

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

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ABSTRACT

The assessment of genetic diversity among the members of a species is of vital importance for successful breeding and adaptability. In the present study 20 old genotypes of maize from Hungary, Union of Soviet Socialist Republics, Poland, Czechoslovakia 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 consistent in reproducibility and showed occasional variation in sharpness and density were not considered. 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. 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 forty registered old maize genotypes according to the similarity index, using UPGMA cluster analysis. The dendrogram was divided into three main clusters. The first cluster contained Czechoslovakia genotype Mikulická and the second cluster contained Czechoslovakia genotypes Celchovicka ADQ. Cluster 3 was divided into 3 subclusters. 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 to have 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).

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 the DNA coding 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; (Ranjana *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); Chňápek *et al.*, 2014; Iqbal *et al.*, (2014); Iqbal *et al.*, (2014); Khan *et al.*, (2014); Petrovičová *et al.*, 2014; AL-Huqail *et al.*, (2015); Chňápek *et al.*, 2015; Gregova *et al.*, (2015); Kačmárová *et al.*, (2016); Kut'ka Hložáková *et al.*, 2015; Tomka *et al.*, (2015); 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.

MATERIAL AND METHODS

Maize genotypes (20) were obtained from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the Gene Bank in Piešťany, the Slovak Republic (Table 1).

SDS-PAGE was carried out according to the standard reference ISTA method (Wrightley, 1992). Storage proteins were extracted from individually ground seeds using extracting using a buffer composed of 6.25 mL Tris (1.0 mol L⁻¹, pH = 6.8), 10 mL glycerol, 12.05 mL H₂O and 2.0 g SDS, diluted with mercaptoethanol and H₂O 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 5000 xg 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.

Table 1 List of 20 analyzed genotypes of maize

	Genotypes	Country of origin	Year of registration
1.	Moldavská	Union of Soviet Socialist Republics	1964
2.	Bučianský Kónský Zub	Slovak Republic	1964
3.	Hodonínský kónský zub žltý	Czechoslovakia	1964
4.	M Silokukurica	Hungary	1964
5.	Valticka	Czechoslovakia	1964
6.	Przebedowska Biala	Poland	1964
7.	Toschevska	Slovak Republic	1964
8.	Šamorínský kónský zub	Hungary	1964
9.	Wielkopolanka	Poland	1964
10.	Czechnicka	Poland	1964
11.	Manalta	Czechoslovakia	1964
12.	Zlota gorecka	Poland	1964
13.	Celchovicka ADQ	Czechoslovakia	1964
14.	Belaja mestnaja	Union of Soviet Socialist Republics	1964
15.	Bučanská žltá	Slovak Republic	1964
16.	Iregszemeseil 2 hetes	Hungary	1964
17.	Dnepropetrovská	Union of Soviet Socialist Republics	1964
18.	Bezuncuškaja	Union of Soviet Socialist Republics	1964
19.	Mikulická	Czechoslovakia	1964
20.	Aranyozon sarga lofogu	Hungary	1964

RESULTS AND DISCUSSION

The number of total scorable protein bands was twentythree as a result of SDS-PAGE technique but those that were not consistent in reproducibility and showed occasional variation in sharpness and density were not considered. Based on these bands twenty accessions of maize (Table 1) 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 (Figure 1). 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 2) 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 cluster contained Czechoslovakia genotype Mikulická and the second cluster contained Czechoslovakia genotypes Celchovicka ADQ. Cluster 3 was divided into 3 subclusters. In subcluster 3a was separated genotype Manalta (Czechoslovakia) from other 17 genotypes (Figure 2). Subcluster 3b is divided into subcluster 3ba and 3bb. Subcluster 3ba contained 12 maize genotypes and subcluster 3bb contained 3 genotypes from Union of Soviet Socialist Republics

(Moldavská, Belaja mestnaja and Bezuncuškaja). Subcluster 3c contained one genotypes from Poland - Zlota gorecka and one genotypes from Union of Soviet Socialist Republics - Dnepropetrovská. 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.

Similar results were detected by other authors (Yoon *et al.*, 2010; Osman *et al.*, 2013; Iqbal *et al.*, 2014; Iqbal *et al.*, 2014; Khan *et al.*, 2014; Merza *et al.*, 2014; AL-Huqail *et al.*, 2015) and these results presented a high level of polymorphism of old maize genotypes detected by SDS-PAGE. Osman *et al.*, (2013) study genetic relationship between some species of Zea mays using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of seed protein. Autors identified 78 bands across the studied species. The number of bands varies from 17 bands in sample number 5 to 6 in sample number 6. Iqbal *et al.*, (2014) analyzed 73 genotypes of maize from China, Japan and Pakistan for the total seed storage proteins using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). A total of 18 protein bands were recorded. Among these 7 (39%) were monomorphic and 11 (61%) polymorphic, with molecular weight varied from 10 kDa to 122 kDa. The aim of Iqbal *et al.*, (2014) was to estimate the genetic diversity across 83 genotypes of maize of Pakistan and Japanese origin using SDS-PAGE. A total of 18 protein subunits were noted out of which 7 (39%) were monomorphic and 11 (61%) were polymorphic, with molecular weight ranging from 10 to 122 kDa. Coefficients of similarity among the accessions ranged between 0.89 and 1.00. The dendrogram obtained through UPGMA clustering method showed two main clusters: 1 and 2. First cluster contained 9 genotypes, while second cluster contained 74 genotypes. Khan *et al.*, (2014) study the variation of zein fraction of seed storage protein in maize by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Variation in terms of absence and presence, intensity and molecular size was observed in zein polypeptides. The study of Merza *et al.*, (2014) was conducted to investigate the ability of total soluble seed protein to discriminate among 21 of maize genotypes through using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). According to SDS analysis , a total of 118 amplified bands were obtained ranging in their molecular size 18-86KDa. Five out of main nine bands were polymorphic and four monomorphic with an average of polymorphism reaches 55.5%. Phylogenetic tree divided 21 of corn genotypes between two major groups each of them divided in to two subgroups .The first main group included 13 genotypes , while the second main group included eight genotypes. AL-Huqail *et al.*, (2015) used SDS-PAGE to detection of 46 polypeptides bands with different molecular weights ranging from 186.20 to 36.00 KDa. It generated distinctive polymorphism value of 84.62%.

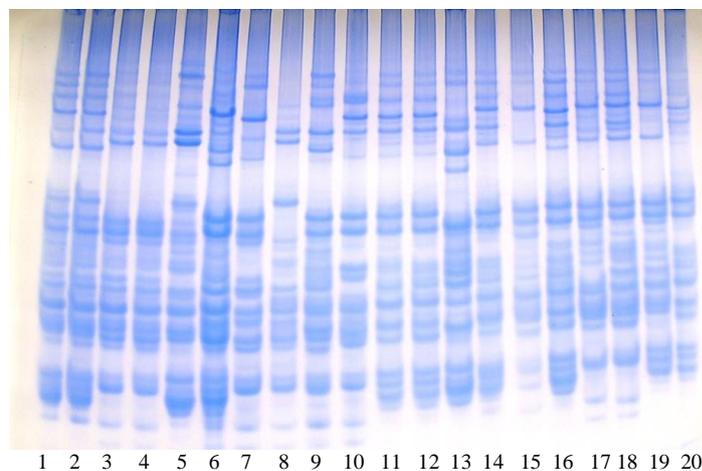


Figure 1 Protein profile showing total seed storage proteins in maize genotypes as a result of SDS-PAGE. Lanes 1- 20 are maize genotypes (Table 1).

Genotypes

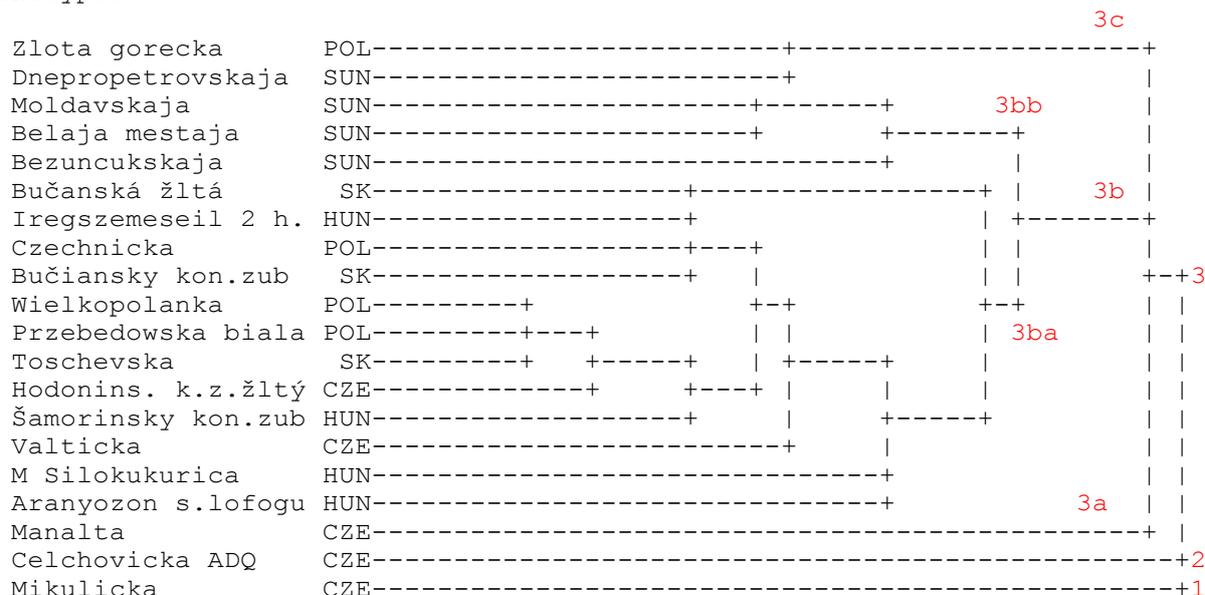


Figure 2 Dendrogram of 20 maize genotypes prepared based on SDS-PAGE markers. CZE - Czechoslovakia, HUN - Hungary, POL - Poland, SUN – Union of Soviet Socialist Republics, SK – Slovakia

CONCLUSION

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 Piesťany, the Slovak Republic were very closely related. The dendrogram was divided into three main clusters. The first cluster contained Czechoslovakia genotype Mikulická and the second cluster contained Czechoslovakia genotypes Celchovicka ADQ. Cluster 3 was divided into 3 subclusters. In subcluster 3a was separated genotype Manalta (Czechoslovakia) from other 17 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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