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Genetic diversity of maize (*Zea mays*) accessions revealed by random amplified polymorphic DNA markers

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ABSTRACT

In the present study, random amplified polymorphic DNA (RAPD) markers were used to assess genetic diversity of the maize genotypes. Five arbitrary random primers were used to determine RAPD polymorphism in the set of 20 maize genotypes. Amplification of genomic DNA of 20 genotypes, using RAPD analysis, yielded 33 fragments, with an average of 6.60 polymorphic fragments per primer. Number of amplified fragments ranged from 5 (OPA-02, OPB-08) to 10 (OPA-13), with the size of amplicons ranging from 250 to 2000 bp. The polymorphic information content value ranged from 0.751 (OPD-02) to 0.872 (OPA-13), with an average of 0.781 and diversity index value varied from 0.718 (OPB-08) to 0.874 (OPA-13) with an average of 0.790. The dendrogram based on hierarchical cluster analysis using unweighted pair group method with arithmetic average (UPGMA) algorithm was prepared. A dendrogram based on UPGMA analysis separated 20 maize genotypes into two clusters. RAPD markers are useful in the assessment of maize diversity, the detection of the duplicate sample in genotypes collection, and the selection of a core collection to enhance the efficiency of genotypes management for use in maize breeding and conservation.

KEY WORDS: Genetic diversity, maize, random amplified polymorphic DNA

INTRODUCTION

Maize (*Zea mays*) is one of the world's most important crop plants after wheat and rice, which provides staple food to a large number of human population in the world (Ahmad *et al.*, 2011). It is belonging to the family of Poaceae. In developing countries, maize is a major source of income to many farmers (Tagne *et al.*, 2008). In addition, a few improved genotypes with narrower genetic variability are quickly replacing maize landraces (Pollack, 2003).

With the beginning of studies that led to the development of polymerase chain reaction (PCR) technology (Mullis and Faloona, 1987), there were amazing advances in the refinement of techniques to obtain specific or non-specific DNA fragments, relevant mainly to research in genetic diversity. The following techniques are those mostly used and are listed in chronological order: Simple sequence repeats (SSR) or just microsatellites (Tautz, 1989), randomly amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990) or arbitrarily primed PCR (Welsh and McClelland, 1990), inter-SSRs (ISSR) (Zietkiewicz *et al.*, 1994), amplified fragment length polymorphism (AFLP) (Vos *et al.*, 1995), single nucleotide polymorphisms (Chen and Sullivan, 2003) and, more recently, diversity array technology (Kilian *et al.*, 2005; Schlotterer, 2004; Schulman, 2007). RAPD has proven to be quite efficient in detecting genetic variations and used for diversity assessment and for identifying germplasm in a number of plant species (Gajeraa *et al.*, 2010; El Kichaoui *et al.*, 2013; Srivashtav *et al.*, 2013; Omalsaad *et al.*, 2014; Vivodík *et al.*, 2014; Žiarovská *et al.*, 2013; Labajová *et al.*, 2011).

The aim of this study was to detect genetic variability among the set of 20 maize genotypes using five RAPD markers.

MATERIALS AND METHODS

Plant Material and Extraction of Genomic DNA

Maize lines (20) were obtained from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the Gene

Bank in Piest'any (Slovakia) [Table 1]. Genomic DNA was isolated from the 14 days leaves with GeneJET Plant Genomic DNA Purification Mini Kit according to the manufacturer's instructions. The maize DNA was stored in a freezer at −70°C.

RAPD Amplification and Gel Electrophoresis

Amplification of RAPD fragments was performed according to Gajeraa *et al.* (2010) [Table 2] using decamer arbitrary primers (Operon Technologies Inc, USA; SIGMA-D, USA). PCR were carried out in 25 µl of following mixture: 10.25 µl deionized water, 12.5 µl Master Mix (Genei, Bangalore, India), 1.25 µl of genomic DNA, and 1 µl of 10 pmol of primer. Amplification was performed in a programmed thermocycler (Biometra, Germany) with initial denaturation at 94°C for 5 min, 42 cycles of denaturation at 94°C for 1 min, primer annealing at 38°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 5 min. Amplified products were separated in 1.5% agarose in ×1 Tris-borate-ethylene-diamine-tetraacetic acid buffer. The gels were stained with ethidium bromide and documented using gel documentation system Grab-It 1D pre-Windows.

Data Analysis

The RAPD bands were scored as present (1) or absent (0), each of which was treated as an independent character regardless of its intensity. A dendrogram based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) with the SPSS professional statistics version 17 software package was constructed. For the assessment of the polymorphism between genotypes ricin and usability RAPD markers in their differentiation, we used diversity index (DI) (Weir, 1990), the probability of identity (PI) (Paetkau *et al.*, 1995) and polymorphic information content (PIC) (Weber, 1990).

RESULTS

Our study dealt with detection of genetic polymorphism in maize cultivars using RAPD markers. For the differentiation of 20 maize genotypes, five RAPD markers [Table 2] were chosen, as Gajeraa *et al.* (2010). PCR amplifications using five RAPD primers produced 33 DNA fragments that could be scored in all genotypes [Figure 1]. The selected primers amplified DNA fragments across the 20 genotypes studied, with the number of amplified fragments varying from 5 (OPA-02, OPB-08) to 10 (OPA-13), and the amplicon size varying from 250 to 2000 bp. Of the 33 amplified bands, all 33 were polymorphic, with an average of 6.60 polymorphic bands per primer. The PIC value varied from 0.709 (OPB-08) to 0.872 (OPA-13), with an average of 0.781 and DI value varied from 0.718 (OPB-08) to 0.874 (OPA-13) with an average of 0.790 [Table 3]. A dendrogram based on UPGMA analysis separated 20 maize genotypes into two clusters. First cluster contained two Slovak

Table 1: List of 20 analyzed genotypes of maize

Genotypes	Country of origin	Year of registration
Feheres Sarga Filleres	Hungary	1965
Mindszentpusztai Feher	Hungary	1964
Zakarpatskaja	Union of Soviet Socialist Republics	1964
Przebedowska Burskynowa	Poland	1964
Krasnodarskaja	Union of Soviet Socialist Republics	1964
Mesterhazy Sarga Simaszemu	Hungary	1964
Slovenska biela perlova	Czechoslovakia	1964
Zuta Brzica	Yugoslavia	1975
Zloty Zar	Poland	1964
Slovenska Florentinka	Czechoslovakia	1964
Juhoslavanska	Yugoslavia	1964
Kostycevskaja	Union of Soviet Socialist Republics	1964
Mindszentpusztai Sarga Lofogu	Hungary	1964
Stodnova	Czechoslovakia	1964
Slovenska žltá	Slovak Republic	1964
Slovenska krajová velkozrná	Slovak Republic	1964
Partizanka	Union of Soviet Socialist Republics	1964
Voroneskaja	Union of Soviet Socialist Republics	1964
Kocovska Skora	Slovak Republic	1964
Milada	Czechoslovakia	1964

Table 2: List of RAPD primers

Primers	Primer sequence (5´-3´)	Molecular weight range (bp)
OPA-02	TGCCGAGCTG	300-2000
OPA-03	AGTCAGCCAC	250-900
OPA-13	CAGCACCCAC	400-2000
OPB-08	GTCCACACGG	400-1700
OPD-02	GGACCCAACC	500-2000

RAPD: Random amplified polymorphic DNA

Table 3: Statistical characteristics of RAPD markers used in maize

Primers	Number of alleles	DI	PIC	PI
OPA-02	5	0.768	0.755	0.041
OPA-03	7	0.826	0.820	0.007
OPA-13	10	0.874	0.872	0.006
OPB-08	5	0.718	0.709	0.032
OPD-02	6	0.765	0.751	0.049
Average	6.6	0.790	0.781	0.027

DI: Diversity index, PIC: Polymorphic information content, PI: Probability of identity, RAPD: Random amplified polymorphic DNA

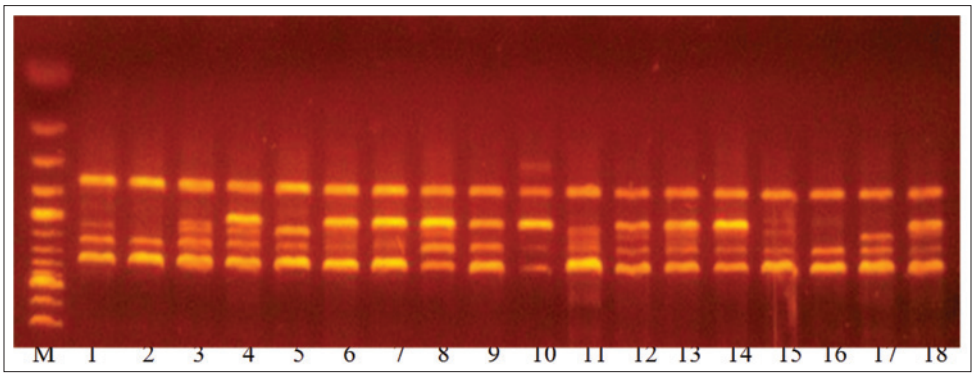

Figure 1: Polymerase chain reaction amplification products of 18 genotypes of maize produced with random amplified polymorphic DNA primer OPA-02. Lane M is 1-kb DNA ladder, and lanes 1-18 are maize genotypes [Table 2]

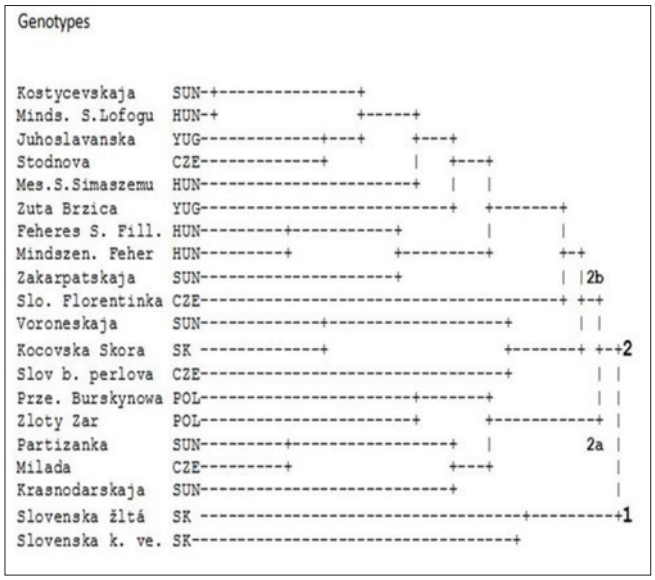

Figure 2: Dendrogram of 20 maize genotypes prepared based on five random amplified polymorphic DNA markers, SUN: Union of Soviet Socialist Republics, HUN: Hungary, YUG: Yugoslavia, CZE: Czechoslovakia, SK: Slovak Republic, POL: Poland

maize genotypes Slovenska žltá and Slovenska krajová velkozrná. Cluster two was divided into two main cluster 2a and 2b. Main cluster 2a contained two Poland genotypes Przebedowska Burskynowa and Zloty Zar, two genotypes from Union of Soviet Socialist Republics - Krasnodarskaja and Partizanka and one Czechoslovakia genotypes Milada. Main cluster 2b contained 13 genotypes of maize. We could not distinguish two genotypes, Kostycevskaja and Mindszentpusztai Sarga Lofogu (subcluster 2b), which can be caused due to close genetic background [Figure 2].

DISCUSSION

Similar values of DI and PIC were detected by other authors (Osipova *et al.*, 2003; De Vasconcelos *et al.*, 2008; Mukharib *et al.*, 2010; Al-Badeiry *et al.*, 2013; Molin *et al.*, 2013; and Mrutu *et al.*, 2014) and these values presented a high level of polymorphism of maize genotypes detected by RAPD markers. Osipova *et al.* (2003) used RAPD and ISSR markers to analyze genetic divergence between the regenerated plants derived from callus cultures and the original maize line A188. Specific polymorphism revealed with random primers was completely confirmed using five sequence characterized amplified regions (SCAR) markers. De Vasconcelos *et al.* (2008) used RAPD technique to evaluate somaclonal variation in maize plants derived from tissue culture from the maize inbred line L48 (derived from Suwan). 47 different decamer oligonucleotide primers generated 221 amplification products, 130 of them being polymorphic. Al-Badeiry *et al.* (2013) used RAPD markers to fingerprint 20 varieties of maize. 20 operon primers generated informative RAPD patterns and selected for further RAPD analysis. The aims of Molin *et al.* (2013) study was to estimate genetic diversity across 48 varieties of maize landraces cultivated at different locations in the States of Rio Grande do Sul (RS) and Paraná (PR) by means of RAPD, SSR, and AFLP markers. The objective of Mrutu *et al.* (2014) study was to assess genetic diversity of maize hybrids grown in Southern highlands of Tanzania by using RAPD markers.

RAPD molecular markers have been used in population genetic studies (Žiarovská *et al.*, 2014; Pawar *et al.*, 2013; Petrovičová *et al.*, 2014; Štefúnová *et al.*, 2014; and Kallamadia *et al.*, 2015). Some researchers have considered RAPD markers to represent segments of DNA with non-coding regions and to be selectively neutral (Penner, 1996; and Vivodík *et al.*, 2015).

CONCLUSION

The analysis showed that RAPD markers present effective molecular markers for assessment of the genetic diversity in maize. A dendrogram based on UPGMA analysis separated 20 maize genotypes into two clusters.

First cluster contained two Slovak maize genotypes Slovenska žltá and Slovenska krajová velkozrná. Cluster two was divided into two main cluster 2a and 2b. We could not distinguish two genotypes, Kostycevskaja and Mindszentpusztai Sarga Lofogu, which can be caused due to the close genetic background.

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