GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Establishment and molecular characterization of a sweet potato germplasm bank of the highlands of Paraná State, Brazil

L.K.P. Camargo[1,2], A.F. Mógor[2], J.T.V. Resende[1] and P.R. Da-Silva[3]

[1]Departamento de Agronomia, Universidade Estadual do Centro-Oeste, Guarapuava, PR, Brasil
[2]Departamento de Fitotecnica, Universidade Federal do Paraná, Curitiba, PR, Brasil
[3]Laboratório de Genética e Biologia Molecular Vegetal, Departamento de Ciências Biológicas, Universidade Estadual do Centro-Oeste, Guarapuava, PR, Brasil

Corresponding author: P.R. Da-Silva
E-mail: prsilva@unicentro.com

Genet. Mol. Res. 12 (4): 5574-5588 (2013)
Received November 1, 2012
Accepted March 11, 2013
Published November 18, 2013
DOI http://dx.doi.org/10.4238/2013.November.18.7

ABSTRACT. The sweet potato (*Ipomoea batatas* L.) is a crop of great importance in developing countries, as a food staple, for animal feed, and potentially for biofuel. Development of cultivars adapted to specific regions within these countries would be useful. To start a breeding program, the first step is the establishment of a germplasm bank. We initiated a sweet potato germplasm bank with accessions collected from the highlands of Paraná State, Brazil. To establish this germplasm bank, we carried out numerous sweet potato-collecting expeditions in regions with an altitude above 700 meters in this region; 116 genotypes currently comprise this collection. The genetic diversity of this germplasm bank was estimated using inter simple sequence repeat (ISSR) markers. Polymorphic information content (PIC), marker index (MI), and resolving power (RP) were calculated to determine the

viability of ISSR markers for use in sweet potato genetic studies. The correlation between PIC and MI ($r^2 = 0.81$) and between MI and RP ($r^2 = 0.97$) were positive and significant, indicating that ISSR markers are robust for sweet potato identification. Two ISSR primers, 807 and 808, gave the best results for all attributes, and thus could be used as representative ISSR primers for the genetic analysis of sweet potato. Cluster analysis and principal component analysis indicated high genetic variability (0.51 of similarity among all genotypes); genotypes collected from different counties grouped together.

Key words: *Ipomoea batatas* L.; Plant breeding; Genetic variability; Biofuel

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is among the most important foods in the world. As a food crop, it ranks fifth in importance in developing countries, after rice, wheat, maize, and cassava (CIP - Centro Internacional de La Papa, 2008). In South America, Brazil is the main producer, and the southern region is responsible for 50.44% of the national production (Suita de Castro et al., 2009). The sweet potato is easy to grow and has high nutritional value; these characteristics make sweet potato cultivation of high social value, contributing decisively to the food supply of poor populations.

The constant search for healthy foods has recently distinguished sweet potato as a nutraceutical food because of its dietary fiber composition and antioxidant compounds, such as beta-carotene and anthocyanins (Lin and Chang, 2005). Another promising niche for sweet potato has emerged with the growing importance of clean fuels that are not derived from petroleum. According to the Brazilian Association of Producers of Cassava Starch, ethanol production from sweet potato promises to not only meet the needs of the biofuel industry, but also the chemical industry, especially pharmaceutical and cosmetic, because of the high-quality alcohol generated from sweet potato (ABAM - Associação Brasileira dos Produtores de Amido e Mandioca, 2007).

Elite cultivars have low genetic variability when compared with wild genotypes. This happens because the breeding process prioritizes the selection of a few genetic characteristics of agricultural importance. One strategy for preserving genetic variability which may be important for future use is the establishment of germplasm banks (Ritschel et al., 2000). A germplasm bank aims to conserve plant genetic resources under semi-natural conditions to ensure the availability of genes for sustainable plant breeding for the purpose of providing food for future generations.

In the State of Paraná, South of Brazil, the second plateau region is located in a strip that bisects the state from northeast to south-central (Figure 1). In this region, topographic relief is very irregular, with altitudes over 700 m (Figure 1). This altitude gives the region a climate different from others of the state. The irregular topography makes it impossible to use machinery, so large cattle farms and family farms cultivated primarily by animal power predominate.

These features make this an agricultural region in need of crops adapted to the local climate and to the available manpower. In addition to differences in climate and topography,

this region has one of the lowest HDIs of the state, and of Brazil. The researchers and breeders of UNICENTRO, a public university maintained by the state government and located on the second plateau of Paraná, have sought to identify crops that influence the economic and social conditions of the region. Being a family crop which requires little technology for cultivation, the sweet potato fits this goal. Regionally adapted cultivars with good quality and yields can help in improving the social conditions of the population. In this context, the need for a sweet potato breeding program is evident; and to start a breeding program it is essential to obtain a core germplasm that has good adaption to the farming region and with a known level of genetic variability.

In breeding programs of sweet potato, understanding the genetic variability within the germplasm bank is important because it can prevent the narrowing of the crop's genetic base and allow breeders to make better use of the available variability. Studies of genetic diversity are considered more reliable when they utilize molecular markers because they cover a large part of the genome and are not influenced by the environment (Goulão and Oliveira 2001; Morales et al., 2011a,b). In sweet potato, the most widely used molecular markers are simple sequence repeats (SSR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and inter-simple sequence repeats (ISSR). ISSR markers have high repeatability compared with RAPD, require no prior knowledge of the genome as do SSR markers (Joshi et al., 2000), and are simpler to perform than AFLP markers, where they are based solely on PCR and resolvable on an agarose gel.

The aim of this study was the establishment and molecular characterization of a sweet potato germplasm bank for the development of cultivars adapted to the highlands. We discuss aspects of the establishment of the germplasm bank, the attributes and efficiency of ISSR markers used in the genetic characterization of the bank, and the observed genetic variability.

MATERIAL AND METHODS

Establishing a sweet potato germplasm bank of the highlands (SPGBH)

Collecting expeditions in various regions of Paraná State, Brazil were conducted for the purpose of starting a sweet potato breeding program for the development of cultivars adapted to high altitude. We collected genotypes of sweet potato from the counties Coronel Vivida, Guarapuava, Imbituva, Laranjeiras do Sul, Palmeira, Pinhão, Prudentópolis, Quedas do Iguaçu, and Turvo (Figure 1). All sites sampled were located over 700 m above sea level. The sampled area corresponds to approximately 37,000 km^2, or 18% of the territory of Paraná State, and 60% of the area above 700 m elevation in the state. In addition to the collected genotypes, part of the germplasm bank is comprised of clones and cultivars from other regions of Brazil (Figure 1). These clones and cultivars were collected and/or developed in regions of high altitude.

The sweet potato genotypes were kept in a greenhouse, planted in pots of 4 dm^3, filled with 50% natural soil, and 50% Plantmax® substrate. Drip irrigation was applied once a day for 20-25 min in summer and for 5-10 min in winter. Phytosanitary control was performed when necessary. Fertilization was 50 g 04-14-08 fertilizer every 60 days as recommended for sweet potato (Silva et al., 2004).

Molecular characterization of the SPGBH

DNA extraction

Genetic characterization with ISSR molecular markers was performed on 40 genotypes of sweet potato from the SPGBH. These genotypes are representative of most areas sampled (Table 1).

Table 1. Genotypes that comprise the Sweet Potato Germplasm Bank from Highlands (SPGBH) of Paraná State.

Code	County of origin	State	Region
UGA19	Coronel Vivida	PR	South
UGA20	Coronel Vivida	PR	South
UGA27	Coronel Vivida	PR	South
UGA31	Coronel Vivida	PR	South
UGA32	Coronel Vivida	PR	South
UGA79	Coronel Vivida	PR	South
UGA80	Coronel Vivida	PR	South
UGA81	Coronel Vivida	PR	South
UGA84	Coronel Vivida	PR	South
UGA89	Coronel Vivida	PR	South
UGA96	Coronel Vivida	PR	South
UGA102	Coronel Vivida	PR	South
UGA112	Coronel Vivida	PR	South
UGA8	Guarapuava	PR	South
UGA9	Guarapuava	PR	South
UGA25	Guarapuava	PR	South
UGA36	Guarapuava	PR	South
UGA62	Guarapuava	PR	South
UGA88	Guarapuava	PR	South
UGA90	Guarapuava	PR	South
UGA93	Guarapuava	PR	South
UGA95	Guarapuava	PR	South
UGA97	Guarapuava	PR	South
UGA101	Guarapuava	PR	South
UGA106	Guarapuava	PR	South
UGA110	Guarapuava	PR	South
UGA16	Imbituva	PR	South
UGA48	Imbituva	PR	South
UGA60	Imbituva	PR	South
UGA67	Imbituva	PR	South
UGA68	Imbituva	PR	South
UGA69	Imbituva	PR	South
UGA71	Imbituva	PR	South
UGA73	Imbituva	PR	South
UGA74	Imbituva	PR	South
UGA1	Laranjeiras do Sul	PR	South
UGA2	Laranjeiras do Sul	PR	South
UGA11	Laranjeiras do Sul	PR	South
UGA12	Laranjeiras do Sul	PR	South
UGA14	Laranjeiras do Sul	PR	South
UGA15	Laranjeiras do Sul	PR	South
UGA18	Laranjeiras do Sul	PR	South
UGA39	Laranjeiras do Sul	PR	South
UGA42	Laranjeiras do Sul	PR	South
UGA43	Laranjeiras do Sul	PR	South
UGA45	Laranjeiras do Sul	PR	South
UGA49	Laranjeiras do Sul	PR	South
UGA52	Laranjeiras do Sul	PR	South
UGA59	Laranjeiras do Sul	PR	South

Continued on next page

Table 1. Continued.

Code	County of origin	State	Region
UGA76	Laranjeiras do Sul	PR	South
UGA78	Laranjeiras do Sul	PR	South
UGA83	Laranjeiras do Sul	PR	South
UGA22	Palmeira	PR	South
UGA41	Palmeira	PR	South
UGA61	Palmeira	PR	South
UGA91	Palmeira	PR	South
UGA98	Palmeira	PR	South
UGA105	Palmeira	PR	South
UGA108	Palmeira	PR	South
UGA23	Pinhão	PR	South
UGA28	Pinhão	PR	South
UGA38	Pinhão	PR	South
UGA44	Pinhão	PR	South
UGA47	Pinhão	PR	South
UGA51	Pinhão	PR	South
UGA53	Pinhão	PR	South
UGA56	Pinhão	PR	South
UGA57	Pinhão	PR	South
UGA58	Pinhão	PR	South
UGA65	Pinhão	PR	South
UGA66	Pinhão	PR	South
UGA70	Pinhão	PR	South
UGA82	Pinhão	PR	South
UGA86	Pinhão	PR	South
UGA87	Pinhão	PR	South
UGA99	Pinhão	PR	South
UGA109	Pinhão	PR	South
UGA111	Prudentópolis	PR	South
UGA113	Prudentópolis	PR	South
UGA114	Prudentópolis	PR	South
UGA115	Prudentópolis	PR	South
UGA116	Prudentópolis	PR	South
UGA34	Quedas do Iguaçu	PR	South
UGA37	Quedas do Iguaçu	PR	South
UGA40	Quedas do Iguaçu	PR	South
UGA72	Quedas do Iguaçu	PR	South
UGA64	Quedas do Iguaçu	PR	South
UGA26	Turvo	PR	South
UGA30	Turvo	PR	South
UGA33	Turvo	PR	South
UGA35	Turvo	PR	South
UGA46	Turvo	PR	South
UGA50	Turvo	PR	South
UGA54	Turvo	PR	South
UGA63	Turvo	PR	South
UGA75	Turvo	PR	South
UGA77	Turvo	PR	South
UGA85	Turvo	PR	South
UGA94	Turvo	PR	South
UGA100	Turvo	PR	South
UGA21	Brasília	DF	Midwest
UGA13	Brasília	DF	Midwest
UGA4	Palmas	TO	North
UGA5	Palmas	TO	North
UGA7	Palmas	TO	North
UGA92	Palmas	TO	North
UGA104	Lavras	MG	Southeast

Continued on next page

Table 1. Continued.

Code	County of origin	State	Region
UGA3	Lavras	MG	Southeast
UGA6	Lavras	MG	Southeast
UGA10	Lavras	MG	Southeast
UGA17	Lavras	MG	Southeast
UGA24	Lavras	MG	Southeast
UGA29	Lavras	MG	Southeast
UGA55	Lavras	MG	Southeast
UGA103	Lavras	MG	Southeast
UGA107	Lavras	MG	Southeast

The shaded genotypes are those used for molecular analyses.

Tissue from young leaves collected before sunrise was used for DNA extraction. The protocol of Sharma et al. (2008), with some modification, was applied. The tissue was ground into a fine powder in liquid nitrogen; 1 mL extraction buffer (20 mM EDTA, 100 mM Tris-HCl, pH 8.0, 2 M NaCl, 2% CTAB, 2% PVP, 2% β-mercaptoethanol) was added to a tube with 100 mg tissue and kept in a water bath at 65°C for 30 min. The DNA was separated from the solution by precipitation with phenol:chloroform:isoamyl alcohol (25:24:1) and centrifugation. Successive rounds of precipitation with PEG (polyethylene glycol) and washes with alcohol were performed to obtain a highly pure DNA. After extraction, the DNA was resuspended in TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), treated with RNase A at 37°C for 30 min, and stored at -20°C until use.

ISSR analysis

For molecular analyses, DNA from each genotype was amplified by polymerase chain reaction (PCR) using 10 ISSR primers (Table 2). PCR was performed in a final volume of 12.5 µL (20 ng DNA, 1X PCR buffer, 50 mM $MgCl_2$, 2.5 mM dNTPs, 10 mM primer, and 1 U Taq DNA polymerase). The programming of the thermocycler for DNA amplification was as follows: 4 min at 94°C; 35 cycles of 30 s at 94°C, 45 s at the primer annealing temperature (which varied according to each primer, Table 2), 90 s at 72°C; and a final step of 7 min at 72°C for complete amplicon extension. The amplification products were separated by electrophoresis on a 1.2% agarose gel. The amplicons were stained with ethidium bromide and visualized under ultraviolet light.

Table 2. ISSR primers used in molecular analyses of sweet potato (*Ipomea batatas* L.) genotypes.

Primer	Repetition 5'-3'[a]	Annealing temperature (°C)
807	$(AG)_8T$	52
808	$(AG)_8C$	50
809	$(AG)_8G$	55
810	$(GA)_8T$	50
811	$(GA)_8C$	53
815	$(CT)_8G$	53
835	$(AG)_8YC$	54
836	$(AG)_8YA$	53
873	$(GACA)_4$	50
878	$(GGAT)_4$	54

[a]Y = C or T.

Data analysis

The reading of gels was performed using the binary system (1 = present band and 0 = absent band). The number of total loci (TL) was determined by counting all the amplified loci, regardless of whether they were monomorphic or polymorphic. The number of monomorphic loci (ML) was calculated by subtracting the number of polymorphic loci (PL) from the number of TL. Loci that were scored in only 1 genotype were considered unique (unique loci - UL). The UL plus the loci scored in up to 15% of genotypes made up the rare loci (RL). The loci scored in 15.1 to 70% of genotypes were considered shared (shared loci -SHL) and the loci scored in more than 70.1% of genotypes were considered similar (similar loci - SL).

The discriminatory power of ISSR primers was evaluated using 3 parameters: i) polymorphism information content (PIC), ii) marker index (MI) and iii) resolving power (RP).

PIC for each ISSR loci was computed as PIC$i = 2fi$ (1 - fi); where PICi is the polymorphic information content of the marker 'i', fi is the frequency of the amplified locus (band present), and 1 - fi is the frequency of the null allele (Roldán-Ruiz et al., 2000).

MI was calculated according Varshney et al. (2007): MI = PIC x EMR, where EMR is "the effective multiplex ratio (E) and is defined as the product of the total number of loci per primer (n) and the fraction of polymorphic loci (β) (EMR = n. β)".

The RP was calculated according Prevost and Wilkinson (1999): RP = $\sum I_b$, where, I_b represents locus informativeness. The I_b can be translated into a 0-1 scale by using the formula: $I_b = 1 - (2 \times |0.5 - p|)$, where, p is the proportion of the 40 genotypes containing the locus.

The genetic distance between genotypes was calculated using the Jaccard coefficient, and clustering was performed with the unweighted pair group method using arithmetic averages (UPGMA). The similarity matrix generated by UPGMA was used for drawing the dendrogram. Clustering analysis was performed using the NTSYS-pc 2.1 software.

The polymorphic loci were also subjected to principal components analysis (PCA), using the Statgraphics Plus 5.1 program, where the principal components were extracted, and the eigenvalues and cumulative variance explained (expressed as percentages).

RESULTS

Establishing a SPGBH

The expeditions for the establishment of a SPGBH resulted in the collection of 116 genotypes from 9 counties of Paraná State, Brazil (Figure 1, Table 1). In addition, 8 genotypes from Universidade Federal de Lavras (UFLA, MG), 4 commercial cultivars from EMBRAPA (DF) and 3 cultivars from Universidade Federal de Tocantins (UFT, TO) (Figure 1) were obtained. The SPGBH was established with the goal of providing a core germplasm for the development of cultivars adapted to high-altitude regions.

This germplasm bank will be characterized to indicate genotype suitability for the main sweet potato applications: food, animal feed and ethanol production. First was the molecular characterization of the SPGBH presented in this study. This characterization aimed to determine the utility of ISSR markers for genetic studies in sweet potato and to estimate the genetic variability available in the SPGBH.

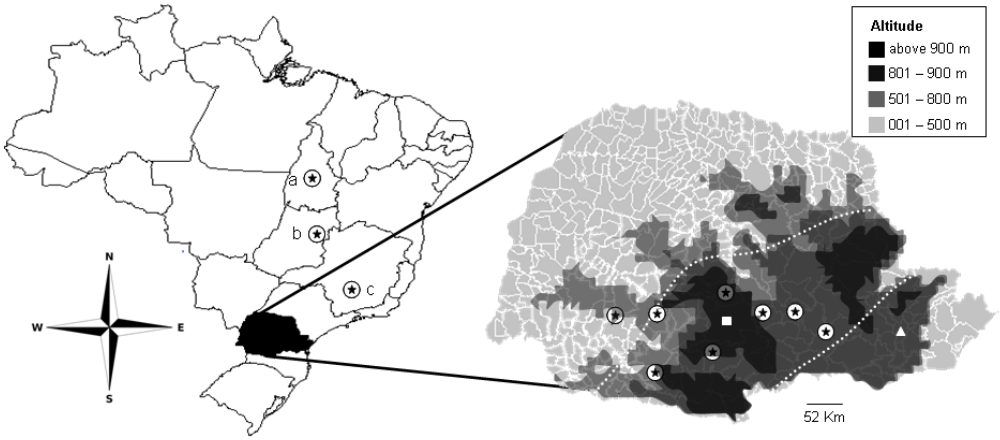

Figure 1. A map of the sampling sites of the 116 genotypes of *Ipomea batatas* L. that comprise the Sweet Potato Germplasm Bank from Highlands (SPGBH) of Paraná State. Stars indicate the counties sampled (**a.** Palmas, TO; **b.** Brasília, DF; **c.** Lavras, MG; counties in the Paraná State: Coronel Vivida, Imbituva, Laranjeiras do Sul, Palmeira, Pinhão, Prudentópolis, Quedas do Iguaçu, and Turvo). The square indicates the county of Guarapuava, where the bank is maintained. The triangle indicates the state capital Curitiba. The dotted line marks the region of high altitude of the State for which the bank is representative of genetic variability.

ISSR features

The information content of the 10 ISSR primers was analyzed using several parameters to determine the potential use of these markers in sweet potato (Table 3). The number of TL generated was 81, and 67 (82.72%) of these were PL and the remaining 14 (17.28%) were ML. The percentage of polymorphism ranged from 66.67% (primer 809) to 100% (primers 808 and 878) with an average of 80.81%.

Table 3. Parameters calculated for the 10 ISSR primers used in evaluating 40 genotypes of sweet potato of a sweet potato germplasm bank of the highlands (SPGBH) of Paraná State.

Primer	TL	ML	PL	% PLP	UL	RL	SL	SHL	PIC	EMR	MI	RP
807	12	1	11	91.67	0	0	7	4	0.38	11	4.14	6.18
808	8	0	8	100.00	0	0	2	6	0.45	8	3.63	6.18
809	9	3	6	66.67	0	0	6	0	0.26	6	1.54	1.88
810	7	1	6	85.71	0	2	3	1	0.21	6	1.28	1.64
811	5	3	2	40.00	0	1	1	0	0.20	2	0.39	0.44
815	6	1	5	83.33	2	0	2	1	0.29	5	1.42	1.10
835	7	2	5	71.43	0	0	1	4	0.45	5	2.25	3.82
836	11	2	9	81.82	0	1	2	6	0.39	9	3.48	5.70
873	8	1	7	87.50	0	0	1	6	0.44	7	3.10	5.02
878	8	0	8	100.00	1	1	3	3	0.35	8	2.78	3.24
Total	81	14	67	82.72	3	5	28	31	-	-	-	-
Mean	8.1	1.4	6.7	80.81	0.3	0.5	2.8	3.1	0.34	6.7	2.40	3.52

TL = total number of loci; ML = number of monomorphic loci; PL = number of polymorphic loci; % PLP = % polymorphism; UL = number of unique loci; RL = number of rare loci; SL = number of similar loci; SHL = number of shared loci; PIC = polymorphic information content; EMR = effective multiplex ratio; MI = marker index; RP = resolving power.

Analysis of the distribution of the frequency of polymorphic loci from the 10 ISSR primers used, showed that the higher numbers of polymorphic loci were in the classes 0.51 to 0.60 (11) and 0.71 to 0.80 (11), followed by 0.81 to 0.90 (10) (Figure 2A). When correlated with the PIC values, it was observed that the most informative locus class was 0.41 to 0.50, when the mean PIC was 0.45 (Figure 2B).

The PL were categorized further into UL, RL, SHL, and SL (Table 3).

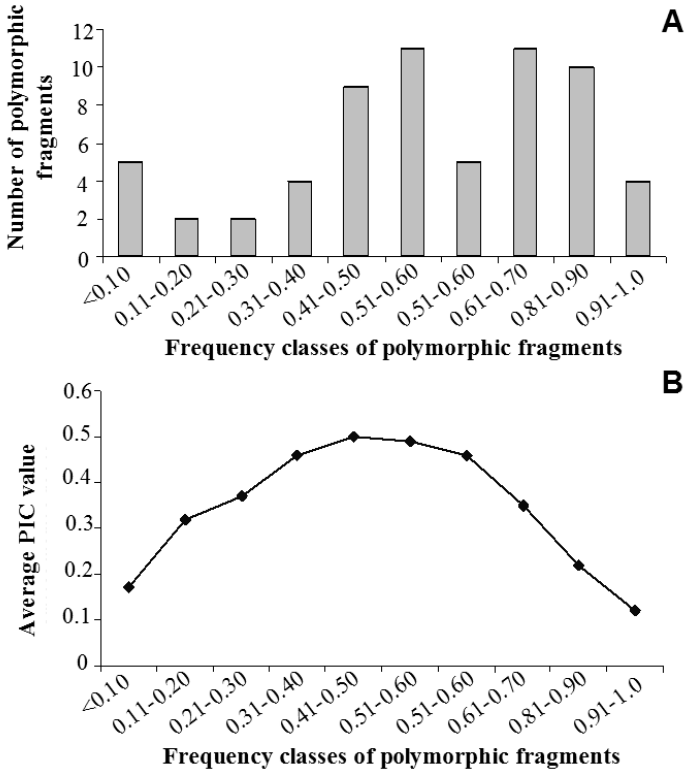

Figure 2. Relationship between the number of polymorphic loci and the polymorphism information content (PIC). **A.** Frequency distribution of polymorphic ISSR loci in *Ipomea batatas* germplasm. **B.** Frequency distribution of PIC value.

UL

UL were those that were present in only 1 genotype. Only 3 UL (4.94%) were obtained: 2 with primer 815 (UGA77 - Turvo and UGA51 - Pinhão) and 1 with primer 878 (UGA51 - Pinhão) (Table 3). The UL were found in genotypes from different origins, Turvo and Pinhão, with UL occurring twice in the UGA51 genotype from the county Pinhão.

RL

RL were those observed in less than 15% of genotypes. A total of 5 RL were obtained:

2 with primer 810 (UGA49 - Laranjeiras do Sul and UGA44 - Pinhão) and 1 with primer 811 (UGA71 - Imbituva), primer 836 (UGA49 - Laranjeiras do Sul) and primer 878 (UGA5 - Palmas, TO) (Table 3).

SHL

SHL were those present in 15.1 to 70% of genotypes. A total of 31 SHL were obtained from the analysis of the 10 ISSR primers (Table 3). The primers that yielded the highest number of SHL were 808 (6 loci), 836 (6 loci), and 878 (6 loci). Primers 809 and 811 showed no SHL.

SL

SL were those that occurred in over 70.1% of genotypes (Table 4). A total of 28 loci were described as similar, averaging 2.8 loci per primer (Table 3). The highest occurrence was found for primers 807 and 809, with 7 and 6 loci, respectively. Primers 811, 835 and 873 produced 1 SL each (Table 3).

Table 4. Results of principal component analysis (PCA) using data from 10 ISSR primers.

Component	Eigen value	% Explained variance	% Cumulative explained variance
1	16.04	40.10	40.10
2	5.56	13.89	53.99
3	3.14	7.86	61.85
4	2.32	5.80	67.66
5	1.91	4.78	72.44
6	1.38	3.45	75.89
7	1.27	3.17	79.07
8	1.15	2.86	81.94
9	1.03	2.58	84.52

Marker attributes

PIC

PIC values ranged from 0.20 to 0.45, with an average of 0.34 per primer (Table 3). The highest mean PIC (0.45) was with primers 808 and 835. The lowest PIC value was 0.05 in a locus with primer 810. Only 12 loci had a PIC below 0.20. Most loci had a PIC ranging from 0.20 to 0.45.

MI

MI was calculated for all 10 ISSR primers. MI values varied from 0.39 to 4.14, with an average of 2.40 (Table 3). The highest marker index (4.14) was obtained using primer 807 and the lowest (0.39) with primer 811. The correlation between MI and PIC was positive ($r^2 = 0.81$).

RP

RP indicates the discriminatory potential of each primer used. RP ranged from 0.44 to 6.18, with an average of 3.52 (Table 3). Primers 807 and 808 had the highest RP (6.18), and primer 811 had the lowest (0.44). The correlation between RP and MI was highly positive (r^2 = 0.97).

Genetic variability

Data from all 67 polymorphic loci generated by the 10 ISSR primers were used to estimate the genetic similarity between genotypes. The genetic distance matrix was calculated using the Jaccard coefficient. The genotype grouping was done by UPGMA. The genetic similarity between pairs of genotypes ranged from 0.09 to 0.88, with an average similarity between all genotypes of 0.51 (Figure 3).

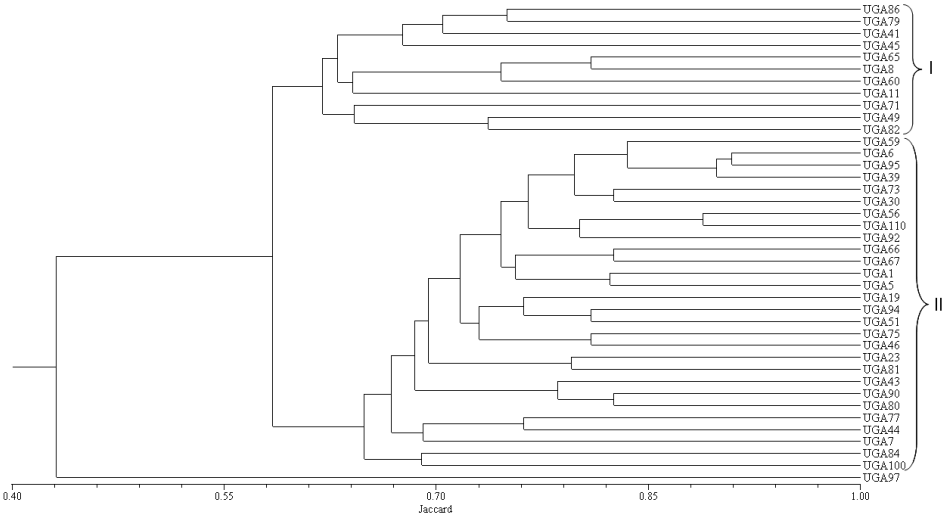

Figure 3. UPGMA dendrogram of 40 *Ipomea batatas* genotypes from the Sweet Potato Germplasm Bank from Highlands (SPGBH) of Paraná State based on data from 67 polymorphic ISSR loci obtained with 10 primers.

In the dendrogram constructed, it is possible to observe 2 groups (Figure 3). Group I is formed by 11 genotypes (3 from Laranjeiras do Sul, 3 from Pinhão, 2 from Imbituva, 1 from Guarapuava, 1 from Coronel Vivida, and 1 from Palmeira). Group II is formed by 28 genotypes (6 from Turvo, 5 from Laranjeiras do Sul, 4 from Coronel Vivida, 4 from Pinhão, 3 from Guarapuava, 2 from Imbituva, 2 from Palmas, 1 from Lavras, and 1 from Palmeira) (Figure 3, Table 1). The genotype UGA97 from Guarapuava did not group with other genotypes (Figure 3).

PCA analysis extracted 9 major components, with eigenvalues greater than or equal to 1.0, which explained 84.53% of variance (Table 4). The graph generated in 2 dimensions by PCA analysis grouped the genotypes into 3 main groups (Figure 4). Group A had 9 genotypes, group B had 28 genotypes and group C had 2 genotypes. The genotype UGA97 from Guar-

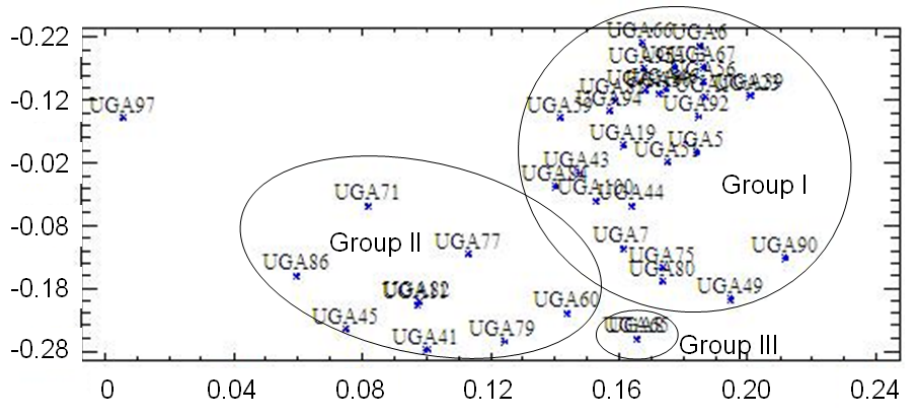

Figure 4. Principal component analysis of 40 *Ipomea batatas* genotypes from the Sweet Potato Germplasm Bank from Highlands (SPGBH) of Paraná State based on data from 67 polymorphic ISSR loci.

apuava was not in any group.

DISCUSSION

Establishing a SPGBH

The establishment of germplasm banks is important for the conservation and cataloging of diversity within a species in a given region. The SPGBH was established with genotypes collected from the regions of high altitude, so it is expected that genotypes within this bank will have features that facilitate the development of elite cultivars adapted to high altitude regions. A preliminary analysis observed varied morphotypes indicative of a high genetic diversity among these genotypes. The genotypes derived from other regions of Brazil will serve for comparison in characterization studies of the SPGBH.

The SPGBH will be characterized using different approaches, each with specific objectives. Morphological characterization of roots and shoots will serve to complement the results obtained in the molecular characterization presented in this work or will be used for comparisons. The physicochemical characterization will be used to analyze the levels of starch, sugars and dry mass of each genotype. Agronomic characterization will evaluate the performance of genotypes in the field for yield and production of roots, root weight, fresh mass and dry shoot mass. Finally, sensory analyses will be conducted to check consumer preferences and acceptability of genotypes.

Attributes of ISSR markers

The proportion of polymorphic loci amplified by 10 ISSR primers, 67 of 81 loci, is similar to those reported in the literature. Hu et al. (2003) evaluated 34 genotypes of sweet potato and wild species of *Ipomoea* using 8 ISSR primers and found 81 polymorphic loci. An average of 10 polymorphic loci per primer has been reported in the literature, while we obtained an average of 8.1. He et al. (2007) found 17 polymorphic bands per primer with 14

ISSR primers.

The percentage of polymorphism in our study ranged from 66.67 to 100%, with an average of 80.81% per primer. This index can be considered satisfactory when compared to the rates determined in other studies available in the literature: 62.2% with 15 ISSR primers (Huang and Sun, 2000), 94.2% with 9 ISSR primers (He et al., 2005) and 94.2% with 7 ISSR primers (Song et al., 2011).

Unlike other studies of the genetic diversity of sweet potato, we described loci as rare, unique, similar or shared. In this study, primer 810 can be considered discriminatory for RL, producing 2 loci that occurred in less than 15% of genotypes. As for the UL, those present in only 1 genotype, it is worth mentioning that genotype UGA51, from the county Pinhão, PR, had UL amplified by 2 primers (815 and 878).

UL and RL can be useful tools in differentiating genotypes within a germplasm bank, and these data can be used in breeding programs for marker-assisted selection and for confirmation of crosses.

Primers 809 and 811 produced no SHL, whereas primers 811, 835 and 873 produced 1 SL. These primers can be considered more discriminatory than others that produced SHL and SL. The information arising from SHL and SL can aid in deducing the probable geographic origin of an unknown genotype or germplasm in breeding programs, and in the characterization of genotypes from different geographical regions (Tatikonda et al., 2009).

The PIC value has been used by many authors in studies of the genetic diversity of several species (Gupta and Varshney, 2000; Varshney et al., 2007). By analyzing the PIC of ISSR primers used in our study, we can affirm that primers 808 and 835 (both with average PIC values of 0.45, close to the maximum value of 0.50 for dominant markers) are the most recommended for future analyses of sweet potato germplasm. According to Tatikonda et al. (2009), the most informative markers, well used in studies of genetic variability, are those with a positive relationship between frequency of higher PIC values and number of polymorphic loci. In our study, no such relationship was observed (Figure 2B). This shows that in sweet potato, a small portion of polymorphic loci contribute most effectively towards differentiation among genotypes. To check whether this is specific to these ISSR primers, it will be necessary to study this relationship using other markers.

The MI of the 10 ISSR primers ranged from 0.39 to 4.14, with an average of 2.40 per primer. According to Tatikonda et al. (2009), there is no value established for MI, and the results should be analyzed by comparing primers in the study. In our work, based on the MI, the most informative primer was 807 (MI = 4.14) and the least informative 811 (MI = 0.39). The correlation between the mean PIC and MI was positive ($r^2 = 0.81$), indicating that the information obtained for *Ipomoea batatas* L. with ISSR markers is robust.

RP values were between 0.44 and 6.18, with an average of 3.52. As with MI, there is no established value for RP. In this work, the primers with the highest RP were 807 and 808 (both with RP = 6.18). Again, it may be noted that these primers were those with high levels in the other parameters evaluated (PIC and MI), indicating that these ISSR primers are the most informative in sweet potato.

The 40-genotype dendrogram drawn using information from the 2 primers with the highest RP (primers 807 and 808) was very similar to the dendrogram topology drawn with all 10 primers, basically changing the values of similarity between individuals (data not shown). This observation indicates that separation of sweet potato genotypes can be done with ISSR

primers 807 and 808, reducing costs and time for analysis. A significant correlation between MI and PIC ($r^2 = 0.81$) and between MI and RP ($r^2 = 0.97$) indicates that any of these parameters can be used to select the most informative ISSR primers for future studies of sweet potato.

Genetic variability of SPGBH

In the dendrogram constructed by UPGMA, the 40 genotypes of sweet potato from the SPGBH were grouped into 2 groups: group I with 11 genotypes, group II with 28 genotypes, and 1 genotype that remained isolated (Figure 3). The largest genetic distance was observed between UGA45 (Laranjeiras do Sul/PR) and UGA6 (Lavras/MG) (0.09 similarity), and the shortest distance between UGA51 (Pinhão/PR) and UGA84 (Coronel Vivida/PR) (0.88 similarity). The average genetic similarity was 0.51. The genetic variability of sweet potato has been widely studied. He et al. (2007) studied 100 genotypes and 8 cultivars from 6 geographic regions of China and found a mean genetic similarity of 0.64. Huang and Sun (2000) studied 40 genotypes of sweet potato and found an average similarity of 0.54. In our study, the average similarity was 0.51. This higher genetic variability can be explained by the fact that Brazil is considered the center of sweet potato dispersion.

PCA identified 9 principal components with eigenvalues equal to or greater than 1.0 and that cumulatively explained 84.53% of the variance. The components extracted were numbered 1 through 9, according to the relative contribution to explaining variance. According to Jolliffe (2002), component 1 is the most important factor. In our analysis, component 1 had an eigenvalue of 16.04, which corresponded to an explained variance of 40.16 (Table 4). In Figure 4, the graph in 2 dimensions shows that genotypes were clustered into 3 groups, namely group A with 9 genotypes, group B with 28 genotypes and group C with 2 genotypes, with 1 genotype remaining isolated (UGA97).

Genotype UGA97 from the county Guarapuava, PR, remained isolated from the others, both by UPGMA and PCA, suggesting that this genotype was the most different among the genotypes of the SPGBH that were analyzed in this work. It can also be inferred that genotypes have a wide genetic diversity within the same county of sampling, since in UPGMA and PCA, genotypes of the same origin clustered in different groups. The association between geographical origin and genetic similarity in asexually propagated species is not common, because the genotypes are moved from one location to another with no exchange of genes through sexual reproduction between local and exogenous materials (Oliveira et al., 2000). This may have occurred in the dispersion of the sweet potato genotypes studied here.

The results obtained in this study lead to the conclusion that ISSR molecular markers are suitable for assessing the genetic diversity of sweet potato. The primers that had higher RP, i.e., 807 and 808, can be used as representatives of ISSR primers in the separation of the germplasm of *Ipomoea batatas* L., reducing the high cost of molecular analysis and minimizing the time needed to obtain results. The 40 genotypes of SPGBH sampled and analyzed with ISSR showed high genetic divergence by cluster analysis (UPGMA) and PCA, indicating high genetic variability for use in genetic breeding programs. These genotypes are representative of the genetic diversity of the highlands of Paraná State.

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