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GENETIC VARIATION OF MAIZE GENOTYPES (*ZEA MAYS* L.) DETECTED USING SDS-PAGE

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In the present study 20 old genotypes of maize from Hungary, Union of Soviet Socialist Republics, Poland, Czech Republic and Slovak Republic were evaluated for the total seed storage proteins using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) through vertical slab unit. The number of total scorable protein bands was twentythree as a result of SDS-PAGE technique but those that were not cosistent in reproducibility and showed occasional variation in sharpness and density were not considered. Out of twenty three polypeptide bands, 6 (31%) were commonly present in all accessions and considered as monomorphic, while 17 (65%) showed variations and considered as polymorphic. On the basis of banding profiles of proteins of different kDa, gel was divided into zones A, B and C. The major protein bands were lied in zones A and B, while minor bands were present in zones C. In zone A out of 10 protein bands, 1 were monomorphic and 9 were polymorphic. In zone B out of 8 protein bands, 3 was monomorphic and 5 was polymorphic and in zone C out of 5 protein bands, 2 were monomorphic whereas 3 polymorphic. The dendrogram tree demonstrated the relationship among the twenty registered old maize genotypes according to the similarity index, using UPGMA cluster analysis. The dendrogram was divided into three main clusters. The first one contained one genotype from maize, the second cluster contained one genotype of maize and third cluster contained 18 genotypes of maize. Similarly the present study of genetic variability in the seed storage polypeptide determined by SDS-PAGE technique proved that it is fruitful to identify genetic diversity among accessions of maize.

Keywords: maize; dendrogram; SDS-PAGE; genetic diversity

Introduction

Maize (*Zea mays* L.) is an annual, cross-pollinated by wind and the only monoecious among cereal crops which has male and female inflorescences on separate branches of the same plant. It belongs to grass family Poaceae (Gramineae) which is leading in importance in the order *Poales* (Bremer et al., 2003). This family contributes to the world economy, food and industry through valuable crops i.e. wheat, rice and maize (Mabberley, 2008). Being most domesticated with controversy in origin and evolution, there is one school of thoughts that maize is the nearest descendant of Mexican teosinte (Dowswell et al., 1996). There is no doubt that human beings directly or indirectly depend on plants for various purposes for which they domesticated these with the passage of time and flourished with spreading communities, undergone through evolution, passing through various cultivating methodologies throughout the world (Larik, 1994).

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Maize seed consists of two types of protein i.e., zein and non-zein protein. The term zein is used for prolamins in maize which is alcohol soluble protein and could be extracted with ethanol (Lawton, 2006). Zein is major seed storage protein of maize (Freitas et al., 2005) and consists of one major and three minor classes and these four classes constitute approximately 50–70% of maize endosperm (Vasal, 1999).

The non-zein protein consists of globulins (3%), glutelins (34%) and albumins (3%). Zein is specific to maize endosperm (Prasanna et al., 2001) and not present in any other part of plant.

Proteins are primary gene products of active structural genes; their size and amino acids sequence are the direct results of nucleotide sequences of the genes; hence, any observed variation in protein systems induced by any mutagen is considered a mirror for genetic variations (Hamoud et al., 2005). Variation in theDNAcoding sequences frequently causes variation in the primary conformation of the proteins. Determination of protein molecular weight (MW) via polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) is a universally used method in biomedical research; (Ranjan et al., 2013) concluded that electrophoresis (SDS-PAGE) of proteins can be economically used to assess genetic variation and relation in germplasm and also to differentiate mutants from their parent genotypes. Some studies used SDS-PAGE for detection of alterations in protein profiles occurring during exposure to electric field (Hanafy et al., 2006; Dymek et al., 2012). So far, several investigations on the discrimination between crop genotypes using SDS-PAGE have been carried out by Yoon et al. (2010), Osman et al. (2013), Iqbal et al. (2014), Iqbal et al. (2014), Khan et al., (2014), AL-Huqail et al. (2015), Gregova et al. (2015), Kačmárová et al. (2016), Socha et al. (2016).

The objectives were to find out the level of genetic variability present in 20 maize germplasm by using the electrophoretic profiles of total seed proteins with different molecular weights through SDS-PAGE.

Materials and methodology

Maize genotypes (20) were obtained from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the GeneBank in Piesťany, the Slovak Republic. SDS-PAGE was carried out according to the standard reference ISTA method (Wrighley, 1992). Storage proteins were extracted from individually ground seeds using extracting using a buffer composed of 6.25 mL Tris (1.0 mol/L, pH = 6.8), 10 mL glycerol, 12.05 mL H_2O and 2.0 g SDS, diluted with mercaptoethanol and H_2O in a 17 : 3 : 40 (v/v) proportion. The buffer was added to flour in a 1 : 25 (w/v) proportion. Extraction was performed at room temperature overnight and heating in boiled water for 5 minutes, centrifugation at 5,000 × g for 5 min. 10 µL of extracts were applied to the sample wells. The gel (1.0 mm thick) consists of two parts: stacking gel (3.5% acrylamide, pH = 6.8 acrylamide) and resolution gel (10% acrylamide, pH = 6.8). Staining of gels was performed in a solution of Coomassie Brilliant Blue R250 dissolved in acetic acid and methanol solution. Gel was scanned with densitometer GS 800 (Bio-Rad) and evaluated with Quantity One-1D Analysis Software.

Results and discussion

The number of total scorable protein bands was twentythree as a result of SDS-PAGE technique but those that were not cosistent in reproducibility and showed occasional variation in sharpness and density were not considered. Based on these bands twenty accessions of maize were screened. Out of twentythree polypeptide bands, 6 (31%) were commonly present in all

accessions and considered as monomorphic, while 17 (65%) showed variations and considered as polymorphic. The size of the protein bands obtained through SDS-PAGE ranged from 20 to 140 kDa.

On the basis of banding profiles of proteins of different kDa, gel was divided into zones A, B and C. The major protein bands were lied in zones A and B, while minor bands were present in zones C. It was noted that different accessions of maize showed more diversity in seed storage proteins in minor bands in comparison to major bands. In zone A out of 10 protein bands, 1 were monomorphic and 9 were polymorphic. In zone B out of 8 protein bands, 3 was monomorphic and 5 was polymorphic and in zone C out of 5 protein bands, 2 were monomorphic whereas 3 polymorphic. By considering these facts zone A and B were more polymorphic.

The dendrogram tree (Figure 1) demonstrated the relationship among the twenty registered old maize genotypes according to the similarity index, using UPGMA cluster analysis. The dendrogram was divided into three main clusters. The first one contained one genotype of maize (Mikulicka), second cluster contained one genotype of maize (Celchovicka ADQ). Third cluster contained 18 maize genotypes and was divided into subcluster 3a, 3b and 3c. Subclaster 3a contained one Czech genotype Manalta and subclaster 3b was divided into subclaster 3ba and 3bb. Subclaster 3c contained 2 genotypes Zlota gorecka and Dnepropetrovskaja (Figure 1).

Similarly the present study of genetic variability in the seed storage polypeptide determined by SDS-PAGE technique proved that it is fruitful to identify genetic diversity among accessions of maize.

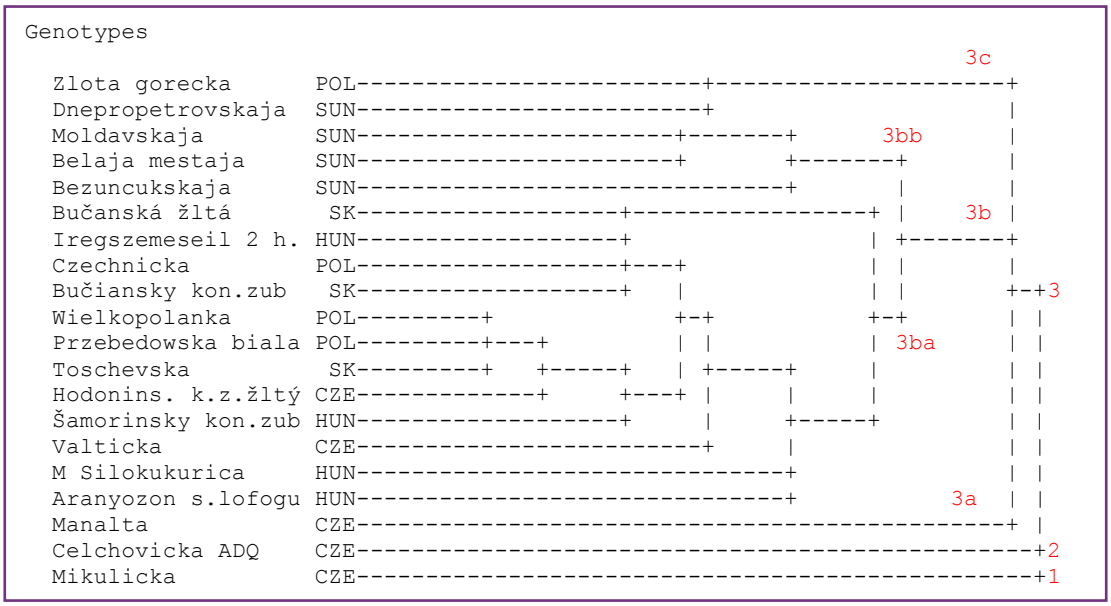

Figure 1 Dendrogram of 20 maize genotypes prepared based on SDS-PAGE
CZE – Czech Republic, HUN – Hungary, POL – Poland, SUN – Union of Soviet Socialist Republics, SK – Slovak Republic

Conclusions

SDS-PAGE techniques may provide useful information on the level of polymorphism and diversity in old maize genotypes. Twenty maize genotypes originated from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the Gene Bank in Piešťany, the Slovak Republic were very closely related. The dendrogram was divided into three main clusters. The first one contained one genotype of maize (Mikulicka), second cluster contained the one genotype of maize (Celchovicka ADQ) and third contained 18 maize genotypes. Result from this study show that protein markers are powerful and efficient in characterising and identifying of old maize genotypes in addition to their usefulness in phylogenetic studies.

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