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GENETIC VARIATION AND RELATIONSHIPS OF OLD 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 40 old genotypes of maize from Hungary, Union of Soviet Socialist Republics, Poland, Czechoslovakia, Yugoslavia 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 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 two main clusters. The first one contained eleven genotypes from maize, while the second cluster contained the twentynine 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 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 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 40 maize germplasm by using the electrophoretic profiles of total seed proteins with different molecular weights through SDS-PAGE.

MATERIAL AND METHODOLOGY

Maize genotypes (40) were obtained from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the GeneBank in Piesťany, the Slovak Republic (Table 1).

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^{-1}, 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 5000 x 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 forty accessions of maize (Table 1) were screened. Out of twentythree polypeptide bands, 6 (31%) were commonly present in all

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. 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

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 forty registered old maize genotypes according to the similarity index, using UPGMA cluster analysis. The dendrogram was divided into two main clusters. The first one contained eleven genotypes from maize, while the second cluster contained the twentynine genotypes of maize. In Cluster I was separated unique genotype Kostycevskaja (Union of Soviet Socialist Republics) from other 10 genotypes (Figure 2). One genotypes in cluster I is from Hungary (Mindszentpusztai Sarga Lofogu) and one genotypes is from Yugoslavia (Juhoslavska), two genotypes are from Union of Soviet Socialist Republics and Czechoslovakia and four genotypes are from Slovak Republic. Cluster I not contained genotype from Poland. Cluster II contained eight genotypes from Hungary (27.6%), six genotypes from Poland (20.7%), six genotypes of maize from Union of Soviet Socialist Republics (20.7%), five genotypes from Czechoslovakia (17.2%), three genotypes from Slovak Republic (10.3%) and one genotype of maize is from Yugoslavia (3.4%) (Figure 2).

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; 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 proteins.

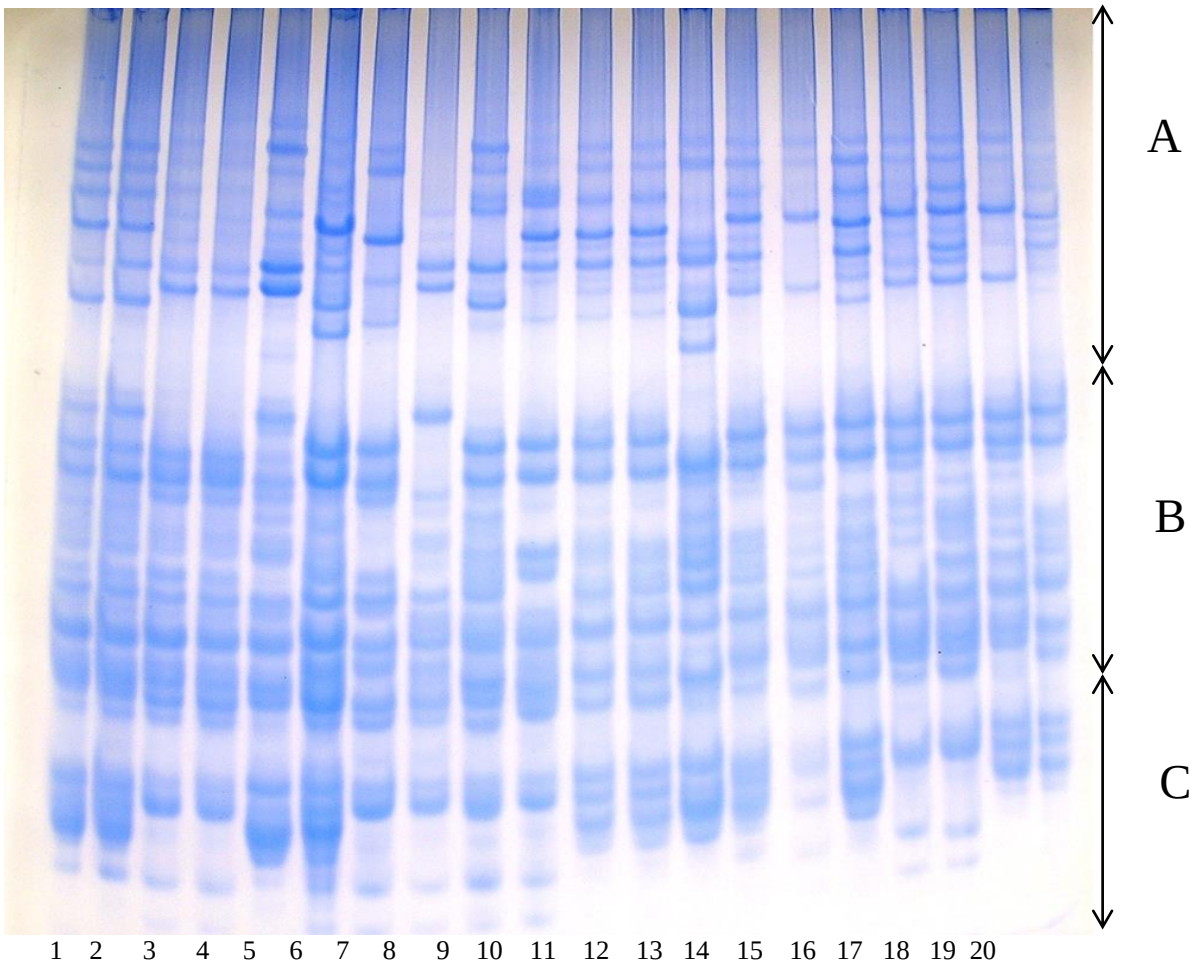

Figure 1 Protein profile showing total seed storage proteins in maize genotypes as a result of SDS-PAGE. Lanes 1 - 20 are maize genotypes (Table 1).

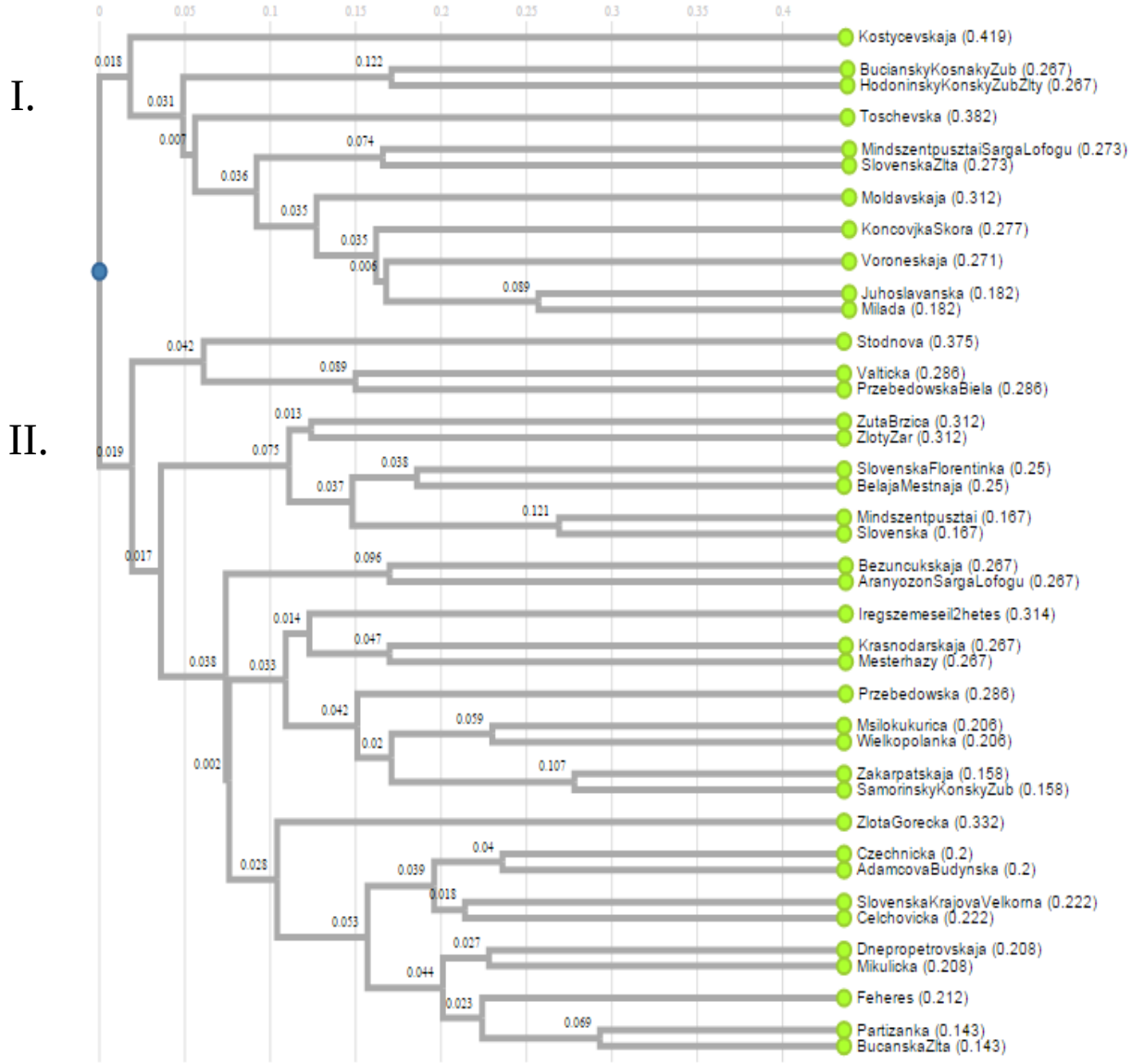

Figure 2 Dendrogram of 40 maize genotypes prepared based on SDS-PAGE markers.

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. **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%.

CONCLUSION

SDS-PAGE techniques may provide useful information on the level of polymorphism and diversity in old maize genotypes. Forty 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 two main clusters. The first one contained eleven genotypes from maize, while the second cluster contained the twentynine genotypes of maize. 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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