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Electrophoretic profiles of storage proteins in selected maize (*Zea mays* L.) genotypes

Elektroforetické profily zásobných bielkovín vybraných genotypov kukurice siatej (*Zea mays* L.)

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ABSTRACT

Maize (*Zea mays* L.) is important cereal and its diversity research is essential from the point of breeding strategy. The aim of this work was to compare the protein profiles of 30 varieties (from the USA and Europe) and 34 lines (from Slovakia) using two electrophoretic methods (SDS-PAGE and A-PAGE) and to construct dendrograms of their genetic similarity. The highest amount of HMW-GS (high molecular weight glutelin subunits) was detected in the European varieties (7.35%), LMW-GS (low molecular weight glutelin subunits) was determined in the Slovak lines (45.42%) and most residual albumins and globulins were found in the American varieties (50.66%). According to the representation of the evaluable protein bands, 4 dendrograms were constructed. In the dendrograms, which were based on the polymorphism of glutelins, Line 15 was separated from other Slovak lines, while European and American varieties split into 2 main clusters. The highest genetic similarity was between varieties Wonderfull and Queen Anna; and Slovak lines 6 and 9. From the point of zein polymorphism, dendrograms separated the varieties and the lines into 2 main clusters. Fruhester Gelber and Maďarská cukrová were the most similar varieties, also the Slovak lines 21 and 22; 13 and 34. The findings of the study will be helpful for proper breeding strategy and further sustainable agricultural development.

Keywords: A-PAGE, dendrogram, maize, SDS-PAGE, storage proteins

ABSTRAKT

Kukurica siata (*Zea mays* L.) je dôležitá obilnina a výskum jej diverzity je z pohľadu šľachtiteľskej stratégie nevyhnutný. Predmetom práce bolo porovnať profily zásobných bielkovín 30 odrôd kukurice siatej (pôvodom z USA a Európy) a 34 línií (zo Slovenska) pomocou dvoch elektroforetických metód (SDS-PAGE a A-PAGE) a zostrojiť dendrogramy na základe ich genetickej podobnosti. Najvyšší obsah HMW-GS (vysokomolekulárnych glutelínových podjednotiek) bol detegovaný v európskych odrodách (7,35%), LMW-GS (nízkomolekulárnych glutelínových podjenotiek) bol určený v slovenských líniách (45,42%) a najviac zvyškových albumínov a globulínov bolo nájdených v amerických odrodách (50,66%). Podľa prítomnosti hodnotiteľných bielkovinových pásikov boli zostrojené 4 dendrogramy. V dendrogramoch, zostrojených na základe polymorfizmu glutelínov, sa oddelila Línia 15 od ostatných línií, kým európske a americké odrody sa rozdelili do 2 hlavných zhlukov. Najvyššia genetická podobnosť bola medzi odrodami Wonderfull a Queen Anna, a medzi slovenskými líniami 6 a 9. Z hľadiska polymorfizmu zeínov dendrogramy rozdelili odrody a línie do 2 hlavných zhlukov. Geneticky najpodobnejšie boli odrody Fruhester Gelber a Maďarská cukrová, a slovenské línie 21 a 22 , a taktiež 13 a 34. Výsledky práce môžu byť nápomocné pre správnu šľachtiteľskú stratégiu a budúci udržateľný rozvoj poľnohospodárstva.

Kľúčové slová: A-PAGE, dendrogram, kukurica siata, SDS-PAGE, zásobné bielkoviny

INTRODUCTION

Maize is an important crop with significant economic value, which is used to produce a wide range of ware. In addition to use in food industry, it is animal feed and also material for other products such as isoglucose or bioethanol. The demand for maize grain is growing throughout Europe, not only because of the intensive pigs breeding in some areas (the Netherlands, Denmark, Belgium, etc.), but especially for the rapid development of the maize industrial processing (Ďuďák, 2016; Pospíšil, 2016). Maize is a model plant in genetics. Heterosis, which is an increase in the quality of hybrids compared to the original forms, is used extensively. Hybrids of the F1 generation created by crossing inbred lines are more fertile than original varieties by up to 30% (Gregová et al., 2015). Storage proteins are found in the germ and in the endosperm of grain. The presence of the individual protein fractions in maize is: the content of albumins is 4%, the globulins is the smallest fraction of 2.8%, the content of the main prolamin proteins is 47.9% and the glutelin content is 45.3%. Prolamins - zeins are predominant proteins of maize seed, which nutritional quality is dependent on its composition. Different molecular weight zeins were identified by the SDS-PAGE method and subsequently classified according to their solubility and structural relationships to α-zeines (22 and 19 kDa), β-zeines (15 kDa), γ-zeins (27,16 and 50 kDa) and δ-zeins (10 and 18 kDa) (Shukla and Cheryan, 2001; Australian Government DHA, 2008; Khan et al., 2014). Glutelins are usually divided into 2 groups according to their molecular weight – high molecular weight glutelin subunits (HMW-GS; the size 80–120 kDa) and low molecular weight glutelin subunits (LMW-GS; the size 30–80 kDa) (Gálová et al., 2011). Genetic diversity plays a key role in crop improvement. SDS-PAGE and A-PAGE markers systems are rapid and reliable methods 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 (Vivodík et al., 2018). The future of maize research is promising. Advances in experimental design and the increased availability of germplasm resources will allow the molecular and functional diversity of maize to split (Buckler et al., 2006).

The goal of this analysis was to compare the protein profiles of maize varieties and lines with use of two different electrophoretic methods and on their basis to construct dendrograms of genetic similarity, which would be help for the future breeding strategy.

MATERIAL AND METHODS

The biological material were grains of maize (*Zea mays* L.) obtained from the Gene bank of RIPP Praha - Ruzyně in the Czech Republic (30 European and American varieties, Table 1) and from Zeainvent Trnava s.r.o. in the Slovak republic (34 Slovak lines, Table 2).

The samples were analysed with 2 electrophoretic methods – polyacrylamide gel electrophoresis in presence of sodium dodecyl sulphate (SDS-PAGE, Wrigley, 1992) and acidic polyacrylamide gel electrophoresis (A-PAGE, Draper, 1987). For the SDS-PAGE method, the stock solution for the extraction of glutelins consisted of Tris-HCl, glycerol, distilled water, SDS (sodium dodecyl sulphate) and Pyronin Y, from which the extraction solution was prepared by adding 2-mercaptoethanol and redistilled water. 8 µl of the extraction solution was added to 1 mg of homogenized grain and the process was carried out for 30 minutes at 100 °C under shaking. For A-PAGE method, 5 µl of extraction solution for prolamins-zeins (chloroethanol, methylgreen, urea and 2-mercaptoethanol in redistilled water) was added to 1 mg of homogenized grain. The content was mixed up and the process was carried out overnight at laboratory temperature. These methods allowed the storage proteins (glutelins and zeins) to separate according to their molecular weight. The triplets from each sample were used to determine the genotype's homogeneity. Electrophoretical separation was run by constant current (SDS-PAGE) or constant voltage (A-PAGE). Protein bands in gel were stained by Coomassie Brilliant Blue R–250. Products in PAGE gels were scanned using the GS-800 Calibrated Densitometer (BioRad) and evaluated using Doc-It LS Image analysis and GelAnalyzer. Hierarchical

Table 1. List of maize varieties from Europe and the USA

Number	Name	Origin
1	Fekete Mazsola	Hungary
2	Black Mexican	USA
3	Black Sugar	USA
4	Howling Mob	USA
5	Rostrata	USA
6	Whiple´s Early White	USA
7	Early King	USA
8	Miniature	USA
9	Fore Most Coss F1	USA
10	Trucker´s Favourite White	USA
11	Ioana	USA
12	Stowell´s F1	USA
13	Illinois Hulles	USA
14	Fruhester Gelber	Germany
15	Maďarská Cukrová	Hungary
16	Zlota Handlowa	Poland
17	Cukrová	Czechoslovakia
18	The Burpee	USA
19	Golden Cross Bantam (Early)	USA
20	Early Evergreen	USA
21	Carmel Cross	USA
22	Golden Harvest	USA
23	Spring Gold	USA
24	Fore Most Extra Early (EE1) F1	USA
25	Golden Beauty F1	USA
26	Extra Early Golden Bantam	USA
27	Barbecue	USA
28	North Star	USA
29	Queen Anna	USA
30	Wonderfull	USA

Table 2. List of Slovak maize lines

Number	Name
1	Line 1
2	Line 2
3	Line 3
4	Line 4
5	Line 5
6	Line 6
7	Line 7
8	Line 8
9	Line 9
10	Line 10
11	Line 11
12	Line 12
13	Line 13
14	Line 14
15	Line 15
16	Line 16
17	Line 17
18	Line 18
19	Line 19
20	Line 20
21	Line 21
22	Line 22
23	Line 23
24	Line 24
25	Line 25
26	Line 26
27	Line 27
28	Line 28
29	Line 29
30	Line 30
31	Line 31
32	Line 32
33	Line 33
34	Line 34

clustering using the UPGMA algorithm (Unweighted Pair Group Method using arithmetic Averages) was used to construct dendrograms in DendroUPGMA program, which is available online (http://genomes.urv.cat/UPGMA/).

RESULTS AND DISCUSSION

SDS-PAGE is considered to be one of the most important methods for identification and differentiation of plant genotypes based on their electrophoretic proteins' profiles. This method has shown to be suitable for detection of the genetic diversity of maize (Shah et al., 2003). Application of constant electric current divided the storage proteins into 3 groups (Figure 1) based on different molecular weights - HMW-GS (high molecular weight glutelin subunits), LMW-GS (low molecular weight glutelin subunits) and zeins, as well as residual albumins and globulins, which had the smallest molecular weight.

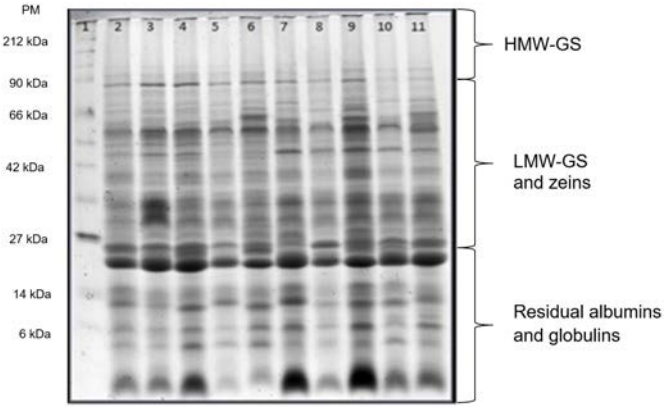

Figure 1. Electrophoretic profiles of Slovak lines obtained with SDS-PAGE method (1, PM – protein marker (range 2-212 kDa), 2 – Line 1, 3 – Line 2, 4 – Line 3, 5 – Line 4, 6 – Line 5, 7 – Line 6, 8 – Line 7, 9 – Line 8, 10 – Line 9, 11 – Line 10; HMW-GS – high molecular weight glutelin subunits, LMW-GS – low molecular weight glutelin subunits)

All samples were homogeneous. In average (Table 3), the highest content of high molecular weight glutelin subunits were found in the European varieties (7.35%), low molecular weight glutelin subunits in the Slovak lines (45.42%), and the most residual albumins and globulins had the American varieties (50.66%). These results are consistent with findings of Chňapek et al. (2014), who, however, observed greater variability within the values.

Table 3. Average representation of weight glutelin groups

	HMW-GS (%)	LMW-GS and zeins (%)	AG (%)
American varieties	5.77	42.58	50.66
European varieties	7.35	43.03	49.62
Slovak lines	6.11	45.42	48.32
Average	6.41	43.68	49.53

HMW-GS – high molecular weight glutelin subunits, LMW-GS – low molecular weight glutelin subunits, AG – albumins and globulins.

The amount of evaluable protein bands in the SDS-PAGE electrophoreogram of the American varieties was 20. Unclear and non-reproducible bands were discarded. Based on these conditions, 6 bands (30%) were present in all samples and considered as monomorphic, while 14 (70%) bands showed variability and were therefore considered as polymorphic. Within the European varieties, 18 bands were detected, out of this number 9 (50%) were monomorphic and 9 (50%) were polymorphic. In the electrophoreogram of the Slovak lines, 22 protein bands were separated, from which 7 (32%) were monomorphic and 15 (68%) were polymorphic. The molecular weight of the rated bands ranged between 5 and 120 kDa.

Based on the presence and absence of protein bands, binary matrices were constructed and the dendrograms of genetic similarity of the varieties and lines were created in terms of glutelin polymorphism with the use of the Jaccard coefficient.

The dendrogram (Figure 2) divided the American and European varieties into two major clusters (I and II), which differentiated into other clusters based on genetic similarity. In the first cluster, there were 8 varieties (in the cluster Ia 4 varieties, in the cluster Ib 4 varieties), in the second cluster were 22 varieties (in cluster IIa 15 varieties, in cluster IIb 7 varieties). The European varieties were in the second cluster. Genetic similarity according to Jaccard ranged from 0.444 to 1.000. Genetically most similar varieties, in terms of glutelin polymorphism, achieved coefficient of similarity 0.933 (30 - Wonderfull and 29 –

Queen Anna). The highest genetic distance was shown by the varieties 26 – Extra Early Golden Bantam and 2 – Black Mexican. Based on the same glutelin composition in the electrophoreogram, two pairs of varieties (5 – Rostrata and 7 – Early King; 15 – Maďarská cukrová and 17 – Cukrová) are unlikely to be distinguished, probably due to their similar genetic basis.

The dendrogram (Figure 3) of the Slovak lines separated the Line 15 (I), the other lines (II) were divided into two main clusters and consequently into smaller clusters. In cluster IIa there were 18 lines, in cluster IIb were 15 lines. Genetic similarity according to Jaccard ranged from 0.389-1.000. Genetically the most similar in terms of glutelin polymorphism were lines with the coefficient of similarity 0.933 (Lines 6 and 9). On the other hand, the most dissimilar were Lines 18 and 8, and also 34 and 19.

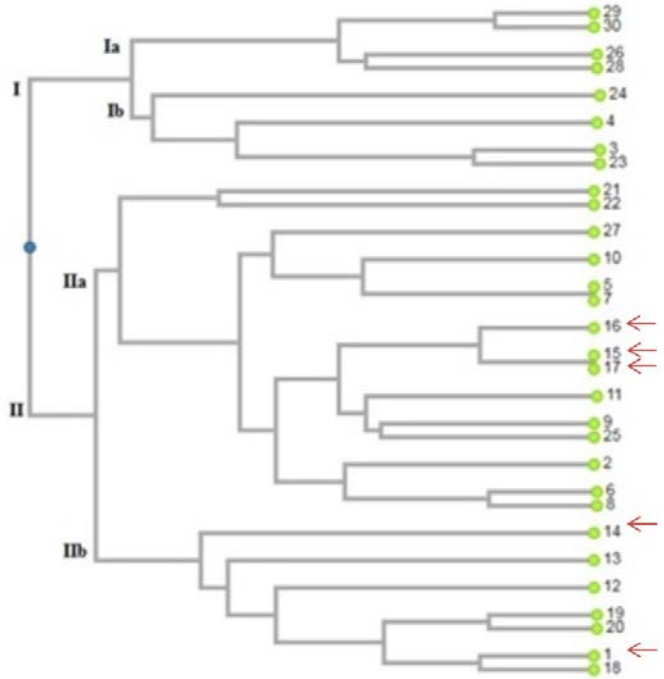

Figure 2. Dendrogram of the European and American varieties (SDS-PAGE) (1–30 - list of maize varieties from Europe and USA (Table 1), European varieties are marked with red arrows)

Iqbal et al. (2014a) observed 18 protein bands within SDS-PAGE method, while 7 (39%) were monomorphic and 11 (61%) were polymorphic with molecular weight from 10 to 122 kDa. Another study from Iqbal et al. (2014b) evaluated 83 genotypes of maize with use of SDS-PAGE method and found out, that from 18 protein

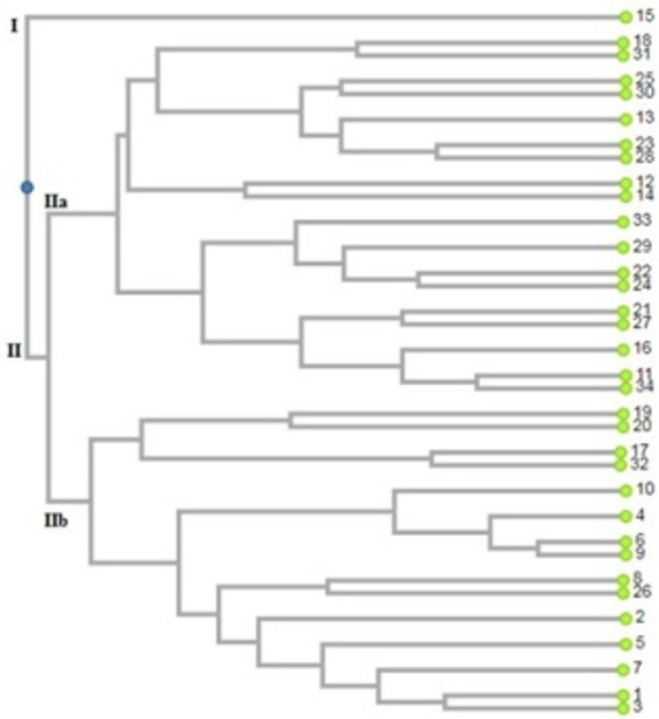

Figure 3. Dendrogram of the Slovak lines (SDS-PAGE) (1–34 – list of Slovak maize lines (Table 2))

bands 7 (39%) were monomorphic and 11 (61%) were polymorphic. Molecular weight varied between 10 to 122 kDa. Coefficient of similarity ranged between 0.89 and 1.00. These studies correspond with results of this work, as well as Vivodík et al. (2016), who rated 40 genotypes of maize with SDS-PAGE and got 23 valuable protein bands, from which 6 (31%) were monomorphic and 17 (65%) were polymorphic. Molecular weight ranged from 20 to 140 kDa.

These findings are also consistent with studies from Pastorello et al. (2009), Khan et al. (2014), AL-Huqail and Abdelhaliem (2015) and Adetumbi (2016).

Polyacrylamide gel electrophoresis in acidic conditions (A-PAGE) allows a more accurate differentiation of proteins with lower molecular weight such as SDS-PAGE, and its main use is the fingerprinting of prolamin fractions, in the case of maize zeins, based on constant electric voltage.

The zeins were separated in the acidic polyacrylamide gel (Figure 4) according to molecular weight, with the biggest zeins in the upper part of the gel and the

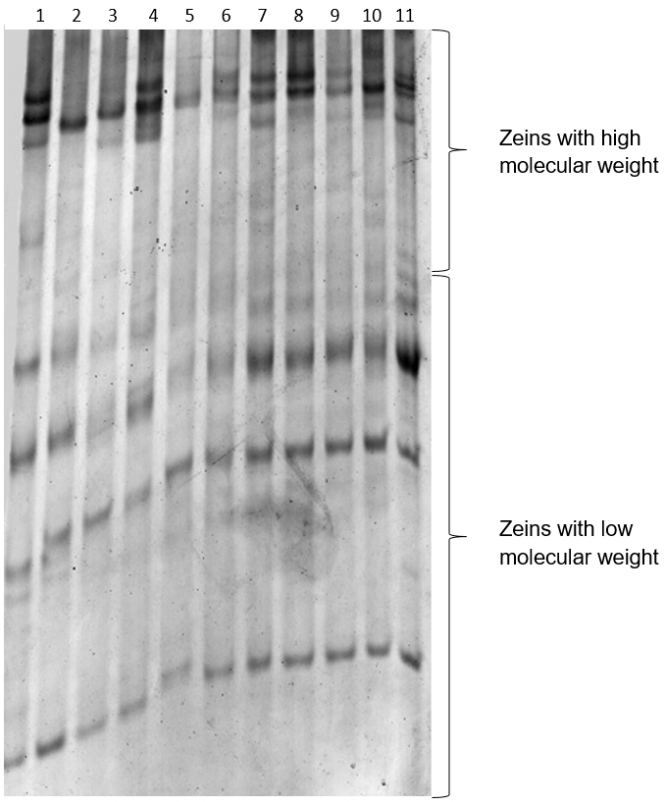

Figure 4. Electrophoretic profiles of selected American varieties obtained with A-PAGE method (1 – The Burpee, 2 – Golden Cross Bantam (early), 3 – Early Evergreen, 4 – Carmel Cross, 5 – Golden Harvest, 6 – Spring Gold, 7 – Fore Most extra Early ee1 F1, 8 – Golden Beauty F1, 9 – Extra Early Golden Bantam, 10 – Barbecue, 11 – North Star)

smallest in the lower part of the gel. On the basis of the presence or, respectively, the absence of protein bands, it was possible to construct dendrograms of varieties/lines to evaluate their genetic similarity in terms of zeins polymorphism. For this purpose, the Jaccard coefficient and UPGMA were applied.

A total of 14 protein bands were detected in European varieties, from which 4 (29%) were monomorphic and 10 (71%) were polymorphic. The American varieties had 19 evaluable protein bands, 4 (21%) were monomorphic, and 15 (79%) were polymorphic. 16 bands were evaluated within the Slovak lines, 4 (25%) were monomorphic and 12 (75%) were polymorphic.

The dendrogram (Figure 5) divided the European and American varieties into two main clusters (I and II). They split into smaller clusters (Ia, Ib, IIa, IIb). In the first cluster (I), 11 varieties were separated and in the

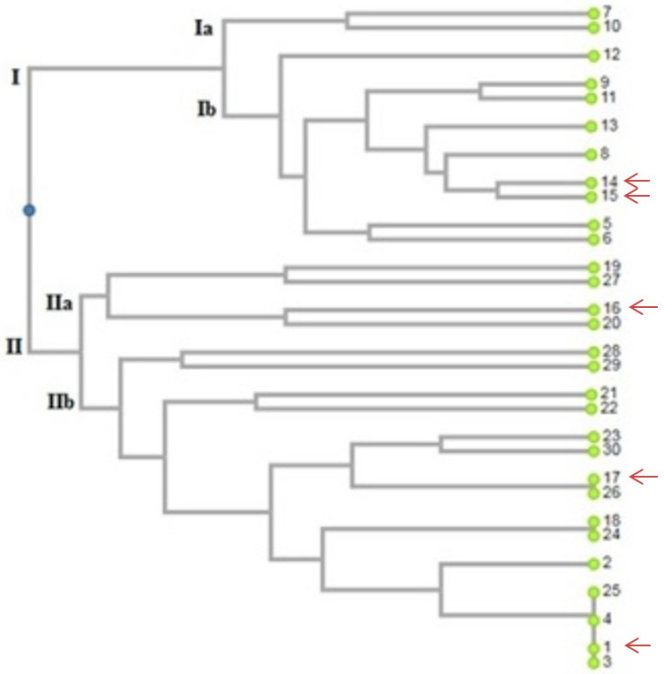

Figure 5. Dendrogram of European and American varieties (A-PAGE) (1–30 - list of maize varieties from Europe and USA (Table 1), European varieties are marked with red arrows)

second cluster (II), there were 19 varieties. In this case, European varieties were divided into both main clusters. Genetically most similar according to zein polymorphism were two varieties with coefficient of similarity 0.923 (14 – Fruhester Gelber and 15 – Maďarská cukrová), the least similar were varieties 20 – Early Evergreen and 8 – Miniature. Genetic similarity by Jaccard ranged from 0.308 to 1.000. Eight varieties could not be distinguished due to the same zein composition in the electrophoreogram (1 - Fekete Mazsola, 3 - Black Sugar, 4 - Howling Mob and 25 - Golden Beauty F1; 18 - The Burpee and 24 - Fore Most Extra Early (EE1) F1; and also 17 - Cukrová and 26 - Extra Early Golden Bantam).

The dendrogram of the Slovak lines (Figure 6) demonstrated the division into two main clusters (I and II), which then differed considerably. In the first cluster (I) there were 8 lines, in the second cluster (II) were 26 lines. There were two pair of lines with the highest coefficient of similarity 0.900 (Lines 21 and 22; 13 and 34), while genetic similarity by Jaccard ranged from 0.357 to 1.000. Genetic distance was the highest between the Lines 28 and 3. Based on the same zein bands in the electrophoreogram, 8 lines (Lines 9, 11 and 15; lines 1, 8

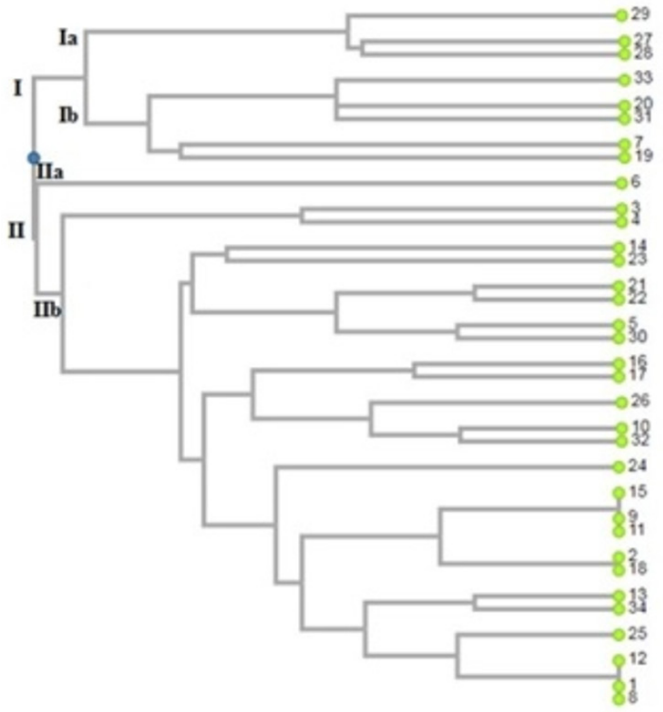

Figure 6. Dendrogram of the Slovak lines (A-PAGE) (1–34 – list of Slovak maize lines (Table 2))

and 12; as well as 2 and 18) could not be distinguished. These findings correspond to the results of Gregová et al. (2015), who evaluated 40 lines of maize, which were separated into two main clusters. In first cluster there were 16 lines and in the second 24 lines. Coefficient of genetic distance according to Jaccard ranged between 0 to 0.88. They also determined the lines, which could not be distinguish.

Acid electrophoresis, as useful tool for separation of various plant genotypes according to their representation of prolamines, was used also by Yan et al. (2003), Pan et al. (2007), Sofalian and Valizadeh (2009), Miháliková et al. (2016) and Petrovičová et al. (2018).

On the basis of the dendrograms, most diverse genotypes were identified and they can be used in future maize breeding program.

CONCLUSION

Electrophoretic methods are excellent tool for assessing the diversity of maize as well as other cereals. Differentiation of individual genotypes was made on the basis of glutelin polymorphism (SDS-PAGE) and zein polymorphism (A-PAGE), which allowed genotypes to be placed differently within dendrograms of genetic similarity. Some genotypes could not be distinguished, because of their same protein profile, which may be explained by a likely common genetic basis. It is important to use several methodologies to their next distinguish. As a consequence of this phenomenon, these genotypes are not recommended to be used within the crossover group, because of the heterologous effect.

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REFERENCES

Adetumbi, J. A. (2016) Genetic purity of maize seed using SDS-PAGE. Applied Tropical Agriculture, 21 (1), 33-38.

AL-Huqail, A. A., Abdelhaliem, E. (2015) Evaluation of genetic variations in maize seedlings exposed to electric field based on protein and DNA markers. BioMed Research International, 2015, 1-15. DOI: http://dx.doi.org/10.1155/2015/874906

Australian Government DHA (2008) The biology of *Zea mays* L. spp. mays (maize or corn). Version 1: September 2008. [Online] Australia: Australian Government DHA. Available at: http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/maize-3/$FILE/biologymaize08.pdf [Accessed 5 September 2018].

Buckler, E. S., Gaut, B. S., McMullen, M. D. (2006) Molecular and functional diversity of maize. Current Opinion in Plant Biology, 9 (2), 172-176. DOI: https://dx.doi.org/10.1016/j.pbi.2006.01.013

Chňapek, M., Tomka, M., Balážová, Ž., Gálová, Z. (2014) Protein complex of wheat, buckwheat and maize in relation to celiac disease. Journal of Microbiology, Biotechnology and Food Sciences, 3 (SI 2), 88–92.

Draper, S. R. (1987) ISTA Variety Committee - Report of the working group for biochemical tests for cultivar identification 1983-1986. Switzerland: Seed science and technology.

Ďuďák, J. (2016) Kukurica na zrno a jej zber. Roľnícke noviny, 86 (36).

Gálová, Z., Balážová, Ž., Chňapek, M., Vivodík, M., Oslovičová, V. (2011) Bielkovinové a DNA markery pšenice. Nitra: Slovak University of Agriculture.

Gregová, E., Hauptvogel, P., Kása, S. (2015) Analýza zeínov v kukurici siatej a kukurici cukrovej elektroforetickými metódami. [Online] Piešťany: VÚRV. http://www.vurv.sk/fileadmin/CVRV/Viac/PUblikacna_cinnost_VURV/Analyza_zeinov_kukurici_siatej_a_kukurici_cukrovej_elektroforetickymi_metodami_-_Gregova.pdf [Accessed 1 October 2018].

Iqbal, J., Shinwari, Z. K., Rabbani, M. A. (2014a) Genetic variability assessment of maize (*Zea mays* L.) germplasm based on total seed storage proteins banding pattern using SDS-PAGE. European academic research, 2 (2), 2144-2160.

Iqbal, J., Shinwari, Z. K., Rabbani, M. A. (2014b) Investigation of total seed storage proteins of Pakistani and Japanese maize (*Zea mays* L.) through SDS-PAGE markers. Pakistan Journal of Botany, 46 (3), 817-822.

Khan, A. H., Khan, N., Minhas, N. M., Rabbani, M. A., Ghafoor, A. (2014) Diversity in seed storage proteins in maize genetic resources: I. Variation in alcohol soluble zein protein fraction. International Journal of Agriculture & Biology, 16 (5), 1015-1018.

Miháliková, D., Gálová, Z., Chňapek, M. (2016) Detection of gliadins and secaline block in wheat genotypes by A-PAGE. In: MMK 2016, International Masaryk conference for Ph.D. students and young reseachers. Hradec Králové, Czech Republic, 12–16 December 2016, Magnanimitas.

Pan, Z. F., Deng, G. B., Zhai, X. G., Wu, F., Yu, M. Q. (2007) Genetic diversity of Acid-PAGE monomeric prolamins in cultivated hulless barley (*Hordeum vulgare* L.) from Qinghai–Tibet plateau in China. Genetic Resources and Crop Evolution, 54, 1691–1699. DOI: https://dx.doi.org/10.1007/s10722-006-9177-2

Pastorello, E. A., Farioli, L., Pravettoni, V., Scibilia, J., Conti, A., Fortunato, D., Borgonovo, L., Bonomi, S., Primavesi, L., Ballmer-Weber, B. (2009) Maize food allergy: lipid-transfer proteins, endochitinases, and alpha-zein precursor are relevant maize allergens in double-blind placebo-controlled maize-challengepositive patients. Analytical and Bioanalytical Chemistry, 395 (1), 93–102. DOI: https://dx.doi.org/10.1007/s00216-009-2945-z

Petrovičová, L., Gálová, Z., Balážová, Ž., Chňapek, M., Vivodík, M., Trebichalský, A. (2018) Genomická a proteomická analýza semena raže a tritikale. Nitra: Slovak University of Agriculture.

Pospíšil, R. (2016) Zber kukurice siatej na zrno. Roľnícke noviny, 86 (36).

Shah, A. H., Khan, M. F., Khaliq, A. (2003) Genetic characterization of some maize (*Zea mays* L.) varieties using SDS-PAGE. Asian Journal of Plant Sciences, 2, 1188-1191.

Shukla, R., Cheryan, M. (2001) Zein: The industrial protein from corn. Industrial Crops and Products, 13, 171–192.

Sofalian, O., Valizadeh, M. (2009) Investigation of seed storage proteins in some wild wheat progenitors using SDS-page and Acid-page. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37 (1), 179-182.

Vivodík, M., Gálová, Z., Balážová, Ž., Petrovičová, L., Kuťka Hlozáková, T. (2016) Genetic variation and relationships of old maize genotypes (*Zea mays* L.) detected using SDS-page. Slovak Journal of Food Sciences, 10 (1), 532–536. DOI: https://dx.doi.org/10.5219/661

Vivodík, M., Balážová, Ž., Gálová, Z., Petrovičová, L. (2018) Study of polymorphism of maize using DNA and protein markers. Slovak Journal of Food Sciences, 12 (1), 157-165. DOI: https://dx.doi.org/10.5219/874

Wrigley, C. W. (1992) Identification of cereal varieties by gel electrophoresis of the grain proteins. Seed Analysis. Berlin: Heilderberg, Springerverlag. DOI: http://dx.doi.org/10.1007/978-3-662-01639-8_2

Yan, Y., Hsam, S. L. K., Yu, J., Jiang, Y., Zeller, F. J. (2003) Allelic variation of the HMW glutenin subunits in *Aegilops tauschii* accessions detected by sodium dodecyl sulphate (SDS-PAGE), acid polyacrylamide gel (A-PAGE) and capillary electrophoresis. Euphytica, 130 (3), 377–385. DOI: http://dx.doi.org/10.1023/A:1023062316439