Notas Científicas

Potato cultivar identification using molecular markers

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Abstract – The objective of this work was to evaluate a set of microsatellite markers for varietal identification and characterization of the most widespread potato cultivars in Brazil. The DNA from 14 potato cultivars was genotyped using microsatellite markers and the alleles were scored in silver-stained polyacrylamide gel. Twenty-four microsatellite markers were evaluated, and only one locus was monomorphic. Based on band patterns, a set of two microsatellites that were able to identify and differentiate all examined cultivars was obtained.

Index terms: *Solanum*, AFLP, genetic certification, varietal protection, variety identification, RAPD.

Identificação de cultivares de batata por marcadores moleculares

Resumo – O objetivo deste trabalho foi avaliar um conjunto de marcadores microssatélites para identificação e caracterização varietal das cultivares de batata mais amplamente utilizadas no Brasil. O DNA das 14 variedades de batata foi genotipado com marcadores microssatélites, e os alelos foram visualizados em gel de poliacrilamida corado com prata. Vinte e quatro marcadores foram avaliados e apenas um loco foi monomórfico. Com base no padrão de bandas, foi obtido um conjunto com dois microssatélites capazes de identificar e diferenciar todas as cultivares analisadas.

Termos para indexação: *Solanum*, AFLP, certificação genética, proteção varietal, identificação varietal, RAPD.

Potato is an herbaceous plant from the Solanaceae family, with a basic set of 12 chromosomes (x = 12). It belongs to the genus *Solanum*, which presents species with different ploidy states, varying from diploid (2n = 24) to hexaploid (2n = 72). *Solanum tuberosum* L., which is tetraploid, is the most commonly cultivated species.

Farmers have used many varieties, and there is a considerable number of cultivated varieties in different countries. Potato breeders provide new cultivars that supply consumer requirements. The commercialized potato must present a pattern of smooth surface, yellow pulp and rounded shape. Many cultivars can have similar traits, and the identification of the variety is important to certify the identity and pureness of a genotype. A new cultivar must represent a single genotype to obtain a certificate of use. The identification descriptors are usually based on morphological traits, such as leaf type, tubercule shape and flower color. These traits are analyzed during different developmental stages, which is time-consuming and can be influenced by environmental factors.

There are methods of potato cultivar identification based on isozymes and total protein extraction (Douches & Ludlam, 1991). However, the results of these analyses are influenced by the developmental stage and growth conditions of the plant. Molecular identification is based mainly on random amplified polymorphic DNA (RAPD) (Demecke et al., 1993; Isenegger et al., 2001; Collares et al., 2004), amplified fragment length polymorphisms (AFLP) (Kim et al., 1998), and microsatellite markers (McGregor et al., 2000; Norero et al., 2002; Ghislain et al., 2004; Moisan-Thiery et al., 2005; Reid & Kerr, 2006; Mathias et al., 2007). The use of RAPD markers provides cultivar discrimination, but difficulties in the reproducibility of the technique are a limiting factor for accurate analysis (McGregor et al., 2000). Many microsatellite studies have used an extensive number of

markers, which increases the cost per assay and the time necessary to complete each identification. The objective of this work was to evaluate a set of microsatellite markers for varietal identification and characterization of the most widespread potato cultivars in Brazil.

A total of 14 potato varieties were used in this study: Ágata, Asterix, Atlantic, Bintje Holandesa, Cupido, Jaette Bintje, Lady Rosetta, Monalisa, Mondial, Vivaldi, Caesar, Marabel, Marjke, and Shepody. The cultivars were obtained from Laboratório de Biotecnologia Biovitrus. Genomic DNA was isolated from lyophilized tubercles, following the method described by Wulff et al. (2002).

Twenty-four microsatellite markers that were described by Ghislain et al. (2004) were selected according to polymorphism and amplification stability (Table 1). Polymerase chain reactions (PCR) were carried out in 25-μL final volumes containing 20 ng of genomic DNA, 10 mmol L^{-1} Tris-HCl, 50 mmol L^{-1} KCl, 150 μmol L^{-1} of each dNTP, 1.5 mmol L^{-1} $MgCl_2$, 0,8 μmol L^{-1} of each primer (forward and reverse) and 1U Taq-DNA polymerase (Invitrogen, São Paulo, Brazil).

PCR for 23 loci was performed using the following cycle conditions: 2 min at 94ºC; 29 cycles of 1 min at 94ºC, 2 min at specific annealing temperature, 1.5 min at 72ºC; and a final extension at 72ºC for 5 min. The only exception was the reaction for the STM1052 locus, which was carried out using the conditions described by Ghislain et al. (2004). After amplification, each PCR reaction was mixed with 5 μL of formamide containing 0.4% (w/v) bromophenol blue and 0.25% (w/v) xylene cyanol FF, and was denatured at 95ºC for 5 min. A total of 4 μL of each mixture was loaded onto a 6% denaturing polyacrylamide gel, which was run at a constant power of 100 W for 1–2 hours. A 10-bp molecular weight ladder (Invitrogen) was used for size determination, and the PCR amplification products were detected via silver staining, according to Creste et al. (2001).

Table 1. Microsatellites used for potato identification (Ghislain et al. 2004) with the number of alleles per locus, variation in size of amplified products and discriminatory power (D).

Locus	Allele number	Size range (bp)	D
STM1049	5	178–190	0.868
STM2022	4	180–200	0.626
STM1053	2	168–170	0.527
STM3023a	8	258–310	0.978
STM3023b	5	107–130	0.813
STM1031	3	160–174	0.692
STPoAc58	5	229–246	0.835
STM0019a	6	172–250	0.989
STM0019b	6	83–118	0.946
STM0031	5	151–195	0.989
STM1052	5	98–250	0.791
STM2013	12	148–176	0.934
STM1104	5	166–175	0.802
STM1016	7	239–258	0.912
STGBSS	6	227–236	0.868
STWAX-2	6	218–240	0.890
STM3012	8	160–197	0.934
STM1106	5	138–156	0.692
STM0037	5	75–90	0.879
STM0030	7	134–167	0.967
STM2030	1	176	0.000
STM1064	5	186–194	0.835
STM1058	4	104–118	0.626
STM1017	2	130–134	0.264

The microsatellites were considered dominant markers in this analysis, with alleles coded as present or absent. This treatment was necessary because the polyploid genome of the potato consequently disabled allele dosage (Moisan-Thiery et al., 2005). The discriminatory power (D) provides an estimate of the probability that two randomly chosen individuals show different banding pattern for the same locus, and it was calculated for each locus (Tessier et al., 1999). Out of the 24 loci analyzed, only STM2030 had a unique monomorphic fragment (Table 1).

The number of alleles detected per locus varied from 1 to 12, with 127 alleles in total. McGregor et al. (2000), using five microsatellite loci, detected 39 alleles in 39 genotypes. Feingold et al. (2005) obtained a range from 1 to 16 alleles using 61 microsatellites in 30 genotypes. Ghislain et al. (2004) reported the number of alleles per locus as varying from 3 to 27 using 935 cultivars. The results of this work are in agreement with other potato studies, considering that only 14 genotypes were studied. The analyzed microsatellite set presented two markers (STM0019a and STM0031) with the highest discriminatory power (D) values of 0.989 each.

The selection of markers for cultivar identification priorized the following: a, visual quality of amplification products in polyacrylamide gel; b, markers with high discriminatory power; and c, the minimum number of markers that could discriminate varieties. According to these criteria, two markers were chosen, STM0030 and STM2013 (Figure 1). The STM0030 marker presented excellent visual band quality and high discriminatory power (0.96), and permit the identification of eight unique profiles,

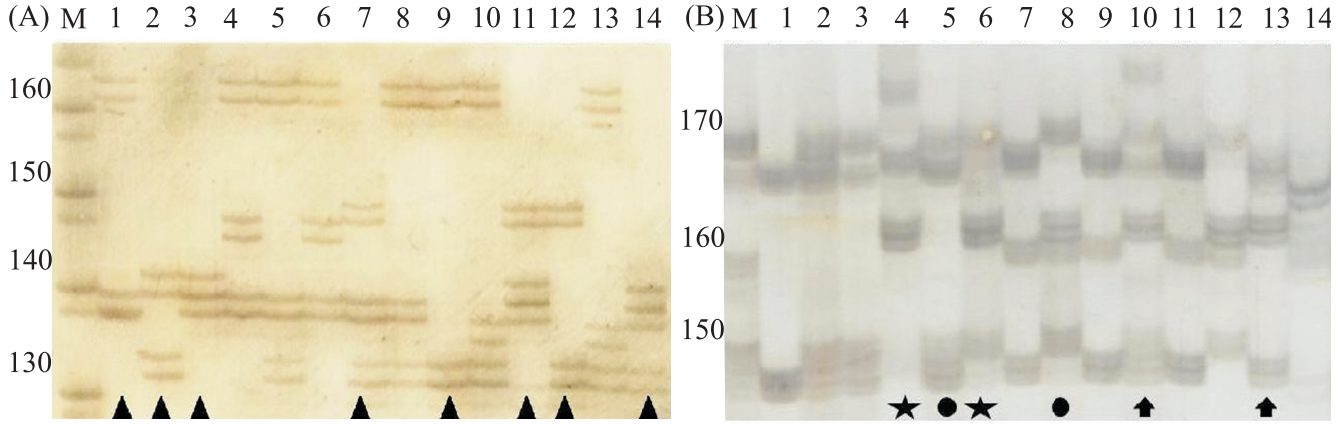

Figure 1. Amplification profile of the 14 cultivars analyzed by polyacrylamide gel 1–14: 1, Ágata; 2, Asterix; 3, Atlantic; 4, Bintje Holandesa; 5, Cupido; 6, Jaette Bintje; 7, Lady Rosetta; 8, Monalisa; 9, Mondial; 10, Vivaldi; 11, Caesar; 12, Marabel; 13, Marjke; and 14, Shepody. A, STM0030 locus, with the arrows marking cultivars 1, 2, 3, 7, 9, 11, 12 and 14 designating unique profiles. B, STM2013 locus, with the same symbol for genotypes 4 and 6, 5 and 8, 10 and 13, which are pairs differentiated by this locus. M, Molecular weight ladder with size in base pairs.

with exclusive band sets for each genotype (Figure 1 A). The remaining six genotypes were represented by three ambiguous profiles. Genotypes 4 and 6, 5 and 8, and 10 and 13 presented the same standard band for STM0030. The STM2013 genotyping permitted differentiation between those loci that were not identified using the STM0030 marker (Figure 1 B), which enabled the molecular characterization of all analyzed cultivars.

Ghislain et al. (2004) developed a set of 18 microsatellite markers for the genotyping of 913 cultivars. Reid & Kerr (2006) used six microsatellites to identify approximately 400 genotypes. The set of two markers described in this study can be widely used to identify cultivars, permitting a straightforward analysis. The microsatellite markers can be transfered between laboratories, are simple to use and the results can be obtained quickly.

The analysis of commercial potato varieties with microsatellite markers is efficient in varietal identification and complements traditional morphological characterization. This microsatellite set can be used for pedigree analysis and to certify the identity of protected varieties.

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