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MOLECULAR VARIABILITY OF OLD MAIZE (*ZEA MAYS* L.) BASED ON GENE SPECIFIC MARKERS

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Maize (*Zea mays* L.) or corn is a plant belonging to the family of grasses and is one of the most important cereal crops worldwide as a 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. The SSR molecular markers were used to assess genetic diversity in 20 old European maize genotypes. Five SSR primers revealed a total of 33 alleles ranging from 5 (UMC1962) to 8 (UMC1370) alleles per locus with a mean value of 6.60 alleles per locus. Variations in DNA sequences lead to polymorphism. Greater polymorphism is indicative of greater genetic diversity. The PIC values ranged from 0.780 (UMC1962) to 0.842 (UMC1370) with an average value of 0.814 and the DI value ranged from 0.794 (UMC1962) to 0.848 (UMC1370) with an average value of 0.823. 100% of used SSR markers had PIC and DI values higher than 0.7 that means high polymorphism of chosen markers used for analysis. Probability of identity (PI) was low ranged from 0.005 (UMC1241 and UMC1370) to 0.011 (UMC1859) with an average of 0.007. A dendrogram based on UPGMA analysis separated 20 maize genotypes into two clusters. The first cluster contained two maize genotypes Bučiansky Konský Zub (SK) and Moldavskaja (SUN). Cluster two was divided into two main clusters 2a and 2b. SSR markers are useful in the assessment of maize diversity, the detection of duplicate samples 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: Old Maize, Genetic diversity, SSR markers, Dendrogram, PIC

Introduction

With the advent of the first maize hybrids, in 1933 in the US and around 1950 in Europe, maize cultivation has undergone a complete change. Numerous open-pollinated landraces adapted to specific regions were substituted by a limited number of hybrids bred from a large genetic basis (Gay, 1984). Today, the main maize hybrids cultivated in the world involve a restricted

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number of key inbred lines. Therefore, the genetic diversity of those cultivars is almost certainly limited, in comparison to the large genetic diversity available in genebanks. A few years ago, the threat of genetic erosion led to a significant interest in the assessment of genetic diversity in germplasm collections and a huge number of studies on various crops (Dubreuil and Charcosset, 1998). Molecular markers based on polymerase chain reaction (PCR) methods, such as simple sequence repeats (SSRs) or microsatellites, have become important genetic markers in a wide range of crop species, including maize. SSRs markers have many advantages over other types of molecular markers, such as co-dominance, abundant in genomes, highly polymorphisms, locus specificity, good reproducibility, and random distribution throughout the genome (Sun et al., 2011). These features, coupled with their ease of detection, make them ideal for identifying and distinguishing between accessions that are genetically very similar (Saker et al., 2005). For the analysis of genetic diversity of maize genotypes were used several dominant molecular markers: amplified fragment length polymorphism (AFLP) (Roy and Kim, 2016), random amplified polymorphic DNA (RAPD) (Balážová et al., 2016; Vivodík et al., 2017a), start codon targeted (SCoT) (Vivodík et al., 2017b), inter-simple sequence repeat (ISSR) (Žiarovská et al., 2013) and sequence-related amplified polymorphism (SRAP) (Abd El-Azeem et al., 2015). And codominant molecular markers were also used for the analysis of maize genotypes: simple sequence repeat (SSR) (Shiri et al., 2014), expressed sequence tag (EST)-SSR (Galvão et al., 2015) and using protein markers (SDS-PAGE) (Vivodík et al., 2017c).

The present study aimed to examine the genetic variability within and among old maize genotypes cultivated in Europe, using 5 SSR markers. The data collected will contribute to identification, rational exploitation and conservation of germplasms of maize genotypes.

Material and methods

Plant material and DNA extraction

Maize genotypes (20) were obtained from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the Gene Bank in Piešťany, the Slovak Republic. DNA of 20 genotypes of maize was extracted from leaves of 10-day old seedlings using the Gene JET Plant Genomic DNA Purification Mini Kit. Maize genotypes were grown in a growth chamber on humus soil.

SSR amplification and statistical analysis

SSR analysis: Amplification of SSR fragments was performed according to (Elçi and Hançer, 2015) (Table 1). Polymerase chain reaction (PCR) was performed in 20 µl of a mixture containing 7.5 µl H2O, 10.0 µl Master Mix (Genei, Bangalore, India), 0.75 µl of each primer (10 pmol) and 1 µl DNA (100 ng). Amplification was performed in a programmed thermocycler (Biometra, Germany) and amplification program consisted of an initial denaturing step at 94 °C for 2 min, followed by 35 cycles of amplification [95 °C (30 s), 1 min at the 55 °C, 72 °C (30 s)] and a final elongation step at 72 °C for 10 min. Amplification products were confirmed by electrophoresis in 7% denaturing polyacrylamide gels and silver stained and documented using gel documentation system Grab-It 1D for Windows.

Table 1 List of 5 SSR primers (Elçi and Hançer, 2015)

SSR marker	F primer	R primer
UMC1859	ATATACATGTGAGCTGGTTGCCCT	GCATGCTATTACCAATCTCCAGGT
UMC1241	TGAAGCAAGTCACTGGTAAGAGCA	TGACACACCCATACTTCCAACAAG
UMC1370	GGGAGCACACACAGTAGTACTCGAT	AGAGGCTCTCCTCCTTCAAGCTC
UMC1962	ATAAGTGGGGGAGGCGAGCTA	GAGAACCAACCACCAAAGAAGTCC
UMC1380	CTGCTGATGTCTGGAAGAACCCT	AGCATCATGCCAGCAGGTTTT

Results and discussion

Five maize SSR primers were used for the identification and estimation of the genetic relations among 20 old European maize genotypes. All 5 SSR primers generated clear banding patterns with high polymorphism (Figure 1). Five SSR primers revealed a total of 33 alleles ranging from 5 (UMC1962) to 8 (UMC1370) alleles per locus with a mean value of 6.60 alleles per locus (Table 2). Variations in DNA sequences lead to polymorphism. Greater polymorphism is indicative of greater genetic diversity. The PIC values ranged from 0.780 (UMC1962) to 0.842 (UMC1370) with an average value of 0.814 and the DI value ranged from 0.794 (UMC1962) to 0.848 (UMC1370) with an average value of 0.823 (Table 2). 100% of used SSR markers had PIC and DI values higher than 0.7 that means high polymorphism of chosen markers used for analysis. Probability of identity (PI) was low ranged from 0.005 (UMC1241 and UMC1370) to 0.011 (UMC1859) with an average of 0.007 (Table 2). A dendrogram based on UPGMA analysis separated 20 maize genotypes into two clusters. The first cluster contained two maize genotypes Bučiansky Konský Zub (SK) and Moldavskaja (SUN). Cluster two was divided into two main clusters 2a and 2b. The main cluster 2a contained genotype Dnepropetrovskaja (SUN) and main cluster 2b was divided into two subclusters 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 2).

Table 2 List of SSR primers, the total number of bands and the statistical characteristics of the SSR markers used in maize

Marker name	Number of alleles	DI	PIC	PI
UMC1859	7	0.808	0.799	0.011
UMC1241	6	0.830	0.823	0.005
UMC1370	8	0.848	0.842	0.005
UMC1962	5	0.794	0.780	0.010
UMC1380	7	0.835	0.827	0.006
Average	6.60	0.823	0. 814	0.007

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

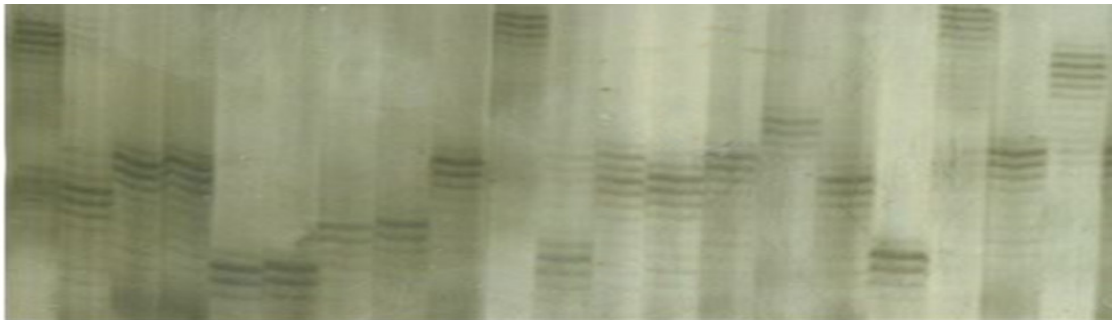

Figure 1 PCR amplification products of 20 genotypes of maize produced with SSR primer UMC1859. Lanes 1–20 are maize genotypes

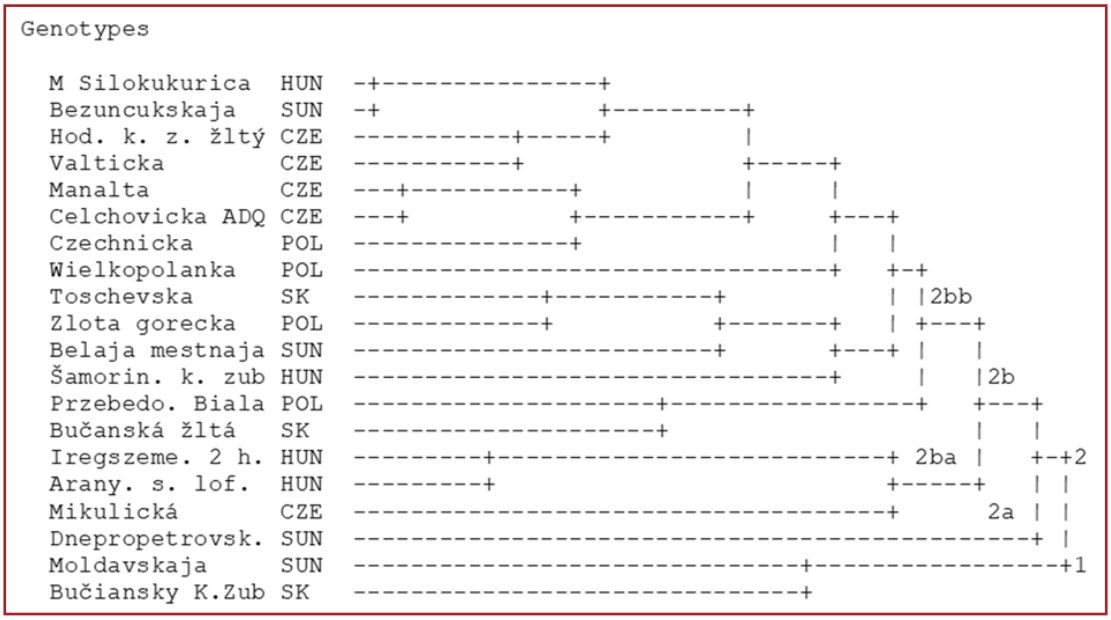

Figure 2 Dendrogram of 20 old maize genotypes prepared based on 5 SSR markers
CZE – Czechoslovakia; HUN – Hungary; POL – Poland; SUN – Union of Soviet Socialist Republics; SK – Slovakia

Efendi et al. (2015) study the level of homozygosity and genetic diversity by codominant SSRs markers. The research aimed to select homozygosity and analyze genetic diversity of 51 maize inbreds using 36 SSRs markers. The research was aimed to select among 51 maize inbreds with high homozygosity and to investigate the genetic diversity using 36 SSRs markers. The result shows that there were 30 inbreds indicating homozygosity level of more than 80%. The diversity of those inbreds was moderately high, with genetic similarity of between 0.22 and 0.87 distributed within six heterotic groups. Genetic diversity of 38 maize hybrids was studied by Shiri et al. (2014) using 12 SSR primers and morphological traits under two different irrigation conditions. The 38 hybrids were evaluated in two trials, one under well-watered (WW) conditions and one under drought-stress (DS) conditions, using an RBCD

design with three replications for two years (2008–2009) in Moghan, Iran. The total number of PCR-amplified products was 40 bands, all of them polymorphic. Primer Phi031 generated the highest number of bands (6). Among the studied primers, UMC2359, PHI031 and UMC1862 showed the maximum polymorphism information content (PIC) and the greatest diversity. The aim of the study Salami et al. (2016) was to evaluate the genetic diversity of Benin's maize accessions by SSR marker. Thus, one hundred eighty seven maize accessions from three areas (South, Center and North) were analyzed using three SSR markers. A total of 227 polymorphic bands were produced and showed high genetic diversity (Shannon index = 0.51). The polymorphic information content (PIC) values for the SSR loci ranged from 0.58 to 0.81, with an average of 0.71. Genetic distance-based UPGMA dendrogram showed a genetic differentiation between accessions and they were grouped into four clusters in each area. Nine flint and nine dent accessions from six agro-ecological groups (races), chosen on the basis of diverse pedigrees, were analyzed by Ignjatovic-Micic et al. (2015) for genetic relatedness using phenotypic and simple sequence repeat (SSR) markers. One of the aims was to establish a reliable set of SSR markers for a rapid diversity analysis using polyacrilamide gels and ethidium bromide staining. In the principal component analysis (PCA) the first three principal components accounted for 80.86% of total variation and separated most of the flint from dent landraces. Ten SSR primers revealed a total of 56 and 63 alleles in flint and dent landraces, respectively, with low stuttering and good allele resolution on the gels. High average PIC value (0.822) also supports informativeness and utility of the markers used in this study.

Conclusion

In conclusion, a high level of genetic diversity exists among the old maize accessions analyzed. According to analysis, the collection of 20 diverse accessions of maize was clustered into two clusters. The first cluster contained two maize genotypes Bučiansky Konský Zub (SK) and Moldavskaja (SUN). Cluster two was divided into two main clusters 2a and 2b. Main cluster 2a contained genotype Dnepropetrovskaja (SUN) and main cluster 2b was divided into two subclusters 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. An SSR marker system is a rapid and reliable method for cultivar identification that might also be used in quality control in certified seed production programs, to identify sources of seed contamination, and to maintain pure germplasm collections.

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