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ASSESSMENT OF RAPD POLYMORPHISM IN MAIZE (*ZEA MAYS* L.) GENOTYPES

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Maize or corn is a plant belonging to the family Poaceae and is one of the most important cereal crops worldwide as human nutrient, a basic element of animal feed and raw material for manufacture of many industrial products. Maize is the oldest plant to have a fully established gene map with the basic genome consisting of 10 chromosomes and is an excellent plant for the detection of genotoxins, mutagenic and clastogenic substances in the environment. 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 twenty maize genotypes. Amplification of genomic DNA of 20 genotypes, using RAPD analysis, yielded 35 fragments, with an average of 7.00 polymorphic fragments per primer. Number of amplified fragments ranged from 5 (OPD-07) to 8 (OPF-14 and SIGMA-D-01), with the size of amplicons ranging from 150 to 2500 bp. The polymorphic information content (PIC) value ranged from 0.723 (OPD-07) to 0.862 (OPF-14), with an average of 0.799 and index diversity (DI) value varied from 0.725 (OPD-07) to 0.865 (OPF-14) with an average of 0.805. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. First cluster contained two maize genotypes Bučiansky Konský Zub (SK) and Moldavskaja (SUN). Cluster two was divided into two main cluster 2a and 2b. Main cluster 2a contained genotype Dnepropetrovskaja (SUN) and main cluster 2b was divided into two subclustrov 2ba and 2bb. 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.

Keywords: maize; genetic diversity; Random amplified polymorphic DNA (RAPD); dendrogram; PIC

Introduction

Maize (*Zea mays* L.) 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 belongs to the family Poaceae. In developing countries maize is a major source of income to many farmers (Tagne et al., 2008). Maize has greater nutritional value as it contains about 72% starch, 10% proteins, 8.5% fiber, 4.8% oil, 3% sugar and 1.7% ash. It is not only an important human nutrient, but also a basic element of animal feed and raw material for manufacture of many industrial products (Farhad et al., 2009). In addition to its agronomic importance, maize has been a keystone model organism for basic research for nearly a century. It is the most thoroughly researched genetic

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system. Several attributes of the maize plant, including a vast collection of mutant stocks, large heterochromatic chromosomes, extensive nucleotide diversity, and genic colinearity within related grasses, have positioned this species as a centerpiece for genetic, cytogenetic, and genomic research (Yim et al., 2007). As a model organism, it is the subject of such far-ranging biological investigations as plant domestication, genome evolution, epigenetics, heterosis, quantitative inheritance, and comparative genomics (Strable et al., 2009).

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).

With the beginning of studies that led to the development of polymerase chain reaction (PCR) technology (Mullis et al., 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 or just microsatellites (SSR) (Tautz, 1989), randomly amplified polymorphic DNA (RAPD) (Williams et al., 1990) or arbitrarily primed PCR (AP-PCR) (Welsh et al., 1990), inter-simple sequence repeats (ISSR) (Zietkiewicz et al., 1994), amplified fragment length polymorphism (AFLP) (Vos et al., 1995), single nucleo-tide polymorphisms (SNPs) (Chen et al., 2003) and, more recently, diversity array technology (DarT) (Kilian et al., 2005; Schulman, 2007). RAPD technique requires only small amounts of DNA sample without involving radioactive labels and are simpler as well as faster. Random amplified polymorphic DNA 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; Srivashtav et al., 2013; Žiarovská et al., 2013; Omalsaad et al., 2014; Vivodík et al., 2014).

The aim of this study was to detect genetic variability among the set of 20 old maize genotypes using 5 RAPD markers.

Materials and methodology

Maize lines (20) were obtained from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the Gene Bank in Piesťany (Slovakia). Maize genotypes were grown in a growth chamber on humus soil. 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.

Amplification of RAPD fragments was performed according to Gajeraa et al. (2010) 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 (Genei, Bangalore, India), 1.25 µl of genomic DNA, 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× TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system Grab-It 1D pre Windows.

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 and discussion

Our study dealt with detection of genetic polymorphism in maize cultivars using RAPD markers. For the differentiation of twenty maize genotypes, five RAPD markers were chosen, as Gajeraa et al. (2010). PCR amplifications using 5 RAPD primers produced 35 DNA fragments, which could be scored in all genotypes. The selected primers amplified DNA fragments across the 20 genotypes studied, with the number of amplified fragments varying from 5 (OPD-07) to 8 (OPF-14 and SIGMA-D-01), with the size of amplicons ranging from 150 to 2500 bp. Of the 35 amplified bands, all 35 were polymorphic, with an average of 7.00 polymorphic bands per primer.

The polymorphic information content (PIC) value ranged from 0.723 (OPD-07) to 0.862 (OPF-14), with an average of 0.799 and index diversity (DI) value varied from 0.725 (OPD-07) to 0.865 (OPF-14) with an average of 0.805 (Table 1).

Table 1 Statistical characteristics of RAPD markers used in maize

Primers	Number of alleles	DI	PIC	PI
OPD-07	5	0.725	0.723	0.026
OPF-14	8	0.865	0.862	0.003
SIGMA-D-01	8	0.854	0.849	0.004
SIGMA-D-14	7	0.741	0.728	0.023
SIGMA-D-P	7	0.839	0.833	0.005
Average	7.00	0.805	0.799	0.012

DI – diversity index; PIC – polymorphic information content; PI – probability of identity

A dendrogram based on UPGMA analysis separated 20 maize genotypes into two clusters. First cluster contained two maize genotypes Bučiansky Konský Zub (SK) and Moldavskaja (SUN). Cluster two was divided into two main cluster 2a and 2b. Main cluster 2a contained genotype Dnepropetrovskaja (SUN) and main cluster 2b was divided into two subclustrov 2ba and 2bb. Subcluster 2ba contained three genotypes- Iregszemeseil 2 hetes (HUN), Aranyozon sarga lofogu (HUN) and Mikulická (CZE) and subcluster 2bb contained other 14 genotypes of maize. We could not distinguish two genotypes, M Silokukurica (HUN) and Bezuncukskaja (SUN) (subcluster 2bb), which can be caused due to close genetic background (Figure 1).

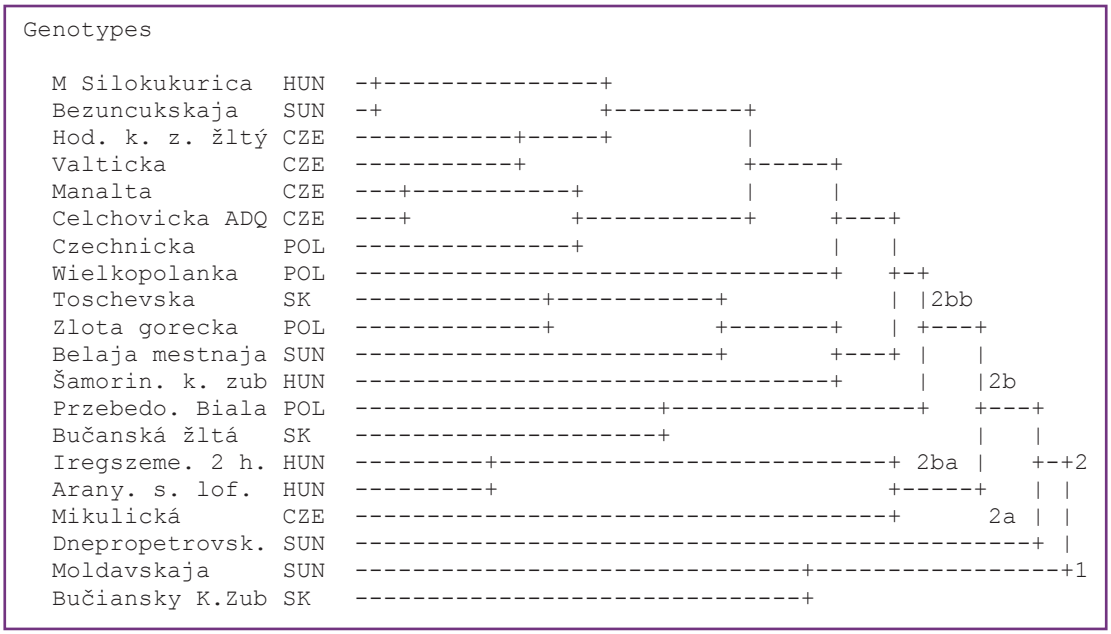

Figure 1 Dendrogram of 20 maize genotypes prepared based on 5 RAPD markers
CZE – Czechoslovakia, HUN – Hungary, POL – Poland, SUN – Union of Soviet Socialist Republics, SK – Slovakia

Conclusions

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 maize genotypes Bučiansky Konský Zub (SK) and Moldavskaja (SUN). Cluster two was divided into two main cluster 2a and 2b. Main cluster 2a contained genotype Dnepropetrovskaja (SUN) and main cluster 2b was divided into two subclustrov 2ba and 2bb. We could not distinguish two genotypes, M Silokukurica (HUN) and Bezuncukskaja (SUN), which can be caused due to close genetic background.

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