Genetic variability in commercial varieties of water yam with microsatellites markers

Marcos V.B.M. Siqueira[1*], Gabriel Dequigiovanni[2], José Carlos Feltran[3], Elizabeth A. Veasey[1]

[1]Genetic Department, "Luiz of Queiroz" College of Agriculture, Piracicaba, Brazil; [2]Rio Grande do Sul Federal University, Bento Gonçalves, Brazil; [3]Agronomic Institute, Campinas, Brazil; *E-mail: mvbms@esalq.usp.br.

Abstract

This study aimed at evaluating the genetic diversity of commercial water yam varieties with microsatellite markers. Yam is the fourth more important crop from the tuber and root crops in the world. We assessed 27 varieties of *Dioscorea alata*, collected in markets of several cities in Brazil, and from the germplasm collection of the Agronomic Institute (IAC), in Campinas, SP. CTAB 3% method was used for DNA extraction. We used 12 microsatellite primers for the amplification reactions. The amplified material was separated on 6% polyacrylamide gels and stained with silver nitrate. We performed clustering analysis and principal coordinates, using the NTSYS-pc software. High polymorphism was found among loci verified by the high PIC values (0.46 - 0.87). The accessions were classified in two main groups, showing great genetic variability, verified by the magnitude of the Jaccard's similarity coefficient (0.29 to 1.0). One of the main groups ranked most of the IAC's collection, with accessions originating from Africa, the Belgian Congo, being similar to varieties marketed today. In the second group, there was a high similarity of an accession originated from Puerto Rico, which led to the Florida variety, the most planted now-a-days, with several commercial varieties. The results, therefore, suggest the hypothesis of separate ancestry of the varieties currently under cultivation in Brazil. Four duplicates were found with accessions acquired in different cities, revealing the marketing of the same variety in different locations. The analysis of principal coordinates, with the first two coordinates explaining 49.2% of total variation, confirmed the groupings formed.

Keywords: *Dioscorea alata*, SRR, genetic diversity, germplasm bank.

Introduction

Yam is one of the most important crops in the tropics and subtropics, being mainly cultivated in the West and Central regions of Africa, where it is also widely consumed (Mignouna et al., 2005). It belongs to the Dioscoreaceae family and *Dioscorea* genus, which contains approximately 600 species, where only 10 are economically viable for consumption (Pedralli, 2002).

The world-wide yam production, estimated in 2005, was approximately 44.3 million tons, with Nigeria being the largest world-wide producer. The remaining is produced mainly in Latin America, the Melanesia and Japan (FAO, 2005). The Brazilian yam production is concentrated in the Northeast and Southeast regions, in the states of Paraíba, Rio de Janeiro, Minas Gerais, Pernambuco, Espírito Santo and São Paulo, responsible for approximately 90% of total national yield (Mesquita, 2001). In the Southeast region, 83.766 tons of *Dioscorea alata* L. were produced in the year 2000, and this number was not higher due to gaps in public and private sector which do not allow the markets supply and a quality standard of these tubers to be reached (Mascarenhas and Resende, 2002).

Studies with molecular markers, including microsatellites (SSR), have become an important tool for the identification of cultivars, and mainly for population genetics studies (Caixeta et al., 2006). Besides, since this marker is, in general, more abundant in the majority of the genomes, possessing a high informative content, it becomes interesting to use it as tool for the assessment of distinct yam (*D. alata*) varieties. Microsatellite primers have already been developed for a few *Dioscorea* species (Hochu et al., 2006; Bousalem et al., 2006), including *D. alata* (Tostain et al., 2006). Fundamentally, SSR markers have been used in studies of segregation patterns and genetic characterization of accessions of *Dioscorea* species (Terauchi and Konuma, 1994; Mignouna et al., 2003a; Mignouna et al., 2003b; Scarcelli et al., 2005).

This study aimed to access the genetic diversity of 27 yam commercial varieties obtained in fairs, markets and germplasm banks using SSR markers, in order to verify the level of genetic vulnerability. The work aimed to contribute for *in situ* and *ex situ* conservation strategies, emphasizing the role of local breeders for the propagation and maintenance of this crop.

Materials and methods

This study used 27 accessions belonging to the germplasm collections from "Luiz of Queiroz" College of Agriculture/São Paulo University (ESALQ/USP), in Piracicaba, SP, and the Agronomic Institute (IAC), in Campinas, SP. The accessions were obtained from different municipalities in Brazil, and also from Puerto Rico, Singapore and Belgian Congo (Table 1).

For DNA extraction, the Siqueira et al. (2009) methodology, based in CTAB extraction buffer, with modifications, was used. Thirteen microsatellite loci (primers), based on Tostein et al. (2006) and Mignouna et al. (2003) were used. The polymerase chain reactions (PCR) were conducted in a final volume of 10.2 µL, in accordance with Siqueira et al. (2009). The reactions were accomplished in the thermocycler BioRad®, MyCycler® model, in the following amplification conditions: initial denaturalization at 94ºC for 5 min, followed by 35 cycles of denaturalization at 94ºC for 30 s, 1 min at the defined annealing temperature for each primer (Table 1), and 1 min at 72ºC, with a final extension at 72ºC for 8 min (Tostein et al., 2006). The amplification products were submitted to electrophoresis in a polyacrylamide gel (6%) using a silver staining procedure (Bassam et al., 1991).

Table 1. List of the 27 *Dioscorea alata* accessions used in this study, including their origin and common names

Germplasm Number[1]	Origin (municipalities/state/country)	Introduction year	Origin	Variety name
SRT 3.0	Campinas – SP - Brazil	1936	IAC	Mimoso
SRT 24.0	Sorocaba – SP – Brazil	1947	IAC	Sorocaba
SRT 29.0	Puerto Rico	1947	IAC	Flórida
DGC 36.0	Iguape – SP – Brazil	2002	Local market	---
DGC 38.0	Araras – SP – Brazil	2002	Local market	---
DGC 40.0	Piracicaba – SP – Brazil	2002	Local market	---
DGC 43.0	Matão – SP – Brazil	2002	Local market	---
DGC 45.0	Campinas – SP – Brazil	2002	IAC	---
DGC 46.0	Piracicaba – SP – Brazil	2002	Local market	---
SRT 66.0	Congo Belga – Jamgambi	1949	IAC	Angola II
SRT 71.0	Congo Belga – Jamgambi	1949	IAC	Bira
SRT 75.0	Congo Belga – Jamgambi	1949	IAC	Leno Dandino
SRT 78.0	Singapore	1949	IAC	Singapura roxo
SRT 80.0	Minas Gerais – Brazil	1949	IAC	Branco Viçosa
SRT 84.0	Campinas – SP – Brazil	1951	IAC	Cova Campinas
SRT 89.0	Araraquara – SP – Brazil	1959	IAC	Araraquara I
DGC 97.0	Cuiabá – Mato Grosso – Brazil	2006	Municipal market	---
DGC 107.0	Botucatu – SP – Brazil	2006	Local market	---
SRT 112.0	Mato Grosso do Sul – Brazil	2000	IAC	Cará do Mato
DGC 115.0	Fortaleza – CE – Brazil	2006	Municipal market	---
DGC 116.0	Cuiabá – Mato Grosso – Brazil	2006	Municipal market	---
DGC 123.0	Mogi-Guaçu – Brazil	2006	Small farmer	---
DGC 124.0	Campo Grande – Mato Grosso – Brazil	2007	Municipal market	---
DGC 127.0	Santa Mercedes – SP – Brazil	2007	Small farmer	---
DGC 128.0	Belo Horizonte – Minas Gerais – Brazil	2007	Municipal market	---
DGC 129.0	Espírito Santo – Brazil	2007	Municipal market	---

DGC 132.0	Fernandópolis – SP - Brazil	2007	Local market	---

[1]SRT – germplasm from the Agronomic Institute (IAC); DGC – germplasm from ESALQ/USP.

For the statistical analysis a similarity matrix was obtained for the 27 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) (Sneath and Sokal, 1973) method, cluster analyzes were accomplished, using the NTSYSpc 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). An analysis of principal coordinates was also accomplished with the NTSYSps software (Rohlf, 1992). The number of alleles per loci as well as the Polymorphism Information Content (PIC) were also determined.

Results and discussion

In this study, all 13 loci (primers) used showed polymorphism between the analyzed accessions, producing well definite and reproducible fragments. A total of 83 alleles (fragments) were amplified with an average of 7 alleles per loci (Table 2). The highest number of alleles (10) was verified for loci Dab2E07, and the lower number (4) was found for primers Da1D08 and YM-28. The polymorphism information content (PIC) values varied from 0.87 to 0.46, with 0.71 on average. The highest value was obtained for primer Dab2E07 and the lower value for primer Da1D08, demonstrating that the SSRs used in the present study presented, on average, a high level of information.

Table 2. Polymorphism detected based on SSR primers, including allele number, annealing temperature, fragment size and polymorphism information content (PIC) when assessing 27 *Dioscorea alata* accessions

Primers	Allele number	Annealing temperature (°C)	Fragments size (pb)	PIC
Dpr3D06	8	56	140 – 180	0.7206
Dpr3F04	8	51	100 – 145	0.7372
Da1A01	7	50	210 – 270	0.7562
Da1C12	5	55	165 – 190	0.6932
Dab2C05	9	50	145 – 205	0.8073
Dab2D06	8	50	260 – 325	0.8041
Da1D08	4	50	310 – 325	0.4629
Dab2E07	10	50	150 – 220	0.8696
Da1F08	9	53	190 – 250	0.6905
YM-19	5	50	230 – 255	0.6538
YM-28	4	50	380 – 440	0.5834
DprF3F12	6	55	155 – 200	0.7836
Dpr3D06	8	56	140 – 180	0.7206
Mean	7	-	-	0.7141

In the cluster analysis (Figure 1), the Jaccard's similarity coefficient varied from 0.29 to 1.0, indicating the existence of a significant genetic variability among the accessions. This genetic variability is divided in two main groups: the first group represented, in its majority, the genetic material of IAC collection, presenting two distinct sub-groups: sub-group 1, including varieties Mimoso, Sorocaba, Branco Viçosa, Bira and Leno Dandino, the two last accessions originating from Belgian Congo; sub-group 2, which includes cultivar Angola II, also originating from Belgian Congo, grouped with 99.8% similarity with two commercial varieties obtained in markets in the state of São Paulo (in the cities of Matão and Piracicaba), indicating that, most probably, these commercial varieties originated from the Angola II cultivar. Sub-group 2 was also constituted by varieties Cova Campinas (mutant variety), and Cará do Mato (originating from Mato Grosso do Sul), both from the IAC germplasm bank, besides a commercial variety collected in Cuiabá.

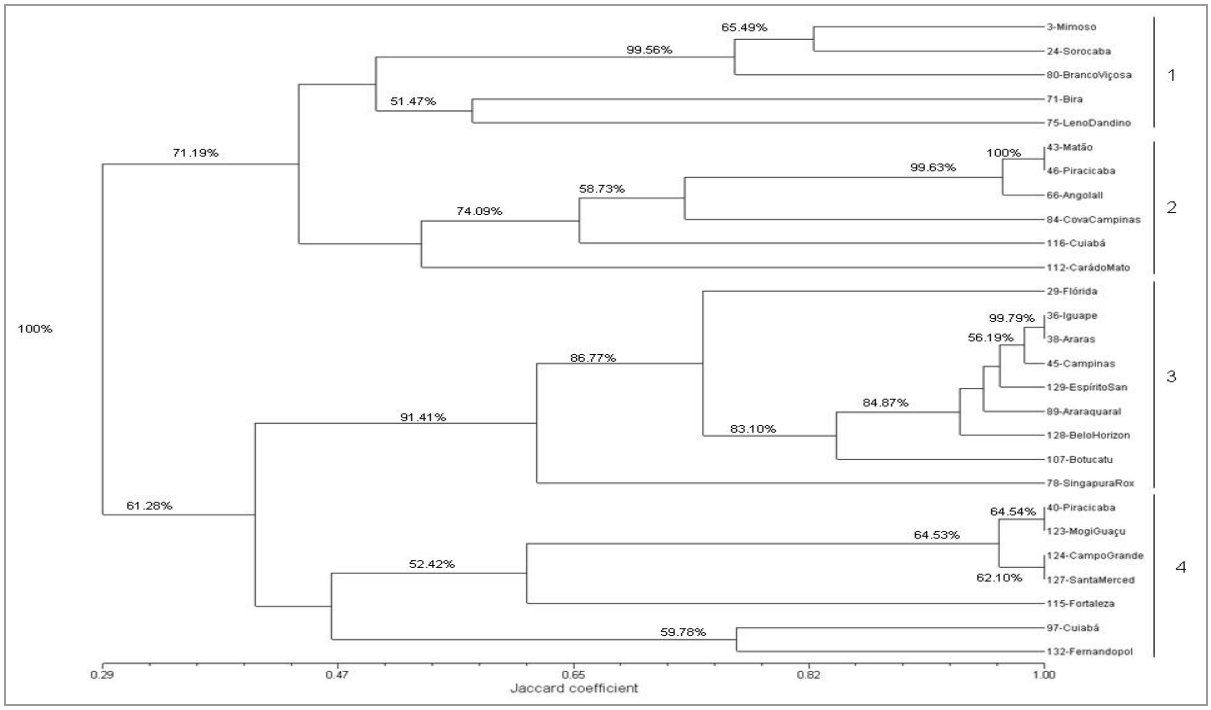

Figure 1. Dendrogram obtained by UPGMA method, Jaccard similarity coefficient and the Bootstrap method (percentages) for 27 *Dioscorea alata* accessions

The second main group, including two sub-groups (sub-group 3 and sub-group 4), is represented in its majority by commercial varieties obtained in Brazilian markets, with the exception of two genetic materials from IAC collection (SRT 29 from Puerto Rico and SRT 78 from Singapore). Sub-group 3 includes Florida variety, introduced in the 1950s and currently the most accepted commercially in São Paulo State (Monteiro and Peressin, 2002), which showed around 70% similarity to a group of commercial varieties obtained in markets in the cities of Iguape, Araras, Campinas, Araraquara, Botucatu, Rio de Janeiro and Belo Horizonte. Therefore, the results indicate that these varieties were probably originated from Florida variety, originally derived from Puerto Rico (Abramo, 1990). This sub-group also includes, although with lower similarity (<65%), the Purple Singapore variety (from IAC), introduced from Singapore in 1949.

Sub-group 4 includes accessions with high similarity level, obtained in Piracicaba, Mogi Guaçu (São Paulo), Campo Grande (Mato Grosso do Sul) and Santa Mercedes (São Paulo), which were grouped, with around 60% similarity, with a variety collected in Fortaleza, Ceará State. This group includes two other commercial varieties, one collected in Cuiabá, Mato Grosso and another in Fernandópolis, São Paulo. Similarly, the principal coordinates scatter graph (Figure 2) agrees with the data presented in the dendrogram, allowing the visualization of the two main groups and the four sub-groups previously mentioned.

Accessions with high similarity level, including materials from distinct regions, were observed in this study, showing the cultivation of the same variety in diverse cities of Brazil or the marketing of the same variety in several places, most probably through CEASA (market supply and distribution center) located in several municipalities of several Brazilian states. Also, it was possible to evidence that materials deriving from other countries, such as Puerto Rico, Singapore and Africa, including Belgian Congo, formed independent groups, which can lead to the hypothesis of distinct ancestry for the varieties commercialized today in Brazil.

High levels of polymorphism were presented by *D. alata* in this study, considering that each allele is considered a unique character and, being a tetraploid species, each individual can present one to four different alleles in each loci. Additionally, the high levels of polymorphism suggest that this molecular marker can be a useful tool with a high precision in the detection of genetic differences between cultivars. However, additional studies, involving a

higher number of accessions, shall be accomplished aiming to a better understanding on how this species germplasm is structured and organized, and on the origins of the cultivated commercial varieties in Brazil.

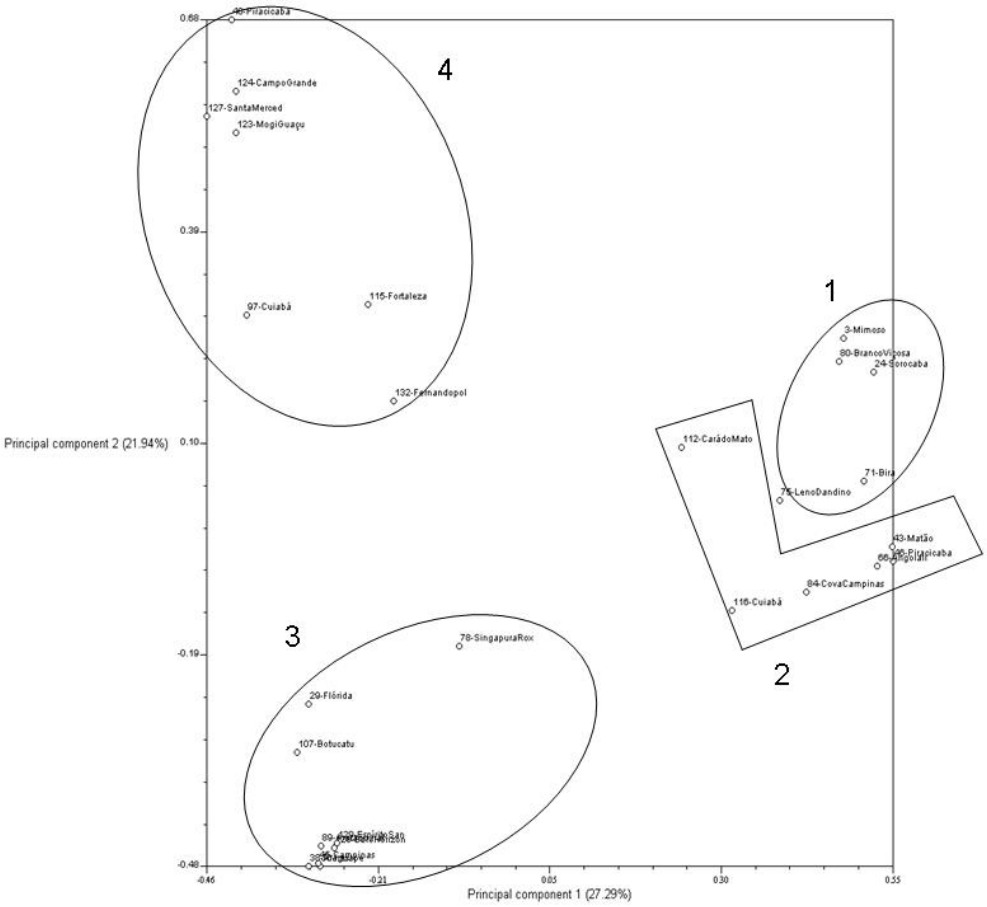

Figure 2. Scatter graph of 27 yam (*Dioscorea alata* L.) accessions obtained from a principal coordinate analysis

Conclusions

Microsatellite markers allowed the identification of high genetic variability in the yam (*D. alata*) accessions originated from two germplasm collections. These markers represent an important tool for the construction of genetic profiles of yam cultivars, showing potential to be used in plant breeding programs and in *ex situ* yam conservation programs as well. The results allowed to infer on the genetic origin of the commercial varieties currently cultivated in Brazil, and on its market distribution.

Acknowledgements

The authors wish to thank FAPESP and CAPES for the financial support and grants given for this research.

References

ABRAMO, M.A. 1990. Taioba, cará, inhame: o grande potencial inexplorado. São Paulo: Ícone. 80p.

AYRES. M.; AYRES,-JR. M.; AYRES, D. L.; SANTOS, A. S. 2005. BioEstat 4.0: Aplicações estatísticas nas áreas das ciências biológicas e médicas. Ed. Sociedade Civil Mamirauá / MCT / Imprensa Oficial do Estado do Pará. Belém. 323p.

Bassam, B.J.; Caetano-Anolles, G.; Gresshoff, P.M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196, 80-83.

Bousalem, M.; Arnau, G.; Hochu, I.; Arnolin, R.; Viader, V.; Santoni, S.; David, J. 2006. Microsatellite segregation analysis and cytogenetic evidence for tetrasomic inheritance in the American yam *Dioscorea trifida* and a new basic chromosome number in the Dioscoreae. Theor Appl Genet 113, 439–451.

Caixeta, E.T.; Oliveira, A.C.B. de; Brito, G.G. de; Sakiyama, N.S. 2006. Tipos de marcadores moleculares. In: Borém, A.; Caixeta, E.T. (Ed.). Marcadores moleculares. Viçosa: UFV.

Coelho, A.S.G. 2001. BOOD: avaliação de dendrogramas baseados em estimativas de distâncias/similaridades genéticas através do procedimento de bootstrap, v. 2.0. Goiânia: Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Biologia Geral.

Fao. 2005. Food And Agriculture Organization Of The United Nations. Disponível em: <www.fao.org>. Acesso em: 28 de Março de 2009.

Hochu, I.; Santoni, S.; Bousalem, M. 2006. Isolation, characterization and cross-species amplification of microsatellite DNA loci in the tropical American yam *Dioscorea trifida.* Mol Ecol Notes, 6, 137–140.

Jatasra, D.S. Paroda, R.S. 1983. Genetic divergence in wheat. Indian J Genet Plant Breed, v. 43, p. 63-67.

Mascarenhas, M.H.T.; Resende, L.M.A. 2002. Situação atual e perspectivas das culturas do taro e do inhame no estado de Minas Gerais**.** In: Carmo, C.A.S. (ed). *Inhame e taro: sistemas de produção familiar*. Vitória: Incaper. p. 61-71.

Mesquita, A.S. 2001. Inhame na Bahia: a produção no caminho da competitividade. Bahia Agrícola, v.4, n.2, p.39-48.

Mignouna, H.D.; Abang, M.M.; Fagbemi, S.A. 2003a. A comparative assessment of molecular marker assays (AFLP, RAPD and SSR) for white yam (*Dioscorea rotundata*) germplasm characterization. Ann Appl Biol 142, 269-276.

Mignouna, H.D; Abang, M.M.; Asiedu, R. 2003b. Harnessing modern biotechnology for tropical tuber crop improvement: Yam (Dioscorea spp.) molecular breeding. Afr J Biotechnol 2, 478-485.

MIGNOUNA, H. D.; ABANG, M.M.; WANYERA, N.W.; CHIKALEKE, V.A.; ASIEDU, R.; THOTTAPPILLY, G. 2005. PCR marker-based analysis of wild and cultivated yams (*Dioscorea* spp.) in Nigeria: genetic relationships and implications for *ex situ* conservation. Genet. Resour. Crop Ev, v. 52, p. 755-763.

Monteiro, D.A.; Peressin, V.A. 2002. Cultura do inhame. In: Cereda, M.P. (Ed.). Agricultura: tuberosas amiláceas Latino Americanas. São Paulo: Fundação Cargill, p. 511-522.

MONTALDO, A. (1991). Cultivo de raíces y tubérculos tropicales. Lima: Instituto Interamericano de Ciências Agrícolas de la OEA, p. 91-127.

PEDRALLI, G. 2002. Distribuição geográfica e taxonomia das famílias Araceae e Dioscoreaceae no Brasil. In: Carmo, C.A.S. (Ed.) Inhame e taro: sistemas de produção familiar. Vitória, ES: Incaper. p.15-26.

Rafalski, D.J.A.; Vogel, J.M.; Morgante, M.; Powell, W.; Andre, S.; Tingey, S.V. 1996. Generating and using DNA markers in plant. In: Birren, B.; Lai, E. Non mammalian genomic analysis: a pratical guide. New York: p. 75-134.

Scarcelli, N.; Daïnou, O.; Agbangla, C.; Tostain, S.; Pham J.L. 2005. Segregation patterns of isozyme loci and microsatellite markers show the diploidy of African yam *Dioscorea rotundata* (2n=40). Theor Appl Genet 111, 226-232.

Siqueira, M.V.B.M., Queiroz-Silva, J.R., Bressan, E.A., Borges, A., Pereira, K.J.C., Pinto, J.G.; Veasey, E.A. 2009. Genetic characterization of cassava (*Manihot esculenta*) landraces in Brazil assessed with simple sequence repeats. Genet Mol Biol, v.32, p.104-110.

SNEATH P.H.A; SOKAL R.R. 1973. Numerical Taxonomy*.* San Francisco: Freeman. 573 pp.

Terauchi, R.; Konuma, A. 1994. Microsatellite polymorphism in *Dioscorea tokoro*, a wild yam species. Genome, 37, 794–801.

TOSTEIN, S.; SCARCELLI, N.; BROTTIER, P.; MARCHAND, J.L.; PHAM, J.L.; NOYER, J.L. 2006. Development of DNA microsatellite markers in tropical yam (*Dioscorea* sp.). Mol Ecol Notes, v. 6, p. 173-175.