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REGULAR ARTICLE

Evaluation of molecular diversity of central European maize cultivars

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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 from Central European countries and Union of Soviet Socialist Republics. Thirteen arbitrary random primers were used to determine RAPD polymorphism in the set of forty maize genotypes. Amplification of genomic DNA of 40 genotypes, using RAPD analysis, yielded 92 fragments, with an average of 7.08 polymorphic fragments per primer. Number of amplified fragments ranged from 5 (OPA-02, OPB-08, OPD-07) to 10 (OPA-13), with the size of amplicons ranging from 100 to 2500 bp. The polymorphic information content (PIC) value ranged from 0.709 (OPB-08) to 0.872 (OPA-13), with an average of 0.801 and index diversity (DI) value varied from 0.718 (OPB-08) to 0.874 (OPA-13) with an average of 0.808. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. The hierarchical cluster analysis divided maize genotypes into 2 main clusters. In the first cluster were included 2 genotypes (Mikulická and Aranyozon sarga lofogu) from Czechoslovakia and Hungary, respectively. Second cluster was subdivided in two subclusters (2a and 2b). Subcluster 2a contained two genotypes of different origin and subcluster 2b was further subdivided into two subclusters, subcluster 2ba with two genotypes and subcluster 2bb with 34 maize genotypes. Two genotypes of 2bb subcluster (Kostycevskaja and Mindszentpusztai Sarga Lofogu) from former Union of Soviet Socialist Republics and Hungary, respectively, were genetically the closest. Clustering only partially reflected geographic origin of studied maize genotypes. In this experiment, RAPD proved to be a rapid, reliable and practicable method for revealing of polymorphism in the maize cultivars.

Key words: Dendrogram; Genetic variability; Molecular markers; Random primers; *Zea mays* L

INTRODUCTION

Maize (*Zea mays*) is one of the world's most important crop plants after wheat and rice, which provides staple food to 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). The genetic diversity observed across landraces is the most important part of maize biodiversity, and local races represent an important fraction of the genetic variability exhibited by this genus. However, few agronomic and genetic data exist for such collections, and this scarcity has limited the use, management, and conservation of this germplasm. In addition, a few improved genotypes with narrower genetic variability are quickly replacing maize landraces (Pollack, 2003).

Since 1990, random amplified polymorphic DNA (RAPD) markers have been successfully applied for identification of DNA polymorphism in various plant species (Williams et al., 1990). They are often used for screening of a wide range of genetic stocks in order to find linkage with traits of agronomic significance (Masojć et al., 2001).

RAPD technique requires only small amounts of DNA sample without involving radioactive labels and are simpler as well as faster. 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., 2014). Suitability of RAPD markers for the construction of genetic maps, fingerprinting and phylogenetic studies has been proved by many authors. In cereal crops, such as wheat (Saleh, 2012; Bibi et al., 2012; Cifci et al., 2012), barley (Bakht et al., 2011), rye (Persson et al., 2002; Petrovičová et al., 2014), the technique has been applied to identify cultivars and revealing phylogenetic

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relationships among them. In the case of maize, there are a lot of papers (Osipova et al., 2003; Carvalho et al., 2004, Asif et al., 2006; Bruel et al., 2007, Abuali et al., 2011, Al-Badeiry et al., 2013, Molin et al., 2013) that have reported the application of the RAPD marker technique for maize molecular identification, and the technique was proved to be effective for or verification of purity and would improve the efficiency of breeding programmes (Asif et al., 2006).

The aim of this study was to detect genetic variability among the set of 40 maize genotypes using 13 RAPD markers and to testify an usefulness of chosen RAPD markers for genetic diversity study.

MATERIAL AND METHODS

Plant material and extraction of genomic DNA

Maize genotypes (40) were obtained from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the Gene Bank in Piest'any, the Slovak Republic (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.

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). Polymerase chain reactions (PCR) were carried out in 25 µl of following mixture: 10.25 µl deionized water, 12.5 µl Master Mix (Promega, USA), 1.25 µl of genomic DNA, 1 µl of 10 pmol of primer. Amplification was performed in a 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× TBE 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 maize 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).

Table 1: List of 40 analyzed genotypes of maize

Genotypes	Country of origin	Year of registration
1. Feheres Sarga Filleres	Hungary	1965
2. Mindszentpusztai Feher	Hungary	1964
3. Zakarpatskaja	Union of Soviet Socialist Republics	1964
4. Przebedowska Burskynowa	Poland	1964
5. Krasnodarskaja	Union of Soviet Socialist Republics	1964
6. Mesterhazy Sarga Simaszemu	Hungary	1964
7. Slovenska biela perlova	Czechoslovakia	1964
8. Zuta Brzica	Yugoslavia	1975
9. Zloty Zar	Poland	1964
10. Slovenska Florentinka	Czechoslovakia	1964
11. C.44 Juhoslavska	Yugoslavia	1964
12. Kostycevskaja	Union of Soviet Socialist Republics	1964
13. Mindszentpusztai Sarga Lofogu	Hungary	1964
14. Stodnova	Czechoslovakia	1964
15. Slovenska žltá	Slovak Republic	1964
16. Slovenska krajová velkozrná	Slovak Republic	1964
17. Partizanka	Union of Soviet Socialist Republics	1964
18. Voroneskaja	Union of Soviet Socialist Republics	1964
19. Kocovska Skora	Slovak Republic	1964
20. Milada	Czechoslovakia	1964
21. Moldavskaja	Union of Soviet Socialist Republics	1964
22. Bučiansky Konský Zub	Slovak Republic	1964
23. Hodoninský konský zub žltý	Czechoslovakia	1964
24. M Silokukurica	Hungary	1964
25. Valticka	Czechoslovakia	1964
26. Przebedowska Biala	Poland	1964
27. Toschevska	Slovak Republic	1964
28. Šamorinsky konský zub	Hungary	1964
29. Wielkopolanka	Poland	1964
30. Czechnicka	Poland	1964
31. Manalta	Czechoslovakia	1964
32. Zlota gorecka	Poland	1964
33. Celchovicka ADQ	Czechoslovakia	1964
34. Belaja mestnaja	Union of Soviet Socialist Republics	1964
35. Bučanská žltá	Slovak Republic	1964
36. Iregszemeseil 2 hetes	Hungary	1964
37. Dnepropetrovskaja	Union of Soviet Socialist Republics	1964
38. Bezuncukskaja	Union of Soviet Socialist Republics	1964
39. Mikulická	Czechoslovakia	1964
40. Aranyozon sarga lofogu	Hungary	1964

RESULTS AND DISCUSSION

Our study dealt with detection of genetic polymorphism in maize cultivars using RAPD markers. For the differentiation of forty maize genotypes thirteen RAPD markers (Table 1)

were chosen according to Gajeraa et al. (2010). PCR amplifications using 13 RAPD primers produced 92 DNA fragments that could be scored in all genotypes (Figure 1). Chosen primers amplified DNA fragments across 40 maize genotypes studied, with the number of amplified fragments ranged from 5 (OPA-02, OPB-08, OPD-07) to 10 (OPA-13), and the amplicon size varying from 100 to 2500 bp. Of the 92 amplified bands, all 92 were polymorphic, with an average of 7.08 polymorphic bands per primer. The polymorphic information content (PIC) values varied from 0.709 (OPB-08) to 0.872 (OPA-13), with an average of 0.801 and index diversity (DI) value varied from 0.718 (OPB-08) to 0.874 (OPA-13) with an average of 0.808 (Table 3).

Similar values of DI and the 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; Mrutu et al., 2014) and these values presented a high level of polymorphism of maize genotypes detected by RAPD markers. Our results based on the values of DI and PIC showed that RAPD markers are suitable marker system to distinguish maize genotypes.

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
OPD-07	TTGGCACGGG	200-1000
OPD-08	GTGTGCCCCA	300-1700
OPD-13	GGGGTGACGA	100-1500
OPE-07	AGATGCAGCC	200-1300
OPF-14	TGCTGCAGGT	150-2500
SIGMA-D-01	AAACGCCGCC	300-2000
SIGMA-D-14	TCTCGCTCCA	400-800
SIGMA-D-P	TGGACCGGTG	200-2500

Table 3: The statistical characteristics of the 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
OPD-07	5	0.725	0.723	0.026
OPD-08	8	0.834	0.829	0.006
OPD-13	9	0.856	0.849	0.005
OPE-07	7	0.835	0.829	0.006
OPF-14	8	0.865	0.862	0.003
SIGMA-D-P	7	0.839	0.833	0.005
SIGMA-D-01	8	0.854	0.849	0.004
SIGMA-D-14	7	0.741	0.728	0.023
Average	7.08	0.808	0.801	0.016

DI: Diversity index; PIC: Polymorphic information content; PI: Probability of identity

Osipova et al. (2003) used RAPD markers to analyse the 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 SCAR markers. De Vasconcelos et al. (2008) used the RAPD technique to evaluate somaclonal variation in maize plants derived from tissue culture from the maize inbred line L48 (derived from Suwan). Forty seven 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. Twenty operon primers generated informative RAPD patterns and selected for further RAPD analysis. The largest number of polymorphic bands (20 bands) was produced by primer OPX-04 while, the lowest number of polymorphic bands (1 band) was produced by primer OPA-03. The primers of the most interest of this purpose were those that produced more variety specific DNA profiles, such as OPD-03, OPE-18, OPF-05, OPL-11 and OPX-04. In our study we have detected 7 polymorphic alleles by primer OPA-03. Much higher number of alleles (7) compared to Al-Badeiry et al. (2013), who detected only one allele, can be caused by diverse set of maize varieties used for analysis. Mrutu et al. (2014) assessed the genetic diversity of maize hybrids grown in Southern highlands of Tanzania by using RAPD markers. Twelve maize samples (six inbreds and six hybrids) were collected and used in this study. A total of 123 bands were produced of which 98 (80%) were polymorphic.

The aim of Molin et al. (2013) was to estimate the 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 different marker system including random amplified polymorphic DNA (RAPD). Maize landrace accessions were genotyped using the 30 RAPD primers. RAPD analysis resulted in amplification of 335 fragments polymorphic fragments and a polymorphic index of 81.9%. Similar level of polymorphism (84.44%) obtained also Bruel et al. (2007).

A dendrogram prepared based on hierarchical cluster analysis using UPGMA algorithm separated 40 maize genotypes into two clusters. First cluster contained two maize genotypes Mikulická and Aranyozon sarga lofogu. from Czechoslovakia and Hungary, respectively. Second cluster was subdivided in two subclusters (2a and 2b).

Subcluster 2a contained two genotypes of different origin from former Czechoslovakia and Yugoslavia, and subcluster 2b was further subdivided into two subclusters, subcluster 2ba with two genotypes from Slovakia and former Union of Soviet Socialist Republics, and subcluster 2bb with 34 maize genotypes. Two genotypes of 2bb subcluster (Kostycevskaja and Mindszentpusztai Sarga Lofogu) from former Union of Soviet Socialist Republics and Hungary, were genetically the closest (Fig 2). Dendrogram partially reflects the country of origin of studied maize genotypes.

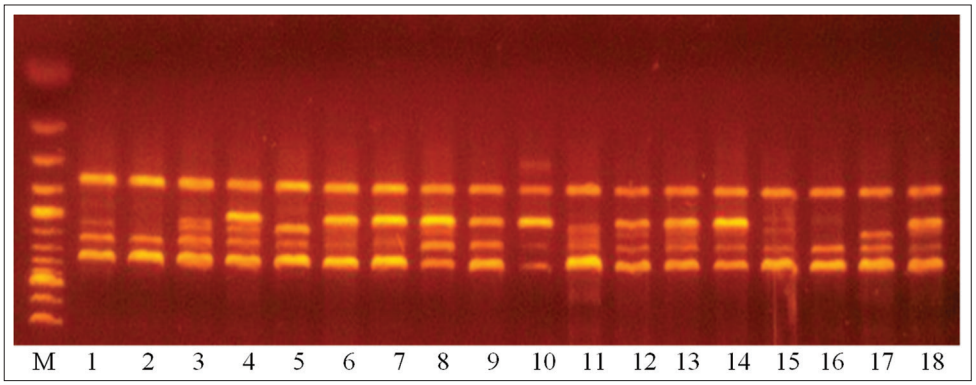

Fig 1. PCR amplification products of 18 genotypes of mayze produced with RAPD primer OPA-02. Lane M is 1-kb DNA ladder and lanes 1- 18 are maize genotypes (Table 2).

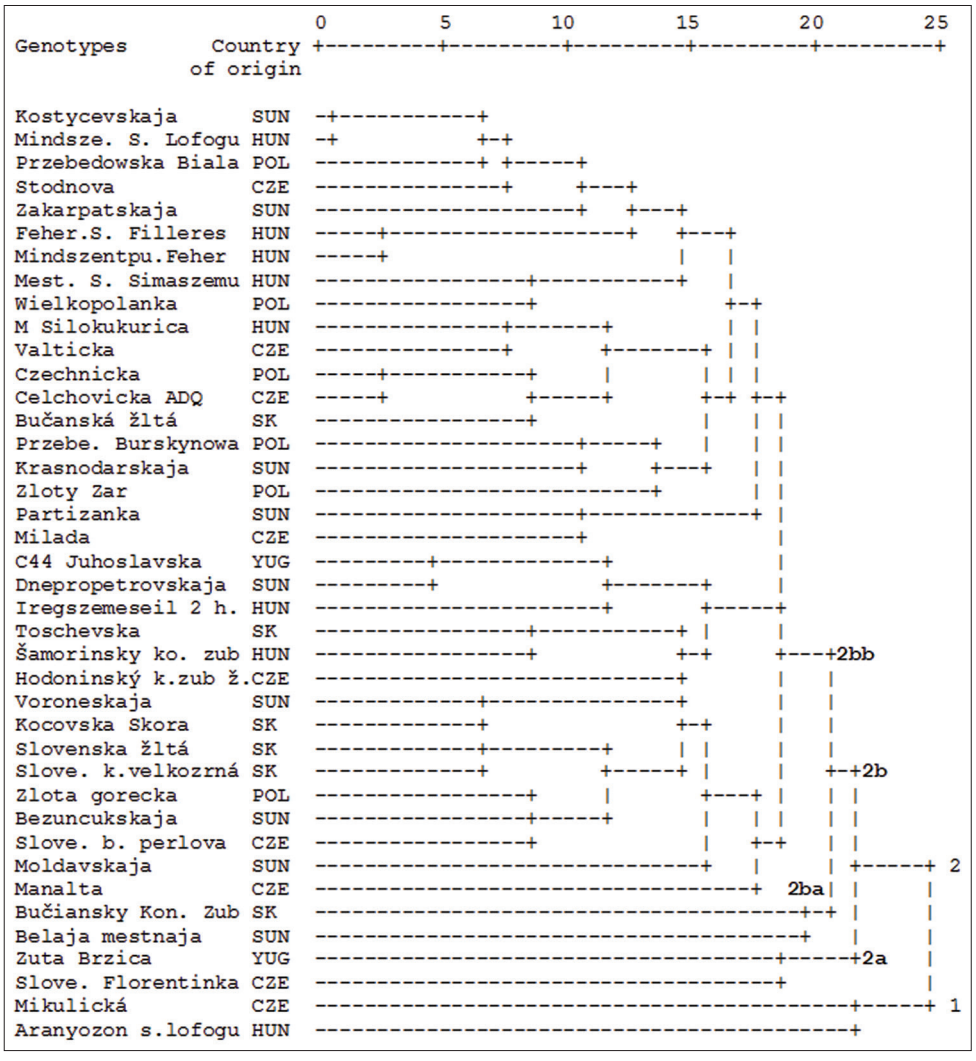

Fig 2. Dendrogram of 40 maize genotypes prepared based on 13 RAPD markers. CZE - Czechoslovakia, HU - Hungary, POL - Poland, SUN - Union of Soviet Socialist Republics, SK – Slovakia, YUG - Yugoslavia

De Vasconcelos et al. (2008) based on cluster analyses divided the maize samples into three distinct groups, considering an upper limit of 0.38 genetic distances. Molin et al. (2013) using 30 RAPD primers studied the 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 different marker system including random amplified polymorphic DNA (RAPD). Regarding the RAPD dendrogram, groups comprising accessions from RS prevailed, whereas SSR comprised varieties from both collection sites. Mrutu et al. (2014) based on RAPD analysis assessed the genetic diversity of maize hybrids grown in Southern highlands of Tanzania. The range of genetic similarity of the studied samples calculating based on Jaccard's similarity coefficient was from 0.32 to 0.95. The UPGMA analysis indicated higher similarity between the hybrids than the inbreds. Bruel et al. (2007) constructed dendrogram using UPGMA clustering method. They found out that 16 lines separated into five distinct groups, which were in agreement with the heterotic patterns described based on the genealogy of the lines.

RAPD molecular markers have been used in population genetic studies (Žiarovská et al., 2013; Pawar et al., 2013; Petrovičová et al., 2014; Kallamadi et al., 2015). Some researchers have considered RAPD markers to represent segments of DNA with noncoding regions and to be selectively neutral (Vivodík et al., 2014), and some studies have shown that RAPD markers are distributed throughout the genome and may be associated with functionally important loci (Penner, 1996).

CONCLUSION

The analysis showed that the RAPD markers are very effective molecular markers for the assessment of the genetic diversity in maize and for differentiation of a set of maize genotypes. A dendrogram based on UPGMA analysis separated 40 maize genotypes into two subclusters. The primers used in our analysis recorded 100 per cent polymorphism. RAPD markers can be used to identify diverse sources in crop germplasm collections or to select groups of genotypes with desirable characters and contrasting phenotypes, if large number are employed. RAPD markers are useful in the assessment of maize diversity, the detection of 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.

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AUTHOR CONTRIBUTIONS

M. V.: was responsible for the molecular analysis and paper writing. Ž. B.: was involved in molecular analysis and evaluating of the results and reviewed the paper. Z. G.: supervised the research.

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