATGGGACGAGAA).

high diversity might be due to the fact that this is a vegetatively propagated crop, which usually maintains high heterozygosity levels (Hancock, 2004). According to Obidiegwu *et al.* (2009b), yams are dioecious plants and spontaneous hybridization must have contributed to the ancestry of some of the accessions, although the selection of somatic mutants might have been the main source of variability used by farmers in their plant improvement practices.

PIC values ranged from 0.57 to 0.77, with an average of 0.66. The highest value was obtained for locus *C5* and the lowest, for locus *9C*. PIC refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency, and has been proven to be a general measure of how informative a marker is (Guo & Elston, 1999). PIC values demonstrated that the SSRs used in the current study presented, on average, high level of information. Similar PIC values were reported by Obidiegwu *et al.* (2009b) when assessing *D. alata* accessions with 13 loci (PIC= 0.65 on average, varying from 0.30 to 0.83) and by Obidiegwu *et al*. (2009c) when assessing *D. cayenensis/D. rotundata* accessions with 15 SSR loci (PIC= 0.65, on average, varying from 0.37 to 0.80).

The 36 accessions were divided in two groups by the cluster analysis, based on the bootstrap analysis (Figure 1). The first group (group I) mostly contained accessions from the IAC germplasm collection, such as SRT 24.0 (Sorocaba, originated from Sorocaba, São Paulo), SRT 80.0 (Branco Viçosa, originated from Minas Gerais), SRT 71.0 (Bira, originated from The Democratic Republic of the Congo), SRT 112.0 (Cará do Mato, originated from Mato Grosso do Sul), and an accession obtained in a market at Cuiabá, Mato Grosso.

The second group (group II) was composed by most of the accessions, including commercial cultivars from two growers in São Paulo (in the municipalities of Mogi Guaçu and Mogi Mirim). This group contained a mix of accessions obtained from markets in São Paulo municipalities, such as Piracicaba, Campinas, Matão, Botucatu, Araraquara, Fernandópolis and Santa Mercedes, and a few accessions from the IAC collection. SRT 29.0 accession (nº 7) originated from Puerto Rico, called Florida variety, was also included in group II. This variety was introduced in Brazil in 1947, and we do not know whether both 'Florida' and 'Florido' are the same variety, although both of them originated in Puerto Rico.

According to Martin *et al.* (1975), apud Lebot (2009), an international *D. alata* germplasm collection was maintained by the US Department of Agriculture in Mayaguez, Puerto Rico, in the 1970s, and more than 300 accessions collected throughout the tropics were evaluated regarding 100 traits in the field, at harvest and in the laboratory. This work resulted in the selection of five elite varieties, one of them being 'Florido'. The others were 'Forastero', 'Veeven', 'Gemelos' and 'Leone Globe'. 'Florido' was introduced into Ivory Coast from Puerto Rico in the early 1950s, and 'Florido' was widely adopted by farmers, spreading to other countries in West Africa. Reasons for Florido's popularity include its flexibility of planting time, good storage ability and postharvest, and tolerance to the internal brown spot disease (causal agent), which usually affects other *D. alata* varieties in Ivory Coast (Lebot, 2009). In the 1940's and 1950's many horticulture crops were launched by 'Instituto Agronômico de Campinas' (IAC). Clones of *D. alata* with resistance to fungal diseases, particularly to leaf blight (*Curvularia maculans*), which decimated the plantings in São Paulo, were made available to farmers. In this context, the clone 'Florida' (SRT 29.0) appealed to farmers for disease resistance and high productivity, which favored the expansion of its cultivation in São Paulo and in Brazil (Feltran, J.C., personal communication).

Historical records from IAC showed that two varieties, 'Florida' and 'Sorocaba', were released, due to their resistance to anthracnose and nematodes, and also commercial value. There are other *D. alata* cultivars commercialized in Brazil, known as 'São Tomé', 'Mandioca', 'Nambu', 'Roxo de Ilheus', 'Caipira', 'Purple de Ceilão', 'Paraná', and 'Nigéria' (Santos, 1996). 'Florida' is currently

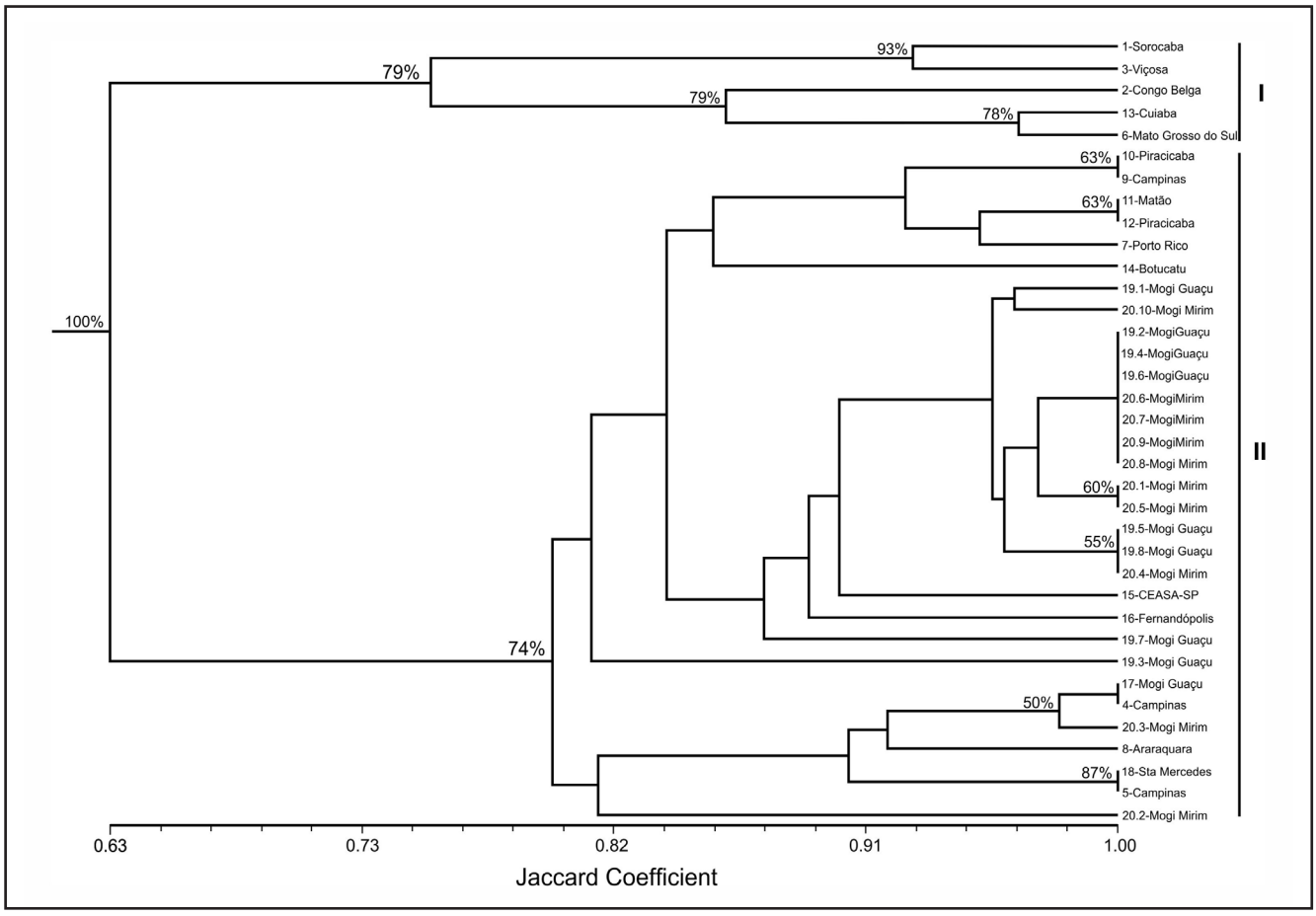

Figure 1. Dendrogram obtained by UPGMA method using Jaccard similarity coefficient and the bootstrap method (%), for 36 *Dioscorea alata* accessions (dendrograma obtido pelo método UPGMA usando o coeficiente de similaridade de Jaccard e o método bootstrap (%)). Piracicaba, ESALQ, 2011.

the most commercially accepted variety in São Paulo (Monteiro & Peressin, 2002). It has an adequate commercial value in Brazil, showing uniform tubers with smooth skin, although culinary characteristics are of lower quality when compared to 'Mimoso' (SRT 3.0; nº 4), which is no longer cultivated in the country (Monteiro & Peressin, 2002), because of its high susceptibility to anthracnose (causal agent *Colletotrichum gloeosporioides*). Therefore, it was no surprise to find in this study that most of the commercial accessions (clustered in group II), collected from several markets in Brazil, showed higher similarity (> 80%) to the most popular 'Florida' variety.

The accession acquired in a market at Cuiabá, classified in group I of the dendrogram (nº 13, Figure 1) bears great similarity to the IAC accession 'Cará do mato' (SRT 112.0; nº 6), originated from Mato Grosso do Sul. Therefore, the commercial variety obtained at a market in Cuiabá might have been originated from this IAC accession. Or it can also be inferred that both of them were originated from 'Sorocaba', which was also released in the country, according to the IAC records mentioned above.

A previous hypothesis was that the tubers collected from farms in Mogi Mirim and Mogi Guaçu would be genetically identical or clones. Agreeing with this hypothesis, several duplicates were found in this study, and the main duplicates belonged to the Mogi Mirim and Mogi Guaçu accessions, within group II. These duplicates were genetically similar (Figure 1) to nº 15 (CEASA) variety, which is a variety acquired at CEASA (Center for Distribution of Agricultural Products), in the city of São Paulo. Usually, water yam growers buy their seed-tubers directly from CEASA, and not from the nearest markets in their own municipalities. Since CEASA has a wider transportation system, it is cheaper for markets to buy yams directly from them rather than from the nearby small-scale farmer.

Another finding was that not all tubers from these two farms were genetically identical, since some of these tubers gave origin to plants that were clustered in distinct sub-groups, but all within group II. One of them (nº 20.3 in the dendrogram, Figure 1) was shown to be highly similar to the 'Mimoso' (SRT 3.0, nº 4 in the dendrogram), which was also shown to be a duplicate to an accession obtained from another farm in Mogi Guaçu (nº 17). These three accessions were genetically closer to SRT 89.0, from Araraquara ('Araraquara I'), from the IAC collection. Another accession from the IAC collection was

included in this sub-group, called variety 'cova Campinas' (SRT 84.0, nº 5), which was shown to be a duplicate of an accession collected in Santa Mercedes, São Paulo state.

Other duplicates were identified in the dendrogram, such as the commercial variety nº 10, from a market in Piracicaba and nº 9 from a market in Campinas. Other duplicates included commercial varieties collected in markets of Matão (nº 11) and Piracicaba (nº 12), also municipalities in São Paulo 170 km apart. These results show that genetically identical varieties are commercialized in different locations, at least in the state of São Paulo.

The cluster analysis conducted by Malapa *et al*. (2005) using AFLPs revealed the existence of three major groups of genotypes within *D. alata*, each assembling accessions from distant geographical origins and distinct ploidy levels. However, Lebot *et al*. (1998), examining the genetic relationship among 269 cultivars of *D. alata* from the South Pacific, Asia, Africa and the Caribbean with isozymes, concluded that the most widespread *D. alata* cultivars exhibited a narrow genetic base. Higher polymorphism was detected by Zannou *et al*. (2009) using RAPD markers, when assessing both *D. alata* and *D. cayenensis/D. rotundata* accessions from the Guinea-Sudan zone of Benin. These polymorphic DNA fragments were used to build dendrograms, clustering all accessions into 18 groups: 12 for *D. cayenensis/D. rotundata* and six for *D. alata*. Obidiegwu *et al.* (2009b), evaluating 89 accessions from nine African countries with SSR found non-distinction between country cultivars of *D. alata.* According to these authors, these accessions must have been distributed over great distances as clones during centuries of human migration and it is possible that some of them share common origins. The authors also stated that the majority of accessions within clusters are most likely clones of a common source. This observation agrees with our data, since most of the accessions in group II are probably clones originated from a common source. We also believe, as pointed by Obidiegwu *et al.* (2009b), that Brazilian farmers had an important role in the selection of somatic mutants, which probably had a great contribution to the range of diversity within these accessions.

The scatter graph resulting from the principal coordinate analysis (data not show), in which the first two principal coordinates accounted for 87.4% of total variance, agreed with the clustering of genotypes presented in the dendrogram, allowing the visualization of the two groups previously mentioned. In a three dimensional plot with 89 *D. alata* accessions from nine countries, Obidiewgu *et al*. (2009b) identified eight groups although the authors underlined that there was no relationship between relatedness of the accessions and their geographical area of collection.

Here, the use of microsatellite markers allowed the identification of considerable genetic variability among 36 *D. alata* accessions. Results provided more information concerning the genetic origin of *D. alata* commercial varieties currently cultivated in Brazil, and about their market distribution. According to Dansi *et al*. (2000), assessment on genetic diversity within *Dioscorea* spp. will serve as an instrument to identify cultivar misclassification, help to understand the relationships between cultivars, and assist in identifying putative duplicates towards the establishment of an accurate core collection. However, additional studies, involving a higher number of accessions and microsatellite markers, should be accomplished to better understand how the germplasm of this species is structured and organized, as well as the origins of commercial varieties cultivated in Brazil. These SSR loci represent an important molecular tool to build genetic profiles of yam cultivars, showing potential to be used in plant breeding programs and in *ex situ* yam conservation programs as well.

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