Molecular characterization of potato cultivars using SSR markers

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ABSTRACT

The potato crop has a very narrow genetic base, so the use of molecular markers is a very important tool in the characterization of germplasm banks and evaluation of genetic divergence. The objective of this study was to identify, using microsatellite or simple sequence repeat (SSR) markers, 38 accessions of potato from two collections of commercial cultivars. For the molecular characterization 10 loci were used, generating a total of 46 alleles, which were analyzed as binary data. A cluster analysis was performed with the Jaccard´s similarity coefficient and the UPGMA method, using the software NTSYSpc. On average, the number of alleles per locus was 4.6, ranging from two alleles for primers STM1049, STM 1053 and STM 1104 to 12 alleles per locus for primer STM0019a. Of the 46 alleles, only five were monomorphic, therefore presenting 89.1% polymorphism. The polymorphism information content (PIC) varied from 0.13 to 0.86, with an average of 0.54. The Jaccard´s coefficient varied from 0.41 to 0.93, showing high genetic variability among accessions. Two possible duplicates [Atlantic (Canada) and Atlantic (Chile), and Colorado and Ágata (EPAMIG) (duplicates with these SSRs, which did not separate them)] were identified. High similarity was also shown by cultivars Chipie and Melodie (EPAMIG), Voyager and Gourmandine (EPAMIG), Eole and Caesar (EPAMIG), and Cupido and Santé (Pirassu). The most genetically divergent accessions (Lady Rosetta and HPC-7B) were also identified.

Keywords: *Solanum tuberosum*, germplasm banks, genetic diversity, microsatellites.

RESUMO

Caracterização molecular de cultivares de batata por marcadores SSR

A batata possui uma base genética estreita, sendo assim a utilização de marcadores moleculares é uma ferramenta muito importante no processo de caracterização de bancos de germoplasma e avaliação de divergência genética. O objetivo deste trabalho foi avaliar por meio de marcadores microssatélites ou *Simple Sequence Repeats* (SSR), 38 acessos de batata de duas coleções distintas contendo cultivares comerciais. Para a caracterização molecular foram analisados 10 loci, gerando um total de 46 alelos, os quais foram analisados como dados binários. Foi obtida uma matriz de similaridade utilizando o coeficiente de Jaccard e com o método aglomerativo UPGMA foi realizada uma análise de agrupamento pelo software NTSYSpc. Em média, o número de alelos por loco foi 4,6, variando de dois alelos para os iniciadores STM1049, STM1053 e STM1104 até 12 alelos por loco para o iniciador STM0019a. Dos 46 alelos identificados, apenas cinco foram monomórficos, observando-se, portanto, 89,1% de polimorfismo. O conteúdo de informação de polimorfismo (PIC) variou de 0,13 a 0,86, com média de 0,54. O coeficiente de similaridade de Jaccard variou de 0,41 a 0,93, mostrando a grande variabilidade genética entre os acessos. Observaram-se duas possíveis duplicatas [Atlantic (Canadá) e Atlantic (Chile), e Colorado e Ágata (EPAMIG) (duplicatas com estes SSRs, que não as separaram)]. Elevada similaridade foi também observada pelas cultivares Chipie e Melodie (EPAMIG), Voyager e Gourmandine (EPAMIG), Eole e Caesar (EPAMIG), e Cupido e Santé (Pirassu). Foram identificados também os acessos mais distantes geneticamente (Lady Rosetta e HPC-7B).

Palavras-chave: *Solanum tuberosum*, bancos de germoplasma, diversidade genética, microssatélites.

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Potato (*Solanum tuberosum* L.), in order of economic importance, is the fourth most important food crop in the world, after wheat, rice and maize, with a global annual production of approximately 300 million tones (CIP, 2011). Worldwide, this crop is undergoing major changes. Until the beginning of 1990, it was the most cultivated and consumed in Europe, North America and in countries of the former Soviet Union. Since then, there has been an increase in potato production and demand in Asia, Africa and Latin America (FAO, 2008). Brazil ranks as a major potato producer in Latin America, with a record harvest in 2006 of around 33.1 million tons. Over the past 15 years, potato production increased, on average, 5% a year and, the average yield increased from 14 to 22 tons per hectare. Current data indicate a total area of 138,852 ha and a total production of 3,438,825 tons with an average yield of 24.7 tons per ha, showing a positive variation of 3.7% (IBGE, 2009; AGRIANUAL, 2009).

Despite the great progress in all these years, it is necessary to search for more productive, adapted and resistant material. Molecular characterization is an important biotechnology tool in plant breeding programs. Microsatellites, also called SSR (Simple Sequence Repeats), are one of the more polymorphic molecular markers available today (Ferreira & Grattapaglia, 1998). Microsatellites also have advantages over other markers based on PCR (Polymerase Chain Reaction), such as RAPD (Random Amplified Polymorphic DNA), because they are co-dominant and easily reproducible, and have a frequent and random distribution, allowing a wide coverage of the genome. The high level of variation detected with microsatellites increases the resolution for genealogy and germplasm genetic diversity studies and reduces the number of markers required to distinguish between genotypes (Borém & Caixeta,

2006).

Rocha (2008), using six RAPD and three SSR primers, identified 16 cultivars of potato. The author observed that SSR markers were more efficient than RAPD markers, since three of the SSR primers allowed the distinction of all cultivars studied, compared with the six primers used for RAPD. Several studies have used SSR markers for the characterization of potato cultivars and accessions, such as Norero *et al.* (2002), Braun & Wenzel (2004), Braun *et al.* (2004), Chimote *et al.* (2004), Ghislain *et al.* (2006), Barandalla *et al.* (2006), Mathias *et al.* (2007), Ispizúa *et al.* (2007), and Fu *et al.* (2009). These last authors evaluated 114 Canadian and 55 exotic potato accessions using 36 SSR loci. However, except for the study conducted by Rocha (2008), no studies have yet been found in Brazil using SSR markers to identify potato cultivars. Within this context, the aim of this study was to characterize, at the molecular level using microsatellite markers, 38 commercial cultivars of potatoes from two collections.

MATERIAL AND METHODS

Thirty-eight potato cultivars from two collections, Pirassu Company, located in Vargem Grande do Sul, São Paulo state, Brazil, and Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), were assessed. Details of the source and country of origin, including the genealogy of each cultivar are described (Table 1).

For the DNA extraction, recently expanded leaves were dried in an oven at 45ºC for a period of 24 hours, after which they were macerated and submitted to a 3% CTAB methodology, as described by Siqueira *et al.* (2009). DNA concentration of each genotype was estimated by running samples in 0.8% agarose gels. The gels were prepared in advance, using 0.8 g of agarose diluted in 1X TBE buffer [100 mL 10X TBE (0.89 M Tris base, 0.89 M boric acid, 20mM EDTA pH 8.0) and 900 mL of distilled water] and stained with 4µL Ethidium bromide. DNA concentration was estimated by comparison to standard undigested lambda DNA, with a range variation of 10, 20, 50, 80 and 100 ng.

Ten potato specific microsatellite primers were used (Ghislain *et al.*, 2006) (Table 2). Polymerase chain reactions (PCR) were performed in 10.2 µL volume containing approximately 15 ng of template DNA, 5 U of Taq DNA Polymerase, 50 mM $MgCl_2$, 1x Polymerase Buffer, 5 pmoles of each forward and reverse primers, and 2,5 mM of dNTPs mix. For these reactions, the thermocycler MyCycler Thermal Cycler model of BioRad was used. PCR reactions were conducted in the following sequence: 3 min at 94°C, followed by 30 cycles of 30 sec at 94°C, 1 min at the annealing temperature for each primer set (Table 2) and 1 min at 72°C, and the final extension of 5 min at 72°C.

The amplification products were separated in 6% polyacrylamide gels under an initial voltage of 60 volts for 30 min, extending it to 120 volts for about 2 h in TBE buffer (0.09 M Tris, 0.09 M boric acid, 2 mM EDTA). Standard molecular weight markers of 10 bp and 100 bp were used. The material was stained with silver nitrate (Bassam *et al.*, 1991) to reveal the microsatellite bands, which were analyzed in a transilluminator and photodocumented using a digital camera.

For the statistical analysis, each SSR locus was characterized as a dominant marker, according to the presence or absence of bands, which were analyzed visually. These data were used in the construction of a binary data matrix, where the value 1 (one) means presence of bands and the value 0 (zero) their absence. With this matrix, the Jaccard similarity coefficients were obtained. Using this coefficient and the cluster method UPGMA (Unweighted pair-group method with arithmetic averages), a cluster analysis was performed using the NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) software (Rohlf, 1992). NTSYSpc was used also to estimate the cophenetic value. The accuracy of the groupings was estimated from simulations with resampling, using 10,000 bootstraps, and the BOOD software, version 2.0 (Coelho, 2001). The polymorphism information content (PIC) was calculated by the formula: $PIC = 1 - \sum_{i=1}^{n} p_i^2$ where: pi = frequency of the allele (band) in each locus and n = number of alleles observed.

RESULTS AND DISCUSSION

All 10 loci used in this study showed polymorphism among the accessions analyzed, producing well-defined and reproducible bands. A total of 46 alleles were amplified with an average of 4.6 alleles per locus, ranging from two alleles for primers STM1049, STM1053 and STM1104 to 12 for the primer STM0019a (Table 2). Only five alleles were present in all the varieties evaluated, while 41 alleles were shown to be polymorphic for all the 38 cultivars, therefore showing 89.1% polymorphism. Milbourne *et al.* (1997), evaluating 14 potato genotypes from northwestern Europe with 17 SSR loci, found 98 alleles (bands) with an average of 5.76 alleles, greater than the value reported in this study, although with a greater number of loci. Braun & Wenzel (2004), evaluating 69 cultivars from Germany with 26 SSR loci, observed 128 alleles (98.4% polymorphism) and a mean number of alleles of 5.12. Mathias *et al.* (2007) observed a variation of two to 17 alleles/locus in the evaluation of 71 genotypes from INIA, Chile, with 21 SSR loci, in agreement with Fu *et al.* (2009) reporting two to 17 alleles per locus in the evaluation of 114 Canadians and 55 exotic potato accessions with 36 SSR loci. The values found in this study, ranging from two to 12 alleles/locus, are therefore consistent with the literature whereas a smaller number of genotypes (38) was evaluated.

The polymorphism information content (PIC) ranged from 0.13 to 0.86, averaging 0.54, with the highest value obtained for primer STM0019a and the lowest value obtained for primer STM1053, showing that the SSR primers in this study presented, on average, a high level of information. Similar values were obtained by Rocha (2008), with PIC values ranging from 0.21 to 0.97 in the evaluation of 16

Table 1. List of the accessions of potato (*Solanum tuberosum*) studied, including their source[1], crossings[2] and origin (lista dos acessos de batata (*Solanum tuberosum*) estudados, incluindo a fonte de obtenção, sua genealogia e origem). Piracicaba, USP-ESALQ, 2009.

Nº	Varieties	Source	Crossings	Origin
1	AGATA	Pirassu	BM 52/72/2206 X Sirco	HOL
2	AGATA	EPAMIG	BM 52/72/2206 X Sirco	HOL
3	ASTERIX	Pirassu	'Cardinal' x 'VE 70-9'	HOL
4	ASTERIX	EPAMIG	'Cardinal' x 'VE 70-9'	HOL
5	ATLANTIC	Pirassu	Wauseon x B 5141-6 (Lenape)	USA
6	ATLANTIC	Pirassu	Wauseon x B 5141-6 (Lenape)	USA
7	ATLANTIC	EPAMIG	Wauseon x B 5141-6 (Lenape)	USA
8	BRS ANA	EPAMIG	'C-1750-15-95' x 'Asterix'	BRA
9	BRS ELIZA	EPAMIG	'Edzina' x 'Recent'	BRA
10	CAESAR	EPAMIG	'Monalisa' x 'Ropta B 1178'	HOL
11	CANELLE	EPAMIG	Not available	FRA
12	CATUCHA	EPAMIG	'2CRI 1149-178'x 'C-999-263-70'	BRA
13	CHIPIE	EPAMIG	'Pilgrim' x ('Saturna' x Pentland Dell')	FRA
14	COLORADO	EPAMIG	'Torridon' x ('Desiree' x 'Pentland Dell')	FRA
15	CUPIDO	Pirassu	'W 72-22-496' x 'Estima'	HOL
16	EDEN	EPAMIG	'Eole' x 'Pentland Dell'	FRA
17	EMERAUDE	EPAMIG	'Estima x 'INRA 75.36.45'	FRA
18	EOLE	EPAMIG	'Ukama' x 'INRA 74.38.12'	FRA
19	FIANNA	Pirassu	'4062-660' x 'AM 66-42	HOL
20	FLORICE	EPAMIG	'Fanette' X 'INRA' 72.68.5	FRA
21	FONTANE	EPAMIG	'Agria' x 'AR 76-34-3'	HOL
22	GOURMANDINE	EPAMIG	'Charlote' x 'Estima'	FRA
23	GREDINE	EPAMIG	Not available	FRA
24	HPC-7B	Pirassu	*S. chacoense* x *S. phureja*	-
25	ITARARÉ	Pirassu	'Arensa'x 'Turma' x 'Leo'	BRA
26	LADY ROSETTA	Pirassu	'Cardinal' x 'SVP(VTN)62-33-3'	HOL
27	MELODIE	EPAMIG	'VE 74-45' x 'W 72-22-496'	HOL
28	MONALISA	Pirassu	'Bierma A 1-287' x 'Colmo'	HOL
29	MONALISA	EPAMIG	'Bierma A 1-287' x 'Colmo'	HOL
30	MONDIAL	Pirassu	'Spunta' x 'VE 66-295'	HOL
31	NATURELLA	EPAMIG	'Sirco' X 'Pentland Squire'	FRA
32	OPALINE	EPAMIG	Non available	FRA
33	PANDA	Pirassu	'UP 0.351/17' x 'W 6858/8'	BRD
34	PIRASSU	Pirassu	Mutant of 'Lady Rosetta'	HOL
35	SANTÉ	Pirassu	'Y 66-13-636' x 'AM 66-42'	HOL
36	SOLÉIA	EPAMIG	Not available	FRA
37	SPUNTA	Pirassu	'BEA' x 'USDA 96-56'	HOL
38	VOYAGER	EPAMIG	'RZ 85-238' x 'Obelix'	HOL

[1] Pirassu –Vargem Grande do Sul, SP; EPAMIG – Empresa de Pesquisa Agropecuária de Minas Gerais, MG.; [2]Hutton *et al.* (2009); Miranda Filho *et al.* (1986); Miranda Filho (1991); Plant de Pomme de Terre (2009); Meijer (2009); Nivap (2009); Pádua (2009).

potato cultivars with 21 SSR primers, Ghislain *et al.* (2006), with the PIC values ranging from 0.0 to 0.67 in the evaluation of 170 potato genotypes with 22 SSR loci, and Mathias *et al.* (2007), with PIC values ranging from 0.42 to 0.90 for a total of 71 genotypes of potato and 21 SSR loci. Fu *et al.* (2009), however, observed much lower PIC values, ranging from 0.01 to 0.49, when assessing 114 Canadian and 55 exotic potato accessions with 36 SSR loci. Therefore, the information level depends on the set of primers used and the material evaluated. These authors concluded that the Canadian accessions have a narrow genetic basis, while the exotic accessions showed greater

Table 2. Potato (*Solanum tuberosum*) primers[1] used in this study. including the number of alleles per locus. the annealing temperature (TºC). the size in bp and the polymorphism information content (PIC) per locus (iniciadores específicos para a batata[1] (*Solanum tuberosum*). utilizados neste estudo. incluindo o número de alelos por loco. a temperatura de anelamento (TºC). o tamanho em pb. e o conteúdo de informação de polimorfismo (PIC) por loco). Piracicaba. USP-ESALQ. 2009.

Locus	Sequence (5' → 3')	N° alleles	T°C	Size pb	PIC
STM0019a	F: AATAGGTGTACTGACTCTCAATG R: TTGAAGTAAAAGTCCTAGTATGTG	12	54.3	160-280	0.8583
STM0037	F: AATTTAACTTAGAAGATTAGTCTC R: ATTTGGTTGGGTATGATA	6	56.1	70-100	0.7149
STM1049	F: CTACCAGTTTGTTGATTGTGGTG R: AGGGACTTTAATTTGTTGGACG	2	61.6	190-200	0.6073
STM1053	F: TCTCCCCATCTTAATGTTTC R: CAACACAGCATSCAGATCATC	2	60	180-190	0.1264
STM1104	F: TGATTCTCTTGCCTACTGTAATCG R: CAAAGTGGTGTGAAGCTGTGA	2	60	170-180	0.3732
STM1106	F: TCCAGCTGATTGGTTAGGTTG R: ATGCGAATCTACTCGTCATGG	4	60	150-170	0.5554
STM2013	F: TTCGGAATTACCCTCTGCC R: AAAAAAAGAACGCGCACG	3	60	145-160	0.5678
STM2022	F: GCGTCAGCGATTTCAGTACTA R: TTCAGTCAACTCCTGTTGCG	6	64	170-230	0.4301
STM3012	F: CAACTCAAACCAGAAGGCAAA R: GAGAAATGGGCACAAAAAACA	3	66	170-210	0.5039
STPoAc58	F: TTGATGAAAGGAATGCAGCTTGTG R: ACGTTAAAGAAGTGAGAGTACGAC	6	63	240-285	0.6997

[1]Ghislain *et al.* (2006).

variability.

The Jaccard's similarity coefficient ranged from 0.41 to 0.93 (Figure 1), showing a significant genetic variability for the commercial varieties assessed in this study, higher than the 16 Brazilian cultivars evaluated by Rocha (2008), where the Jaccard's coefficient ranged from 0.57 to 0.73. Braun & Wenzel (2004) found a total of 128 SSR bands and 98.4% polymorphism when assessing 47 genotypes of the potato breeding program in Germany, with the similarity coefficient of Nei & Li (1979) ranging from 0.57 to 0.79, showing less variability than the cultivars used in this study. Similar results to our study were obtained by Barandalla *et al.* (2006) in the evaluation of 41 cultivars of the Tenerife Island using 19 SSR loci, with the Jaccard's coefficient ranging from 0.57 to 1.00.

The 38 cultivars were classified in three groups, according to the Bootstrap criterion, in the cluster analysis (Figure 1), showing a cophenetic value of 0.7423. The first group, with a 53% confidence degree, was composed of all the varieties except for Lady Rosetta and HPC-7B varieties. The second group included Lady Rosetta variety, with a 53% degree of confidence obtained by the Bootstrap method, while the third group classified the HPC-7B variety, with 100% reliability. Within the first group, the data suggests the presence of a duplicate for the cultivars Atlantic (Canada) and Atlantic (Chile) from Pirassu collection, as expected, since they are same variety, but originated from different places. They presented 93% similarity by Jaccard's coefficient and a confidence degree of 97%. However, the Atlantic cultivar from EPAMIG collection, was not genetically identical to these two accessions. The Atlantic cultivar is also known to be originated from different parents (Pepita x Desireé) (Hutten *et al.*, 2009), which could be an explanation.

Varieties Colorado and Agata (EPAMIG) also showed high similarity with approximately 91% similarity by the Jaccard's coefficient and a 67% confidence degree. Rocha (2008) also observed high similarity (69% by the coefficient of Jaccard) between these two cultivars (Agata and Colorado). However, these cultivars do not have parents in common (Table 1), and also do not have similar morphological and horticultural traits. It is interesting to emphasize that cultivar Agata, from the two collections (EPAMIG and Pirassu), were genetically distinct, although both of them are part of the large group (group I). Cultivar Agata (Pirassu) was closer to Mondial, with about 75% similarity and 47% reliability. The same result was found for cultivar Monalisa from both collections, both of them with the same genealogies. Further SSR analyses were conducted to confirm these results, and therefore we concluded that these cultivars could not be considered genetically identical. Possible explanations are the occurrence of somatic mutations at the various

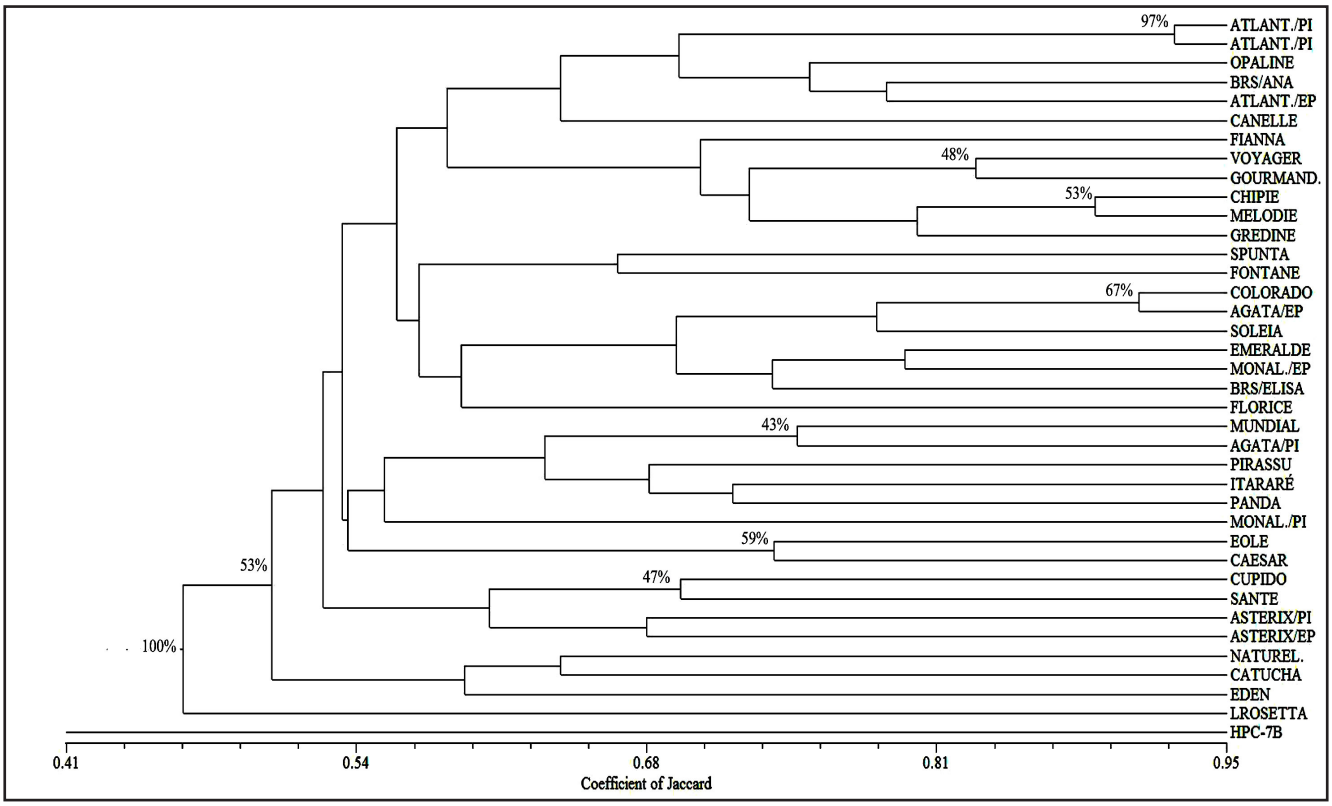

Figure 1. Dendrogram obtained from the Jaccard´s similarity coefficient, the UPGMA cluster method and confidence degree using the Bootstrap method for 38 cultivars of potato (*Solanum tuberosum*) (dendrograma obtido a partir do coeficiente de similaridade de Jaccard, pelo método aglomerativo UPGMA e grau de confiança pelo método Bootstrap, para 38 cultivares de batata (*Solanum tuberosum*)). Piracicaba, USP-ESALQ, 2009.

phases of multiplication since its origin in Brazil.

Cultivars Chipie and Melodie, both from EPAMIG collection, were also very similar, with about 90% similarity and 53% reliability in this grouping, followed by Voyager and cultivar Gourmandine (EPAMIG) with 48% reliability, and cultivars Eole and Caesar (EPAMIG), with a 59% confidence degree. These two cultivars are similar in relation to the tuber traits, which are oval, large, with a moderately smooth yellow skin and shallow eyes. Varieties Cupido and Santé (Pirassu) were also similar, with approximately 70% similarity, but with a degree of reliability of 47%. It is worth considering that there are similarities found in the tubers traits of these two cultivars, which are large, oval to round-oval and uniform, with a smooth and yellow skin and light yellow flesh.

Variety HPC-7B (Pirassu), derived from the cross between *Solanum phureja* and *S. chacoense*, was the most divergent accession from this collection, which was already expected considering it to be a diploid and originated from two different species, and was followed by Lady Rosetta (Pirassu), originated in Holland from the crosses 'Cardinal' x 'SVP(VTN)62-33-3'. HPC-7B cultivar is used mostly in plant breeding programs as a parent in crosses, as it shows high resistance to late blight (*Phytophtora infestans*) and nematodes (Silva *et al*., 2010).

The polymorphism levels presented in this study are high, considering that in the analyses each allele is a unique character and, as potato is a tetraploid species, each individual may present from one to four different alleles in one locus. This contributes to a high level of genetic diversity. Associated with the high reproducibility of the SSR markers, the results obtained in this study support the use of these markers as an important tool in the molecular characterization of potato varieties in germplasm banks, in the identification of duplicates, in the correct identification of cultivars and of genetically divergent potential parents to be used in breeding programs.

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