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AFLP markers for the assessment of genetic diversity in european and North American potato varieties cultivated in Iran

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ABSTRACT – Information about the genetic diversity of potato germplasm in Iran is important for variety identification and to enhance the classification of germplasm collections and exploit them in breeding programs and for the development and introduction of new varieties. AFLP fingerprinting was applied to a group of cultivated potato varieties to find if there is any geographical differentiation in potato diversity from Europe and North America. The high level of polymorphism within potato varieties and the high number of variety-specific bands suggest that AFLPs are powerful markers for diversity analysis in potato varieties. No region-specific AFLP markers were found (present in varieties from the same origin and absent in others). The UPGMA dendrogram revealed four distinct clusters corresponding almost to the geographical origin of the varieties. However, the bootstrap support for branches was rather weak. No clusters clearly distinguished varieties from Europe and North America. Varieties from the same geographical origins however tended to group together within each cluster. The mean similarity and the UPGMA dendrogram both suggest that North American varieties have nearly identical genetic diversity to European varieties. The results of AMOVA revealed large within-region variations which accounted for 94.5% of the total molecular variance. The between-region variation, although accounting for only 5.5% of the total variation, was statistically significant. AFLP technology was successfully used to evaluate diversity between different geographical groups of potatoes and is recommended for potato genetic studies.

Key words: Solanum tuberosum, genetic diversity, AFLP, Europe, North America.

INTRODUCTION

The cultivated potato types grown for world trade are collectively designated *Solanum tuberosum*. In total, there are seven cultivated species (including *Solanum tuberosum*), with seven subspecies, according to the latest comprehensive taxonomic treatment of Hawkes (1990). In addition to the cultivated species there are 199 related wild tuber-bearing species, distributed from the southwestern United States to south-central Chile (Hijmans and Spooner 2001). The European cultivated potato is known to have arisen from

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a limited number of introductions (Glendinning 1983), resulting in a low level of genetic diversity, compared to the potato gene pool of the American countries. Moreover, the selection of genotypes which produced tubers under long day-length conditions, combined with selection for superior agronomic traits, further narrowed the European gene pool (Provan et al. 1999).

By 1975 potato was planted in nearly all provinces. of the Iran and ranked nationally as the third most important crop, after wheat and rice (Avval 1976, Shamoradi 1985). Although Iran is clearly one of the largest potato producers in the Middle East, reliable data on recent production and on the history of former and current varieties is rare or non-existent and production statistics published by different sources vary largely. According to the Iranian Ministry of Agriculture, the total potato production in the 2004/2005 and 2005/ 2006 growing seasons was 4,830,124 and 4,218,522 tons and the mean potato yield was 25.76 and 26.20 t/ha, respectively. Before 1986 no basic seed potato was produced in Iran and most farmers used to either save a portion of their harvest, buy uncertified seed tubers at local markets or trade seed potatoes with other farmers to provide seed for the following crop season. A program was established in the mid-1970's to multiply and distribute certified seed imported from Europe. Thereafter, despite occasional interruptions, almost all potatoes cultivated commercially and used in breeding and screening programs in Iran, were introduced from European countries, particularly from the Netherlands and Germany. In recent years, some national research centers and producers began to introduce some North American and compare them with the traditional European varieties, to replace degenerating European by novel American varieties. The recently introduced North American varieties had been selected as commercially more profitable in their countries of origin. Actually almost all studied varieties are globally known as outstanding varieties.

The success of any genetic conservation and breeding program depends largely on the identification of the amount and distribution of genetic diversity in the gene pool of the concerned plant. Knowledge on the genetic diversity and relationships among plant varieties is important to recognize gene pools, to identify gaps in germplasm collections and to develop effective conservation and management strategies. In this way, molecular evaluations can provide insights into the genetic structure and diversity within and among varieties from different geographical origins, producers and distributors. Without this information, breeders have no means of selecting appropriate plant material for the participation in screening and breeding programs, with a view to the introduction of novel varieties into a country (Russell et al. 1997).

Information about the genetic diversity of potato germplasm in Iran is particularly important for variety identification, to enhance the classification of germplasm collections and exploiting them in breeding programs and for the development and introduction of new varieties. The uniformity and genetic purity of potato varieties are also highly important at the different cultivation stages and for maintenance, at harvest and for the processing industries. A lack of attention to this important issue may lead to the presence of tubers of different colors and sizes in a collection of potato tubers, indicating low genetic purity and the presence of more than one variety in a tuber collection.

In this study the main objective was to assess the level of genetic diversity within and among cultivated potatoes introduced from different origins in order to specify primary sources of germplasm for variety improvement programs. Particularly, it was investigated if there is any geographical differentiation in potato diversity between Europe and North America. Additionally, the AFLP ability was evaluated for potato genetic studies as well as for a discrimination of potato varieties, based on their geographical origin and producers. Apart from the regional importance of our studies (in Iran), since most of the studied varieties are commercially important in their countries of origin as well as in many potato producing countries, the results may serve as relevant source of information about the general value of cultivated potato germplasm.

AFLP has already been used successfully with a number of crops such as rice (Mackill et al. 1996), tea (Paul et al. 1997), almond (Sorkheh et al. 2007), barley (Russel et al. 1997), Cynodon species (Wu et al. 2005), and has been shown to reveal significant levels of DNA polymorphism in plants (Vos et al. 1995). The advantages of this technique include the large number of loci analyzed, high polymorphism levels, high reproducibility without prior sequence knowledge, and genome-wide marker distribution (Powell et al. 1996).

MATERIAL AND METHODS

Plant material and DNA extraction

The 25 accessions of Solanum tuberosum analyzed are presented in Table 1. Leaf material from each of 25 plant varieties were harvested from healthy seedlings grown in a glasshouse. The leaf tissue was immediately immersed in liquid N and stored until use. Total genomic DNA was extracted using the CTAB method, as described by Murray and Thompson (1980) and modified by Weising et al. (1995). The purified total DNA was quantified by gel electrophoresis and the quality verified by spectrophotometry. DNA samples were stored at 4 °C. Two independent extractions were performed for each sample. (1995) and Sorkheh et al. (2007). Genomic DNA was restricted with *Pstl/Tru*11 enzyme combination, doublestranded adapters specific to each site were ligated, and pre-selective amplification was performed with primers complementary to the adaptors and with one selective base at the 3° end. Selective amplification was carried out with 15 primer combinations which were synthesized by MWG (Germany) (Table 2). Fragments were resolved using acrylamide sequencing gels (Gibco-BRL, Biometra, Germany) containing 7 M urea in 1 × TBE buffer. Gels were run for 1.5–2 h at 100 W until the forward running dye (Bromophenol blue) reached the end of the gel. The DNA bands were visualized by silver staining, as described by Bassam and Caetano-Anolles (1993).

Scoring and data analysis

AFLP analysis

Details of AFLP assay, adapter and primer sequences, PCR conditions for pre-selective and selective amplifications, and PCR product electrophoresis were performed according to Vos et al. For the genetic relationship studies, only distinct, reproducible, well-resolved AFLP fragments in the size range of 67-501 bp were scored as present (1) or absent (0), and a binary data matrix was constructed based on band scores. Different polymorphic fragments produced

Table 1. Name, country of origin, and geographical origin of 25 potato varieties studied in AFLP analysis

Variety No.	Variety name	Country of origin	Geographical origin	Variety No.	Variety name	Country of origin	Geographical origin
1	Cosima	Germany	Europe	14	Sierra	United kingdom	Europe
2	Concourd	Netherlands	Europe	15	Araicana	Netherlands	Europe
3	Marfona	Netherlands	Europe	16	Atlantic	United States	North America
4	White Desire	Netherlands	Europe	17	Umo	United States	North America
5	676079		Europe	18	Russet Burbank	United States	North America
6	Achirana	Peru	South America	19	Ranger	United States	North America
7	Agria	Germany	Europe	20	Shepody	Canada	North America
8	Loman	Netherlands	Europe	21	Umatilla Russet	United States	North America
9	Serrana	Argentina	South America	22	Yukon Gold	Canada	North America
10 ,	Aracy	Brazil	South America	23	Wallowa	United States	North America
11	Americana	Netherlands	Europe	24	Ayg-2	Canada	North America
12	Maine	Germany	Europe	25	Mazama	United States	North America
13	Surrena	United kingdom	Europe				

Name	Sequence				
Msel Adapter	5'-GACGATGAGTCCTGAGTACTCAGGACTCAT -3'				
PstI Adapter	5'-GACTGCGTAGGTGCAGAGCATCTGACGCATCC -3'				
MseI + 1	5'-GATGAGTCCTGAGTAA/N -3'				
PstI +1	5'-GACTGCGTAGGTGCAG/N-3'				
MseI + 3-CAA	5'-GATGAGTCCTGAGTAA/CAA -3'				
MseI + 3-CAC	5'-GATGAGTCCTGAGTAA/CAC-3'				
MseI + 3-CAG	5'-GATGAGTCCTGAGTAA/CAG -3'				
MseI + 3-GAG	5'-GATGAGTCCTGAGTAA/GAG -3'				
MseI + 3-GCA	5'-GATGAGTCCTGAGTAA/GCA -3'				
MseI + 3-GCT	5'-GATGAGTCCTGAGTAA/GCT -3'				
MseI + 3-GTC	5'-GATGAGTCCTGAGTAA/GTC -3'				
MseI+3-GGG	5'-GATGAGTCCTGAGT AA/GGG-3'				
PstI + 3-GCA	5'-GACTGCGTAGGTGCAG/GCA-3'				
PstI + 3-GCC	5'-GACTGCGTAGGTGCAG/GCC-3'				
PstI + 3-GTA	5'-GACTGCGTAGGTGCAG/GTA -3'				
PstI + 3-ACC	5'-GACTGCGTAGGTGCAG/ACC-3'				
PstI + 3-ACT	5'-GACTGCGTAGGTGCAG/ACT-3'				
PstI + 3-AGC	5'-GACTGCGTAGGTGCAG/AGC-3'				
PstI + 3-ACG	5'-GACTGCGTAGGTGCAG/ACG-3'				
PstI + 3-AAC	5'-GACTGCGTAGGTGCAG/AAC -3'				

Table 2. Oligonucleotide adapter and primer names and sequences for 16 selective amplified fragment length polymorphism primer combinations (assay units)

by each primer were treated as unit and numbered sequentially. Monomorphic fragments and those with low intensity were not taken into account. Similarity indices were calculated using the coefficients of Jaccard and Simple Matching (SM) to estimate relationships between cultivars. A dendrogram of genetic relationship was produced by clustering the data using the unweighted pair group method with arithmetic average (UPGMA). The cophenetic correlation coefficient was calculated, and the Mantel test (Mantel 1967) was performed to check the goodness of fit of cluster analysis to the similarity matrix on which it was used. By the Mantel test, the correlation between the similarity matrix and the cophenetic matrix obtained by the Jaccard and SM coefficients, respectively, is calculated separately. Then the method by which the correlation coefficient is higher is selected for analysis. All above steps were performed using the NTSYS-pc 2.02 software package (Rohlf 1998). The relative support for the different groups and stability of the dendrogram, was assessed by bootstrap analysis (5,000 replicates), using the TREECON software package version 1.3 (Van

de Peer and De Wachter 1994). The information content of each AFLP marker was computed as $PIC_i = 1-\sum pi^2$, where pi is the frequency of the ith band. The Mean polymorphic information content (PIC) was calculated for AFLP markers across assay units by applying the above formula, proposed by Powell et al. (1996). The discrimination power of each AFLP marker was evaluated by the polymorphism information content (PIC). Finally, the partitioning of molecular variance within and among groups and accessions was calculated by the AMOVA technique (Excoffier et al. 1992) using ARLEQUIN software (Schneider et al. 2001). All significance tests were performed with 1023 permutations.

RESULTS AND DISCUSSION

Levels of AFLP polymorphism

In this study we applied AFLP fingerprinting to evaluate diversity in potato. The 16 primer combinations generated 564 polymorphic and clearly

scorable fragments across the 25 varieties (Figure 1). The number of polymorphic fragments ranged from 16 for the primer combination T-GAG/P-ACC to 52 for T-CAC/P-GCC, T-GCA/P-AGC and T-GCT/P-ACT, with a mean of 35.25 fragments per primer combination.

PIC values ranged between 0.48 and 0.723, with a mean of 0.606. PIC provides an estimate of the discriminatory power of a marker by taking into account not only the number of alleles, but also theire relative frequencies. The distribution of PIC scores was nearly uniform (random) for all 564 polymorphic AFLP markers. Results show that most of the markers have a high discrimination power.

The high number of polymorphic bands and the high level of polymorphism within potato varieties suggest that AFLPs are highly discriminatory and powerful markers for classification, fingerprinting and diversity analysis in cultivated potato varieties and most likely in wild relatives and populations as well. Furthermore, the high polymorphism produced make AFLP markers a powerful tool for genotyping a large number of accessions and suitable for the evaluation of genetic diversity in large potato gene banks. There were no region-specific (diagnostic) markers (present in varieties from the same geographical origin and absent in others) and no AFLP marker could clearly discriminate European from North American potato varieties. The variation range of genetic similarity (GS) coefficients in two groups differed only slightly, where the values varied from 0.697 to 0.881 (irrespective of GS=0.923 between Ayg-2 and Yukon Gold) in North American and from 0.701 to 0.845 in European varieties. This may indicate potentially identical diversity in European and North American potato gene pools.

Cluster and bootstrap analysis

Cluster analysis using Jaccard and simple matching coefficients led to near agglomerations. By the SM coefficient the absence of bands (score 00) was recorded as well. However, such cases do not necessarily imply an identity between two genomes (Bornet et al. 2001). The Jaccard coefficient on the other hand only considers matches between bands that are present (11) and ignores pairs in which a band is absent in both individuals (Mohammadi and Prassana 2001), so it is recommended for dominant markers such as AFLP (Link et al. 1995).



Figure 1. Electrophoretic pattern of primer combinations M-GCA/P-AGC (left) and M-GCA/P-AAC (right). The first and last lines in both images represent the ladder (VIII marker) pattern and the 25 middle lines correspond to varieties in Table 1 with the same arrangement (1-25, from left to right)

Data clustering using the Jaccard matrix showed a clear separation of the 25 cultivated potato varieties (Figure 2). The Mantel test resulted in a 0.99 cophenetic coefficient for the Jaccard coefficient. The UPGMA dendrogram revealed clusters that almost corresponded to the geographical origin of the varieties. However, rather weak bootstrap supports were revealed for branches separating varieties from the same origin, particularly those from Europe (Figure 3). The reason was most likely the homogeneous nature of European varieties and the intrinsic similarities between potato varieties since these originally shared a limited gene pool exploited in European countries. No clusters distinguished varieties from Europe and North America clearly. Within each cluster, varieties from the same geographical origins however tended to group together and the overall geographic proximity was rather high, as shown by the AFLP dendrogram. This agrees with Bornet et al. (2001), who constructed a dendrogram which revealed two main clusters corresponding to potatoes from Argentina and from Europe (Bornet et al. 2002).

North American varieties tended to group together and this cluster was more clearly distinguished and

more strongly supported than the European (Figure 3). However the mean similarity and the UPGMA dendrogram both suggested that the genetic diversity in North American is rather similar to that in European varieties. Although the European cultivated potato is known to have arisen from a limited number of introductions (Glendinning 1983) resulting in a lower level of genetic diversity compared to the American potato gene pool, the slight difference between Europe and North America in genetic diversity indicates here that the smaller geographical distance of North American countries to the main origin of potatoes (South America) does not necessarily contribute to a higher genetic diversity in the potato gene pool. Therefore the more diverse potato germplasm from South American countries is still preferable for introduction into breeding programs and further improvement of potato varieties in a country.

Among the studied verities, there are three (Serrana, Achirana and Aracy) native to South American countries (Table 1). They were however selected from European collections and introduced in Iran via Europe. Thereafter they were cultivated in the country for many years along with those originated from Europe. As







Figure 3. Bootstrap tree of 25 studied potato varieties revealed by AFLP data based on Jaccard's coefficient. Bootstrap support value is given above each branch

indicated in the dendrogram, in genetic analyses these three varieties tend to group with European rather than with North American varieties (Figure 2). These results may indicate the role of factors other than origin in the determination of genetic similarities, which may contribute to a further separation of genetically homogeneous potato germplasm. Bornet et al. (1997) reported that in their studies Argentinean potato varieties could be further subdivided into two groups, corresponding to some of the most cultivated varieties in South America and to three different dates of creation (Bornet et al. 1997).

In the cluster obtained here, Yukon Gold and Ayg-2 were considerably similar and not distinguishable from each other (GS=0.9233). Ayg-2 is a selection from Yukon Gold germplasm and had been selected due to morphological differences observed in tissue culture assays at the Iranian Potato Research Center, and was compared with newly introduced germplasm. Results indicate however that Yukon Gold and its selection were not genetically divergent enough to be considered as two different varieties and their similarity is firmly supported (Figure 3). Besides, in other field experiments carried out by the author, no obvious phenotypic dissimilarity in apparent characteristics could be observed (data not shown). These results may be considered as a confirmation of the suitability of AFLP in discriminating potato varieties based on their effective genetic differences.

The most surprising result of this analysis was the very high genetic diversity observed between an unreleased new accession 676079 and other studied varieties (Figure 2). This variety is separated from the others by a distance of 0.97 and the genetic difference to other varieties is considerable. The far genetic distance is strongly supported by bootstrap analysis and clearly observed in AFLP electrophoretic patterns (Figure 1). The DNA extraction and AFLP

experiments with this variety were repeated several times to ensure that the large genetic distance observed had not been caused by a laboratory error or by errors from other sources. In field experiments conducted by the authors however, no extreme dissimilarities with other varieties were observed in the phenotypic traits and the appearance was completely normal (data not shown). Due to this large genetic distance, the accession may serve as a valuable genetic source and promising parental material in potato breeding programs, on the basis of further research in future genetic, physiological and morphological studies.

The slightly lower mean genetic similarities and the higher genetic diversity among North American potatoes reflected the potential larger number of wild and cultivated potatoes from North America than from Europe. This agrees with Bornet et al. (2001) who suggested that European potatoes were quite homogeneous and their genetic diversity was very low compared with the Argentinean varieties, but corresponded to data from potato history. Indeed the European and North American potato gene pools both originate from South America, known as the main origin of potato plants, however only a few accessions out of the enormously rich germplasm of domesticated and wild potaoes were introduced into Europe (and North America). Therefore the potato genetic background in Europe and North America is much more limited than in South America. This should however be further evaluated and confirmed based on a larger number of more divergent varieties from geographically more diversified regions.

Analysis of molecular variance (AMOVA)

The results of AMOVA based on the geographical origin of potato varieties revealed large within-region variations which accounted for 94.5% of the total molecular variance (Table 2). This high within-region variation may have been caused by a number of reasons. One is the fact that potato is a highly heterozygous tetraploid species with a rich gene pool and wide parental diversity and the variation in genetic traits in the individual varieties is therefore expected to be high. The other within-region variation source is the between-country variation within a geographical region. Geographical

regions studied here include more than one country, since North American varieties came from the United States and Canada and European varieties from the Netherlands, Germany and England. The betweencountry component in molecular variance analysis was not separately analyzed in this study due to extreme differences in the sample sizes from different countries and producers. Furthermore, the extensive European and North American variety production may not be clearly represented by the limited number of genotypes sampled here.

The between-region variation, although accounting for only 5.5% of the total variation, was statistically significant (Table 2). This means there is measurable divergence between two regions. This result may also have been caused by differences in genetic resources exploited in breeding and variety production programs in different regions. However, it remains to be determined if the variation between two regions is due to the more divergent gene pool that exists potentially in North American countries. It was concluded here that potato varieties from the same region were more divergent, on average, than those from different regions. In fact, a large contribution of potato diversity in the studied geographical regions is based on the divergence in plant material of the respective regions.

In conclusion, AFLP technology was used to investigate genetic diversity within and among different geographical groups of potatoes and to discriminate varieties based on their geographical origin. The results show that the two studied groups of varieties are almost equally divergent and the potato gene pools of Europe or North America do not appear to be genetically more diverse in either. The varieties originated in both regions could therefore be used as a source of germplasm for further improvement of cultivated potatoes in Iran. The small sample sizes in this study, however, restrict the relevance of the analysis and the credibility of results for more generalized conclusions. Further studies should therefore be carried out, using larger variety samples derived from more extended geographical regions to clarify the general attitude of potato genetic variation and define valuable germplasms for improvement of this important crop. This study also showed that the AFLP technique could not perfectly discriminate potato varieties based on their origin.

Primer combination	Number of selective nucleotides	Total number of bands	Number of polymorphic bands	POL (%) ^a	PIC ⁺	MI ⁺⁺
M-CAA + P-GCA	3+3	40	40	100	0.554	55.40
M-CAA + P-GCC	3+3	39	37	94.87	0.596	56.54
M-CAC + P-GCA	3+3	26	25	96.15	0.723	69.52
M-CAC + P-GCC	3+3	53	52	98.11	0.681	66.81
M-CAG + P-GTA	3+3	32	32	100	0.487	48.70
M-GAG + P-ACC	3+3	19	16	84.21	0.643	54.17
M-GAG + P-ACT	3+3	38	36	94.77	0.684	64.82
M-GCA + P-AAC	3+3	47	46	97.87	0.663	64.89
M-GCA + P-ACT	3+3	19	18	94.74	0.550	52.10
M-GCA + P-AGC	3+3	55	52	94.55	0.496	46.89
M-GCT + P-ACC	3+3	35	33	94.29	0.701	66.10
M-GCT + P-ACT	3+3	52	52	100	0.634	63.40
M-GCT + P-AGC	3+3	26	25	96.15	0.480	46.15
M-GGG + P-AAC	3+3	31	30	96.77	0.629	60.86
M-GGG + P-ACG	3+3	47	45	95.74	0.621	59.45
M-GTC + P-AAC	3+3	25	25	100	0.551	55.10
Mean		36.5	35.25	96.14	0.606	58.18

AFLP markers for the assessment of genetic diversity in european and North American potato varieties cultivated in Iran Table 3. Degree of polymorphism and information content for 16 AFLP primer combinations applied to 25 cultivated potato varieties

Mean polymorphic information content for polymorphic bands (see Materials and Methods). Marker index calculated as MI = POL - PIC (POL is polymorphism percentage)

Table 4. Results of AMOVA analysis of 25 potato varieties based on two geographical origins (Europe and North America)

Source of Variation	Df	SSD ^a	MSD ^b	Variance component	Total ^c (%)	P value ^d
Between regions	1	91.847	91.847	3.145	5.50	< 0.002
Individuals within	23	1244.233	54.097	54.097	94.50	
regions Total	24	1336.080		et Rhele Manada	at the second to	and the second second

a= Square Sum of Deviations

b= Mean Square of Deviations

c= Percent of total molecular variance

d= Probability of obtaining a larger component estimate. Number of permutations=1023

Although the varieties originated from a same geographical region tended to group together, no diagnostic marker for the origin of potatoes was denoted in AFLP-based genetic analysis. In general, considering the high polymorphism and data frequency revealed by AFLP markers, the technique is recommended for potato genetic studies and for the identification of potato varieties.

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Marcadores de AFLP na avaliação da diversidade genética de variedades europeas e norte americanas de batata-inglesa cultivadas no Irã

RESUMO - As informações sobre a diversidade genética do germoplasma de batata no Iran são importantes para a identificação de variedades a fim de melhorar a classificação da coleção de germoplasma e utilizar estas variedades nos programas de melhoramento genético para o desenvolvimento e introdução de novas variedades. O fingerprinting de AFLP foi aplicado a um grupo de variedade de batatas cultivadas para verificar a existência de diferenciação geográfica na diversidade da Europa e da América do Norte. O alto nível de polimorfismo dentro das variedades e o alto número de bandas específicas sugerem que os AFLPs são marcadores úteis para a análise da diversidade nas variedades de batata. Nenhum marcador AFLP específico a determinada região foi encontrado (presente nas variedades da mesma origem e ausente em outras). O dendrograma UPGMA revelou quatro grupos distintos correspondendo à origem geográfica das variedades da Europa e da América do Norte. A similaridade média e o dendrograma UPGMA sugerem que as variedades da América do Norte possuem diversidade genética muito semelhante às variedades européas. Os resultados da AMOVA revelaram ampla variação dentro das regiões as quais apresentaram 94,5% da variância molecular total. A variação entre regiões, embora represente somente 5,5% da variação total, foi estatisticamente significante. A tecnologia AFLP foi utilizada com sucesso para avaliar a diversidade entre diferentes grupos geográficos de batatas e é recomendada para estudos genéticos nessa espécie.

Palavras-chave: Solanum tuberosum, diversidade genética, AFLP, Europa, América do Norte.

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