# 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 in the identification of the most promising choice of parents to be used in plant breeding programs. The objective of this study was to assess, using microsatellite or simple sequence repeat (SSR) markers, 38 accessions of potato from two separate collections of commercial cultivars, aiming at the genetic characterization, identification of duplicates and possible parents for breeding programs. For the molecular characterization 10 primers were used, generating a total of 46 alleles (bands), which were analyzed as binary data. A cluster analyses 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 a high genetic variability among accessions. Two possible duplicates [Atlantic (Canada) and Atlantic (Chile), and Colorado and Agata (EPAMIG)] were identified. High similarity was also shown by cultivars Chipie and Melodie (EPAMIG), Voyager and Gourmandine (EPAMIG), Eole and Caesar (EPAMIG), and Cupido and Santè (Pedro Hayashi). The genetically most divergent accessions (Lady Rosetta and HPC-7B) were also identified. The high levels of polymorphism observed for *Solanum tuberosum* suggest that microsatellite markers represent a useful tool to detect genetic differences between cultivars and can be used in potato breeding programs.

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

## Introduction

Potato (*Solanum tuberosum* L.), in order of economic importance, is the fourth agricultural crop, planted at least in 125 countries and consumed by more than a billion people around the world (Pastorino et al., 2003). 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, where production rose less than 30 million tons in the 60s to over 165 million tons in 2007 (FAO, 2008).

Brazil ranks as a major potato producer in Latin America, with a record harvest in 2006 of around 33.1 million tonnes. The highest yield was observed in regions of southeast and south with 1,962,045 and 1,188,416 tons respectively. Current data indicate a total area of 138,852 hectares and a total production of 3,438,825 tonnes with an average yield of 24.7 tonnes per hectare, giving a positive variation of 3.7% (Brazilian Institute of Geography and Statistics - IBGE , 2009; FNP, 2009).

Despite the great progress in all these years, it is necessary to search for more productive, adapted and resistant material. Molecular characterization is one of the biotechnology tools to help, remarkably, plant breeding programs in a period of time considerably shorter compared to traditional methods of breeding. Microsatellites, also called SSR (Simple Sequence Repeats), are one of the more polymorphic molecular markers available today (Grattapaglia and Ferreira, 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 and 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 Ghislain et al. (2000), using two SSR primers to identify 20 varieties of native potatoes of the Andes, Norero et al. (2002) using four SSR loci for discriminating 37 commercial potato cultivars from INTA (National Institute of Agricultural Technology) in Argentina, Braun and Wenzel (2004) using 26 SSR loci to evaluate 47 genotypes from the potato breeding program in Germany, Braun et al. (2004) evaluating 75 cultivars of North America, Europe and Japan with 15 SSR loci, Chimote et al. (2004) assessing 32 potato cultivars in India with 16 SSR loci, Barandalla et al. (2006) evaluating with 19 SSR loci 41 cultivars from Tenerife Island, Mathias et al. (2007) using 21 SSR loci to evaluate 71 potato genotypes from the Institute of Agricultural Research (INIA) of Chile, Ispizúa et al. (2007) assessing 155 accessions from INTA in Argentina using four SSR loci, and Fu et al. (2009) evaluating 114 Canadian and 55 exotic potato accessions using 36 SSR loci.

Within this context, the aim of this study was to characterize, at the molecular level using microsatellite markers, 38 commercial cultivars of potatoes used in Brazil, originating from two separate collections, identifying possible duplicates and different materials with potential for use as parents, to assist in the breeding programs.

## Material and methods

In this study, 38 potato cultivars from two collections, Pedro Hayashi Company, located in Vargem Grande do Sul, SP and Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), were assessed (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 individual was estimated by running samples in 0.8% concentration agarose gels. The gels were prepared 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.

Ten specific microsatellite primers were used (Ghislain et al., 2006). The total volume for each polymerase chain reaction (PCR) was 10.2 µL, including the following components: 0.2 µL of Taq-Polymerase (5U/µL), 1.0µL of buffer (10x Amplification Buffer), 1.0µL $MgCl_2$ (50mM); 0.5µL of Primer F (5pmoles/µL), 0.5 µL of Primer R (5pmoles/µL); 1.0µL of dNTP's (2.5mM each); 3µL of Milli-Q $H_2O$ and 3µL of DNA in each microtube. For these reactions, the thermocycler MyCycler Thermal Cycler model of BioRad was used. The 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 a 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 two hours 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 and photodocumented.

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). 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.

**Table 1. Potato (*Solanum tuberosum*) accessions evaluated and origin (source) of the accessions**

| Nº | Comercial Varieties | Source |
|---|---|---|
| 1 | ATLANTIC | Pedro Hayashi |
| 2 | CUPIDO | Pedro Hayashi |
| 3 | ASTERIX | Pedro Hayashi |
| 4 | SANTÈ | Pedro Hayashi |
| 5 | MONDIAL | Pedro Hayashi |
| 6 | ATLANTIC Chile | Pedro Hayashi |
| 7 | FIANNA | Pedro Hayashi |
| 8 | PIRASSU | Pedro Hayashi |
| 9 | ITARARÉ | Pedro Hayashi |
| 10 | SPUNTA | Pedro Hayashi |
| 11 | HPC-7B | Pedro Hayashi |
| 12 | LADY ROSETTA | Pedro Hayashi |
| 13 | PANDA | Pedro Hayashi |
| 14 | AGATA | Pedro Hayashi |
| 15 | MONALISA | Pedro Hayashi |
| 16 | AGATA | EPAMIG |
| 17 | ASTERIX | EPAMIG |
| 18 | ATLANTIC | EPAMIG |
| 19 | BRS ANA | EPAMIG |
| 20 | BRS ELISA | EPAMIG |
| 21 | CAESAR | EPAMIG |
| 22 | CANELE (FR) | EPAMIG |
| 23 | CATUCHA | EPAMIG |
| 24 | CHIPIE | EPAMIG |
| 25 | COLORADO | EPAMIG |
| 26 | EDEN | EPAMIG |
| 27 | EMERALDE | EPAMIG |
| 28 | EOLE | EPAMIG |
| 29 | FLORICE | EPAMIG |
| 30 | FONTANE | EPAMIG |
| 31 | GOURMANDINE | EPAMIG |
| 32 | GREDINE | EPAMIG |
| 33 | MELODIE | EPAMIG |
| 34 | MONALISA | EPAMIG |
| 35 | NATURELLA | EPAMIG |
| 36 | OPALINE | EPAMIG |
| 37 | SOLÉIA | EPAMIG |
| 38 | VOYAGER | EPAMIG |

## Results and discussion

In this study, all 10 loci used showed polymorphism among the accessions analyzed, producing well-defined and reproducible bands. A total of 46 alleles (bands) 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 and 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.

**Table 2. Potato (*Solanum tuberosum*) primers[1] used in this study, including the number of alleles per locus, the annealing temperature (T°C), the size in bp and the polymorphism information content (PIC) per locus**

| *Locus* | Sequence (5' → 3') | Alleles number | T°C | Size pb | PIC |
|---|---|---|---|---|---|
| **STM0019a** | F: AATAGGTGTACTGACTCTCAATG | 12 | 54,3 | 160-280 | 0,8583 |
| | R: TTGAAGTAAAAGTCCTAGTATGTG | | | | |
| **STM0037** | F: AATTTAACTTAGAAGATTAGTCTC | 6 | 56,1 | 70-100 | 0,7149 |
| | R: ATTTGGTTGGGTATGATA | | | | |
| **STM1049** | F: CTACCAGTTTGTTGATTGTGGTG | 2 | 61,6 | 190-200 | 0,6073 |
| | R: AGGGACTTTAATTTGTTGGACG | | | | |
| **STM1053** | F: TCTCCCCATCTTAATGTTTC | 2 | 60 | 180-190 | 0,1264 |
| | R: CAACACAGCATSCAGATCATC | | | | |
| **STM1104** | F: TGATTCTCTTGCCTACTGTAATCG | 2 | 60 | 170-180 | 0,3732 |
| | R: CAAAGTGGTGTGAAGCTGTGA | | | | |
| **STM1106** | F: TCCAGCTGATTGGTTAGGTTG | 4 | 60 | 150-170 | 0,5554 |
| | R: ATGCGAATCTACTCGTCATGG | | | | |
| **STM2013** | F: TTCGGAATTACCCTCTGCC | 3 | 60 | 145-160 | 0,5678 |
| | R: AAAAAAAGAACGCGCACG | | | | |
| **STM2022** | F: GCGTCAGCGATTTCAGTACTA | 6 | 64 | 170-230 | 0,4301 |
| | R: TTCAGTCAACTCCTGTTGCG | | | | |
| **STM3012** | F: CAACTCAAACCAGAAGGCAAA | 3 | 66 | 170-210 | 0,5039 |
| | R: GAGAAATGGGCACAAAAAACA | | | | |
| **STPoAc58** | F: TTGATGAAAGGAATGCAGCTTGTG | 6 | 63 | 240-285 | 0,6997 |
| | R: ACGTTAAAGAAGTGAGAGTACGAC | | | | |

[1]Ghislain et al. (2006)

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) were 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 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 their 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 Canadians 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 Canadians accessions have a narrow genetic basis, while the exotic accessions showed greater 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 with 16 SSR loci by Rocha ( 2008), where the Jaccard's coefficient ranged from 0.57 to 0.73. Braun and 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 and 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.

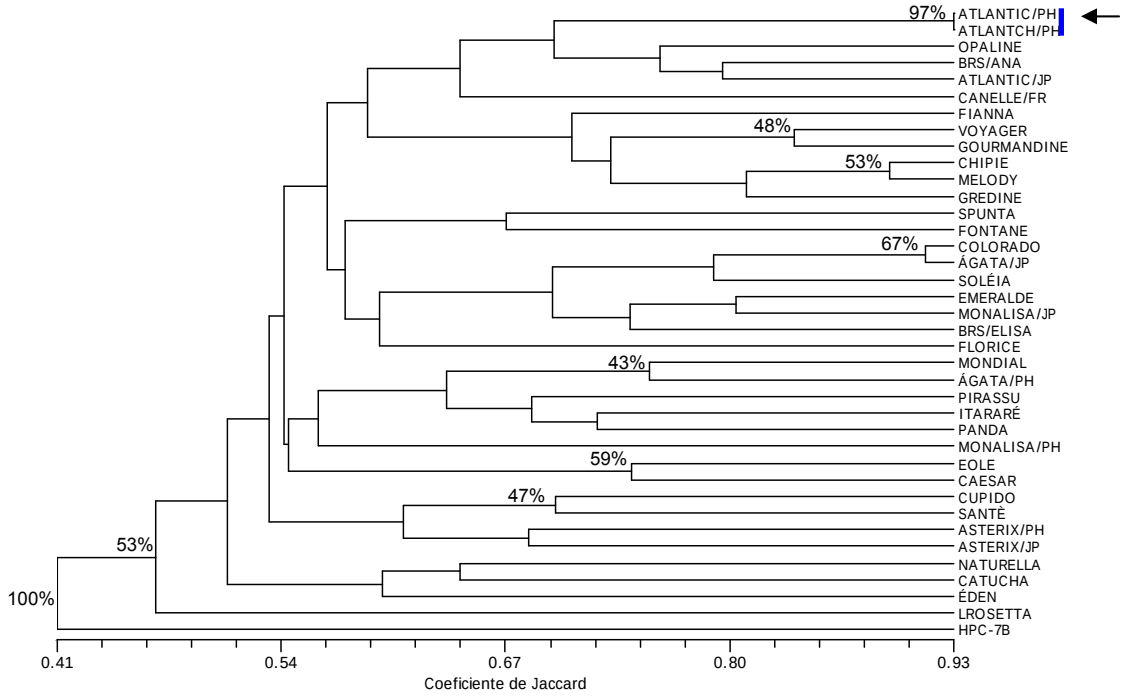


**Figure 1. Dendrogram obtained from the Jaccard´s similarity coefficient, the UPGMA cluster method and confidence degree using the Bootstrap method for 38 cultivars of potato (*Solanum tuberosum*)**

The 38 cultivars were classified in three groups in the cluster analysis (Figure 1). 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), which is the same variety but originating from different places, presenting 93% similarity by Jaccard's coefficient and a confidence degree of 97%, with great possibility of being the same genetic material.

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), using 21 SSR loci, 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, and also do not have similar morphological and agronomic characteristics, that is, tubers of Colorado present elongated oval shape and skin-red-flat tubers, while Agata tubers show oval shape

and yellow skin. It is interesting to emphasize that the cultivars named Agata, from two collections (EPAMIG and Pedro Hayashi), were genetically distinct, although both of them are part of the large group (group I). Cultivar Agata (Pedro Hayashi) was closer to Mondial cultivar, with about 75% similarity and 47% reliability.

It is worth noting that the greatest similarities tended to occur between accessions from each collection (Pedro Hayashi and EPAMIG). Cultivars Chipie and Melodie (both from EPAMIG) were also very similar, with about 90% similarity and 53% reliability in this grouping, followed by Voyager and cultivars Gourmandine (EPAMIG) with 48% reliability and cultivars Eole and Caesar (EPAMIG), with a 59% confidence degree. These two cultivars (Eole and Caesar) are similar in relation to the tuber characteristics, which are oval, large, with a moderately smooth yellow skin and superficial eyes. Varieties Cupido and Santa (Pedro Hayashi) were also similar, with approximately 70% similarity, but with a degree of reliability below 50% (47%). It is worth considering that there are similarities found in the tubers 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 (Pedro Hayashi), obtained by crossing *Solanum phureja* and *Solanum chacoense*, was the most divergent accession from this collection, followed by Lady Rosetta (Pedro Hayashi). These two varieties differ with respect to some characteristics, especially in relation to disease susceptibility, that is, Lady Rosetta is susceptible to late blight (*Phytophtora infestans*), while HPC-7B shows high resistance.

The polymorphism levels presented in this study are high, considering that in the analyses each allele is considered 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 and breeding programs.

## Conclusions

Although potatoes have a narrow genetic base because of its propagation, in this study the molecular characterization of microsatellites showed a significant genetic variability and high levels of polymorphism.

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