

Comparison of American and European maize (*Zea mays* L.) protein profiles

Porovnanie bielkovinových profilov americkej a európskej kukurice siatej (*Zea mays* L.)

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Abstract

Maize is an important crop with great use in agriculture, food and other industries. The aim of this work was to compare 30 varieties of *Zea mays* L., that were grown in the USA and Europe in terms of nutritional quality and to genetically distinguish them by creating a dendrogram of relationship. The content of total nitrogen, crude protein content, fractional composition of proteins and the calculated coefficient of nutritional quality were all determined. Compared to American varieties, higher nutritional quality was shown by the varieties of European origin with the coefficient of nutritional quality higher about 17.55%. However, the highest value was achieved by the American variety Howling Mob (331.91%). Storage proteins - glutelins were divided according to molecular weight into HMW-GS (high molecular weight glutelin subunits), LMW-GS (low molecular weight glutelin subunits) plus zeins and residual albumins and globulins by electrophoretic separation. The biggest difference was recorded among HMW-GS content - 5.77% (American varieties) and 7.35% (European varieties), while the LMW-GS plus zeins and residual albumins and globulins content was similar. 20 protein bands (15 polymorphic and 5 monomorphic) in American varieties and 18 protein bands (10 polymorphic and 8 monomorphic) in European varieties were evaluated in the electrophoretograms. Based on the presence of individual proteins, a dendrogram was constructed, using the UPGMA (Unweighted Pair-Group Method Using Arithmetic Averages) method and the Jaccard coefficient of similarity. In the dendrogram, 2 clusters of varieties with the dissimilarity 0.481 were formed. In the subgroups, from the point of glutelin polymorphism, the varieties Illinois Hulles and Queen Anna were separated from the others. The European varieties were segregated within the second cluster and created the common subgroup of 2 varieties - Madarska cukrova (Hungary) and Zlota Handlowa (Poland).

Keywords: dendrogram, maize, proteins, SDS-PAGE

Abstrakt

Kukurica siata je významnou plodinou s veľkým využitím v poľnohospodárstve, potravinárstve, ako aj v rôznych priemyselných odvetviach. Cieľom práce bolo porovnať 30 odrôd kukurice siatej (*Zea mays* L.), ktoré boli vyšľachtené v USA a Európe z hľadiska nutričnej kvality a geneticky ich odlíšiť zostrojením dendrogramu príbuznosti. Stanovený bol obsah celkového dusíka, hrubých bielkovín, frakčná skladba bielkovín a vypočítaný koeficient nutričnej kvality. Vyššiu nutričnú kvalitu vykazovali odrody európskeho pôvodu v porovnaní s americkými odrodami, pričom koeficient nutričnej kvality bol vyšší o 17,55%. Avšak najvyššiu nutričnú kvalitu dosiahla americká odroda Howling Mob (331,91%). Elektroforetickou separáciou bielkovín sa glutelíny rozdelili na základe molekulovej hmotnosti na HMW-GS (vysokomolekulárne glutelínové podjednotky), LMW-GS (nízkomolekulárne glutelínové podjednotky) plus zeíny a zvyškové albumíny a globulíny. Najväčší rozdiel bol zaznamenaný v obsahu HMW-GS – 5,77% (americké odrody) a 7,35% (európske odrody), pričom obsah LMW-GS a zeínov a reziduálnych albumínov a globulínov bol podobný. V elektroforeogramoch sa vyhodnotilo 20 bielkovinových pásov (15 polymorfných a 5 monomorfných) v amerických odrodách a 18 pásov (10 polymorfných a 8 monomorfných) v európskych odrodách. Na základe prítomnosti jednotlivých bielkovín bol zostrojený dendrogram genetickej podobnosti s použitím metódy UPGMA (metóda skupín nevážených dvojíc využívajúca aritmetický priemer) a Jaccardovho koeficientu podobnosti. V dendrograme vznikli 2 zhluky odrôd s disimilaritou 0,481, pričom v podskupinách sa od ostatných odrôd z hľadiska polymorfizmu glutelínov oddelili odrody Illinois Hulles a Queen Anna. Európske odrody sa oddelili v rámci druhého zhluku a spoločnú podskupinu vytvorili 2 odrody - Madarska cukrova (Maďarsko) a Zlota Handlowa (Poľsko).

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

Introduction

Maize, a classical model plant for genetic studies, is an important plant species and also the most diverse crop species known (Zhao et al., 2006). After wheat and rice, maize is on the third place among the most cultivated crops in the world. The largest production is in the USA, China, Brazil, Mexico and France. Most of the world's maize production (70%) is used to feed livestock, about 20% in food consumption, the industry uses about 5% of maize and the rest is for seed (Uher et al., 2012). Cereals are important sources of proteins in human nutrition, but have low protein quality due to limitations in the amounts of essential amino acids, notably lysine. These deficiencies result from the low levels of these amino acids in the prolamin storage proteins (Shewry, 2007). Maize seeds contain ~10% proteins and ~70% of them are classified as storage proteins. Based on their solubility in different solvents, endosperm proteins are divided into four groups: albumins, globulins, glutelins, and prolamins (Flint-Garcia et al., 2009; Wu and Messing, 2014). Genetic purity and verity identification of maize can be carried out effectively through sodium dodecyl sulphate polyacrylamide gel electrophoresis – SDS-PAGE (Khan et al., 2013). It has been studied that besides physiochemical and molecular study, the SDS-PAGE is

fruitful to study the genetic variability in seed storage proteins (Zeb et al., 2006). Zeins accumulate in rough endoplasmatic reticulum of endosperm cells. A number of different molecular weight zeins have been identified by SDS-PAGE and have been further classified, based on their solubility and structural relationships, into α -, β -, γ - and δ - zeins. (Australian Government DHA, 2008). Glutelins are usually divided into 2 groups according to their molecular weight – high molecular weight glutelin subunits (HMW-GS) with the size of 80–120 kDa and low molecular weight glutelin subunits (LMW-GS) with the size of 30–80 kDa. In practice, the most widely used type of plant proteins for the purpose of identifying and differentiation of genotypes are storage grain proteins. By electrophoretic methods, these proteins can be easily and rapidly split, visualized and genetically interpreted, because this type of proteins has characteristic polymorphism given by the existence of multiple discrete forms as a consequence of heterozygosity (Gálová et al., 2011). Comparison a lot of marker systems showed, that molecular marker systems could generally be considered to be independent of environmental influences and have greater genome coverage than others. Molecular markers are consistent in the entire plant body and that is why these markers are not influenced by pleiotropic, epistatic, nor environmental factors. Genetic diversity in crops is essential to make improvements related to superior germplasms. Implementation of molecular markers to identify suitable genotypes speeds up the breeding progress by enhancing selection efficiency (Agarwal et al., 2008; Muhammad et al., 2017).

Materials and methods

Plant material

Samples of 30 maize varieties (*Zea mays* L.) were provided by the Genbank VURV Prague-Ruzyne. The grains of 25 analyzed samples originated from the USA (Miniature, Queen Anne, Spring Gold, Early King, Golden Harvest, Golden Beauty F1, Stowell's F1, Golden Cross Bantam (early), Extra Early Golden Bantam, Fore Most Extra Early (EE1) F1, Rostrata, Black Sugar, Wonderfull, North Star, Illinois Hulles, Ioana, The Burpee, Whiple's Early white, Early Evergreen, Barbecue, Black Mexican, Howling Mob, Carmel Cross, Fore Most Coss F1, Trucker's favourite white), the remaining 5 varieties (Cukrova, Zlota Handlowa, Madarska cukrova, Fekete Mazsola and Fruhester Gelber) were of European origin (from Czechoslovakia, Poland, Hungary and Germany).

Crude protein content and fractional composition of proteins

Protein fractions were extracted from ground grains according to their solubility in various solutions. Determination of total nitrogen content by Kjeldahl method (Michalík, 2002) is based on the combustion of the plant sample or fraction of protein in a Kjeldahl flask in the presence of concentrated sulfuric acid and the suitable catalyst. After distillation and titration, the total nitrogen content of the sample was calculated. This value was multiplied by the conversion factor for maize (6) and as final values, the crude protein content and coefficient of nutritional quality were obtained.

SDS-PAGE and dendrogram

Glutelins were isolated from the endosperm of dry and mature grains. Proteins after grain homogenization were extracted by standard ISTA method. Next electrophoresis with presence of sodium dodecylsulphate was performed (Wrigley, 1992) using the vertical discontinual electrophoretic system Hoefer SE 600 DeLuxe by Thermo Fisher Scientific. Then protein bands were stained by Coomassie Brilliant Blue R – 250. Electrophoretic profiles were scanned by GS-800 Calibrated Densitometer (BioRad). The separated glutelin subunits (HMW-GS – high molecular weight glutelin subunits and LMW-GS – low molecular weight glutelin subunits) were identified in Doc-It LS Image analysis UVP software and the dendrogram was constructed using Jaccard similarity coefficient and the method UPGMA (Unweighted Pair - Group Method using Arithmetic Averages). Results were evaluated by mathematical - statistical methods (average value, standard deviation and coefficient of variation).

Results and discussion

The collection of 25 American maize varieties had in average 2.21% of total nitrogen content (Table 1). Most albumins and globulins were detected in the variety Wonderfull (34.57%), least in the variety Fore Most Coss F1 (20.83%). Overall, albumins and globulins are important from the point of nutrition, because they have high content of essential amino acids. Average content of the prolamins was 22.71%. The most significant fraction were the glutelins (31.61%), their highest content was detected in the variety Rostrata (40.38%). Similar to these results is research of Socha et al. (2010), who determined total nitrogen content in maize at 1.19%. Subsequently they detected 17.2% representation of albumins and globulins, 23.57% of prolamins and 32.05% of glutelins, which are in agreement with results of this research.

Table 1. Fractional composition of proteins in American varieties

	AG ^a (%)	P ^b (%)	G ^c (%)	N _{TOT} ^d (%)	CP ^e (%)	CNQ ^f (%)
Average value	25.06	22.71	31.61	2.21	13.26	209.26
Standard deviation	3.72	4.39	5.05	0.24	1.42	54.79
Minimum	20.83	13.82	20.88	1.68	10.08	126.82
Maximum	34.57	31	40.38	2.77	16.63	331.91
Coefficient of variation	14.83	19.34	15.99	10.67	10.67	26.19

^aAG – albumins and globulins, ^bP – prolamins, ^cG – glutelins, ^dN_{TOT} – total nitrogen content, ^eCP – crude protein content, ^fCNQ – coefficient of nutritional quality.

The 5 European varieties (Table 2) achieved in average more albumins and globulins (26.56%), more prolamins (20.27%) and total nitrogen content was also higher (2.39%) than in American varieties. However, the content of prolamins was lower (20.27%).

Table 2. Fractional composition of proteins in European varieties

	AG ^a (%)	P ^b (%)	G ^c (%)	N _{TOT} ^d (%)	CP ^e (%)	CNQ ^f (%)
Average value	26.56	20.27	34.47	2.39	14.31	226.81
Standard deviation	2.2	2.1	3.12	0.31	1.89	41.9
Minimum	23.68	17.61	30.61	1.99	11.93	169.08
Maximum	29.58	23.87	38.11	2.74	16.46	291.94
Coefficient of variation	8.3	10.38	9.04	13.18	13.18	18.48

^aAG – albumins and globulins, ^bP – prolamins, ^cG – glutelins, ^dN_{TOT} – total nitrogen content, ^eCP – crude protein content, ^fCNQ – coefficient of nutritional quality.

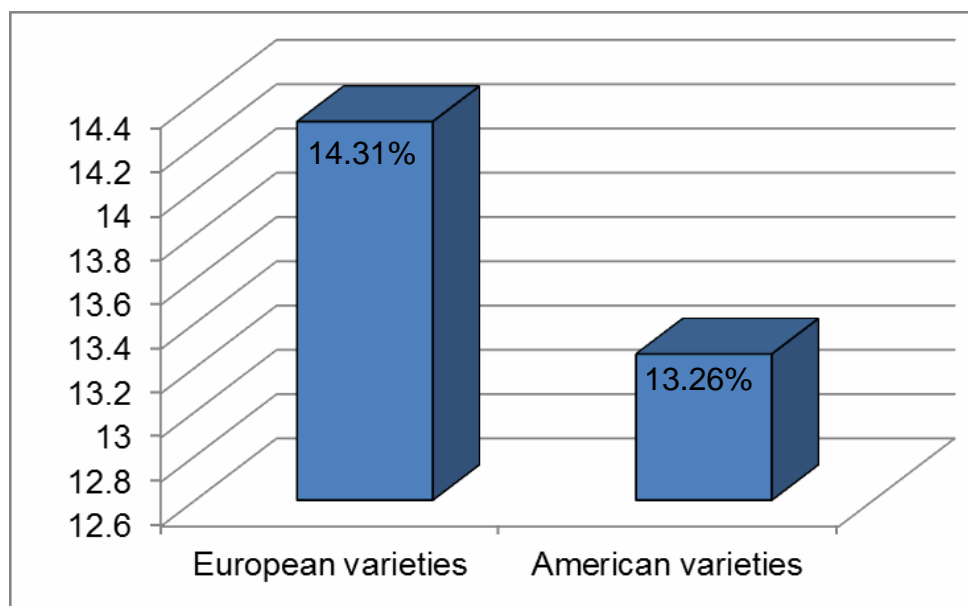


Figure 1. Crude protein content – comparison of European and American varieties

After calculation from the value of total nitrogen content, average crude protein content (Figure 1) in American varieties was determined to be 13.26% and in European varieties to be 14.31%. The distinction among the average values was 1.05%.

The values ranged from 10.08% (variety Fore Most Coss F1) to 16.63% (variety Spring Gold), which are in agreement with the results of Cazares-Sanchez et al. (2015), who determined the crude protein content from 9.35% to 12.5% in 41 Mexican maize populations, even though the maximum in the variety Spring Gold was about 4.13% higher. Crude protein content in maize was only 7.15% according to results of Socha et al. (2010), which is lower than the detected value in the varieties from the USA and Europe.

Coefficient of nutritional quality (CNQ) is a useful factor, which serves as a tool for assessing the suitability of the variety for nourishment. It was obtained (Figure 2) by calculating the proportion of the albumins, globulins and residual fractions to the prolamins content. The maize varieties from the USA achieved an average 209.26% and from Europe 226.81%. Highest CNQ was achieved by the variety Howling Mob (331.91%), so it can be concluded, that it has the highest nutritional value. On the other hand, the least nutritious was the variety Black Mexican (126.82%).

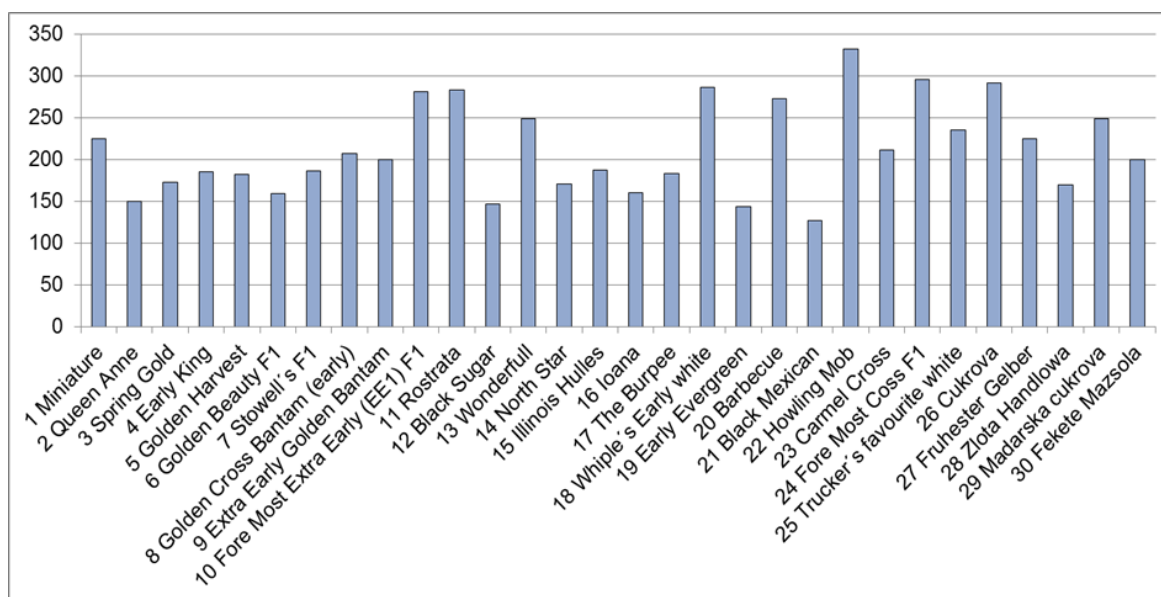
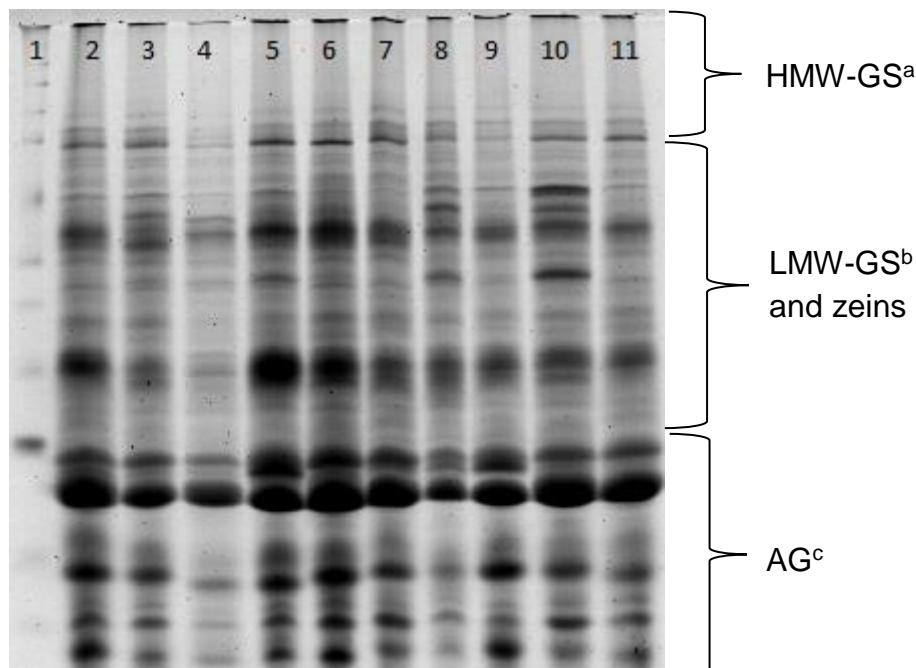


Figure 2. Coefficient of nutritional quality (%)

Separation of maize storage proteins with SDS-PAGE method showed as a result electrophoretic spectrum (Figure 3). Split proteins served as means to differentiation of varieties and to construction of the dendrogram of genetic relatedness.

According to presence of protein subunits, the electrophoretogram differentiated the varieties. Based on molecular weight, proteins were separated to high molecular weight glutelin subunits (HMW-GS), low molecular weight glutelin subunits (LMW-GS) including prolamins (zeins) and residual albumins and globulins (AG), which are the smallest parts. The results showed, that average value of high molecular weight glutelin subunits (Figure 4 and Table 3) was 5.77% in American varieties and 7.35% in European varieties. The varieties from the USA achieved 42.58% of LMW-GS plus zeins and 50.65% of albumins and globulins, while

European varieties showed the content of LMW-GS plus zeins at 43.03% and albumins and globulins at 49.62%.



1 – protein marker (range 2-212 kDa), 2 - Ioana, 3 – Stowell's F1, 4 - Illinois Hulless, 5 - Fruhester Gelber, 6 - Madarska cukrova, 7 - Zlota handlowa, 8 – Cukrova, 9 - The Burpee, 10 - Golden Cross Bantam (early), 11 - Early evergreen, ^aHMW-GS – high molecular glutelin subunits, ^bLMW-GS – low molecular glutelin subunits, ^cAG – albumins and globulins.

Figure 3. Electrophoretogram of selected maize varieties

From the point of comparison between American and European varieties, the most significant was the difference in the content of HMW-GS. European varieties achieved about 1.58% higher amount of HMW-GS than the American varieties.

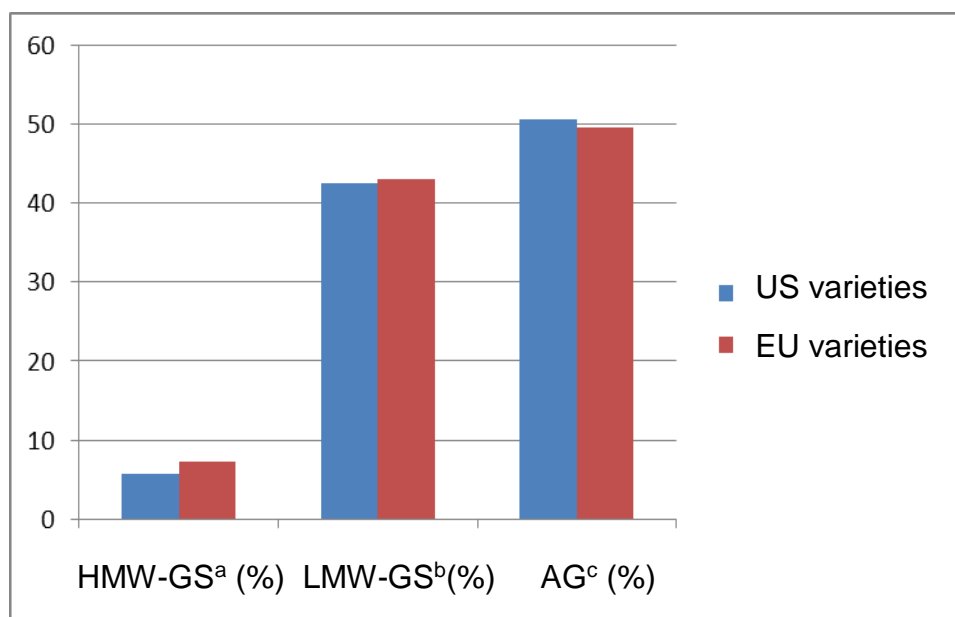
The content of LMW-GS plus zeins and albumins and globulins were more similar in both groups of varieties. These results are consistent with the results of Chňapek et al. (2014), who recorded average content of HMW-GS in analyzed maize samples at 4.71% (1.28%-12.13%).

Table 3. Comparison of presence HMW-GS, LMW-GS and AG in American and European varieties

	HMW-GS ^a (%)	LMW-GS ^b and zeins (%)	AG ^c (%)
American varieties	5.77	42.58	50.65
European varieties	7.35	43.03	49.62

^aHMW-GS – high molecular weight glutelin subunits, ^bLMW-GS – low molecular weight glutelin subunits, ^cAG – albumins and globulins.

The value of LMW-GS and maize prolamins were represented with an average of 44.06%, with high variability, which ranged from 21.15% to 88.59% and is similar to detected LMW-GS content in this work - 42.58% and 43.03%. Eventually, the content of residual albumins and globulins was high in both cases (in this analyze 50.65% (American) and 49.62% (European) to 51.23% (Chňapek et al., 2014)).



^aHMW-GS – high molecular weight glutelin subunits, ^bLMW-GS – low molecular weight glutelin subunits, ^cAG – albumins and globulins.

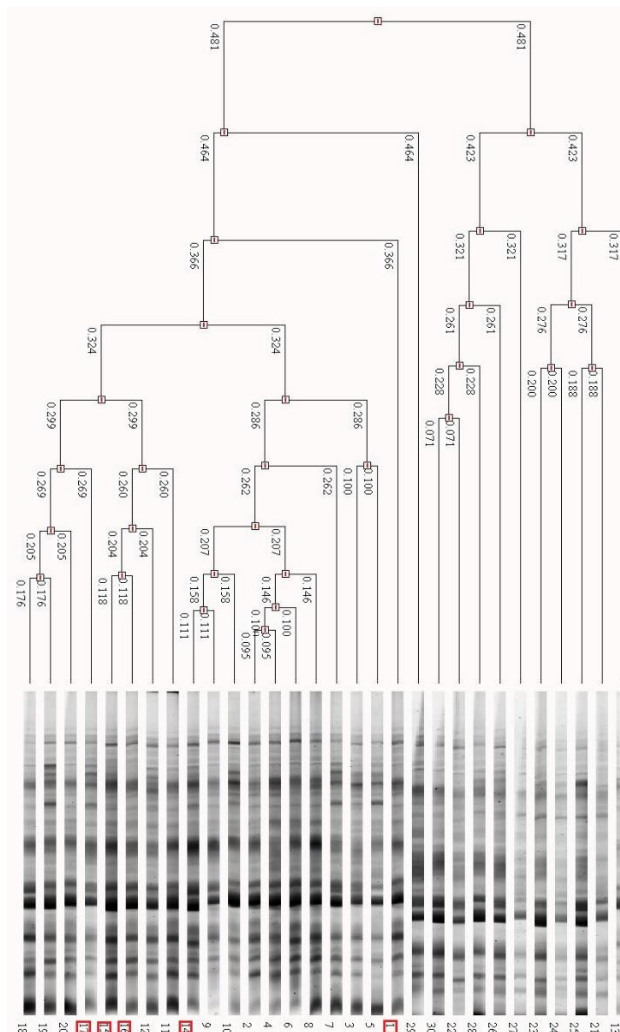
Figure 4. Average values of glutelin subunits in American (US) and European (EU) maize varieties

As a result of SDS-PAGE method, there were 20 scorable protein bands in electrophoretogram of the American varieties. The bands, which were not consistent in reproducibility and showed variation in sharpness and density, were excluded. Based on these terms, 5 bands (25%) were present in all samples and can be considered as monomorphic, while 15 bands (75%) showed variations and can be

considered as polymorphic. In the European varieties, there were 18 protein bands, from which 8 were considered as monomorphic (44.44%), while 10 were polymorphic (55.56%).

The dendrogram (Figure 5) demonstrated the relationships among 30 varieties of maize according to the Jaccard similarity index, using UPGMA cluster analysis. The dendrogram was based on glutelins polymorphism and divided varieties into 2 main clusters with dissimilarity index 0.481. The first cluster contained 10 maize varieties, while the second included 20 varieties. The unique variety Illinois Hulles was separated from other varieties in the first cluster, while in the second cluster was the variety Queen Anna in the same position. European varieties were located in the second cluster. Genetically closest relatedness showed the varieties Madarska cukrova and Zlota Handlowa from Hungary and Poland, which created the common subgroup.

The size of the protein bands, which were obtained from SDS-PAGE, ranged from 5 to 120 kDa. Similar results were detected by Vivodík et al. (2016), who evaluated 23 protein bands in 40 maize European genotypes, while 6 bands were monomorphic and 17 were polymorphic. Molecular weight ranged between 20 and 140 kDa. Other results were demonstrated by Iqbal et al. (2014), who recorded 18 protein bands – 7 (39%) were monomorphic and 11 (61%) were polymorphic, while molecular weight varied from 10 to 122 kDa. Gregová and Hauptvogel (2015) evaluated 109 lines of maize with the SDS-PAGE and obtained 14 polymorphic bands, which divided the lines into 2 major clusters. The extent of their genetic distance by Jaccard was 0 - 0.88. The glutelin spectrum contained proteins with a relative molecular weight from 9 to 97 kDa.



1 - Fekete Mazsola (European), 2 - Black Mexican, 3 - Black Sugar, 4 - Howling Mob, 5 – Rostrata, 6 – Whiple’s Early White, 7 - Early King, 8 – Miniature, 9 - Fore Most Coss F1, 10 – Trucker’s favourite white, 11 – Ioana, 12 – Stowell’s F1, 13 - Illinois Hulless, 14 - Fruhester Gelber (European), 15 - Madarska cukrova (European), 16 - Zlota Handlowa (European), 17 – Cukrova (European), 18 - The Burpee, 19 - Golden Cross Bantam (early), 20 - Early Evergreen, 21 - Carmel Cross, 22 - Golden Harvest, 23 - Spring Gold, 24 - Fore Most Extra Early (ee1) F1, 25 - Golden Beauty F1, 26 - Extra Early Golden Bantam, 27 - Barbecue, 28 - North Star, 29 - Queen Anna, 30 – Wonderfull

Figure 5. Dendrogram of maize varieties

Conclusion

Maize is an important global commodity and therefore it is important to monitor and analyze the parameters that are significant for its wide-scale use. From the point of nutrition, the content of albumins and globulins is important. However, the analysis showed, that the most represented fraction in the varieties was the glutelins. The European varieties had more albumins and globulins, glutelins and higher content of total nitrogen and coefficient of nutritional quality. On the other hand, the highest coefficient of nutritional quality (331.91%) was achieved by the variety Howling Mob. The American varieties had more prolamins. SDS-PAGE is useful tool for differentiation varieties according to genetic relationships. There were 20 scorable

bands in electrophoretogram of the American varieties, which were monomorphic (25%) and polymorphic (75%). In electrophoretic spectrum of the European varieties there were 18 protein bands – 8 monomorphic (44.44%) and 10 polymorphic (55.56%). Based on them, 2 main clusters with the dissimilarity of 0.481 were created in the dendrogram, while in the first cluster were 10 varieties and in the second cluster were 20 varieties, including European varieties. Illinois Hulles and Queen Anna were separated from other varieties, so they can be considered as unique. Close group was created from European varieties Madarska cukrova (Hungary) and Zlota Handlowa (Poland). Application of molecular markers to identification suitable genotypes can speed up the breeding programs and increase biodiversity.

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References

- Agarwal, M., Shrivastava, N., Padh, H. (2008) Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Reports*, 27, 617-631. DOI: <https://dx.doi.org/10.1007/s00299-008-0507-z>
- Australian Government DHA (2008) The biology of *Zea mays* L. spp. Mays (maize or corn). Australia: Office of the Gene Technology Transfer, Australian Government. Available at: [http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/maize-3/\\$FILE/biologymaize08_2.pdf](http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/maize-3/$FILE/biologymaize08_2.pdf) [Accesed 20 September 2017].
- Cazares-Sanchez, E., Chavez-Servia, J.L., Salinas-Moreno, Y., Castillo-Gonzalez, F., Ramirez-Vallejo, P. (2015) Grain composition variation among native maize (*Zea mays* L.) of Yucatan, Mexico. *Agrociencia*, 49 (1), 15-30.
- 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 (S12), 88-92.
- Flint-Garcia, S.A., Bodnar, A.L., Scott, M.P. (2009) Wide variability in kernel composition, seed characteristics, and zein profiles among diverse maize inbreds, landraces, and teosinte. *Theoretical and Applied Genetics*, 119, 1129–1142. DOI: <https://dx.doi.org/10.1007/s00122-009-1115-1>
- 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. (2015) Analýza zeínov v kukurici siatej a kukurici cukrovej elektroforetickými metódami. [Online] Piešťany: VÚRV. Available at: http://www.vurv.sk/fileadmin/CVRV/Viac/Publikacna_cinnost_VURV/Analyz_a_zeinov_kukurici_siatej_a_kukurici_cukrovej_elektroforetickymi_metodami_-_Gregova.pdf [Accessed 26 September 2017].
- Iqbal, J., Shinwari, Z.K., Rabbani, M.A., Khan, S.A. (2014) 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.
- Khan, S.A., Shinwari, Z.K., Rabbani, M.A. (2013) Study of total seed protein of rice (*Oryza sativa* L.) breeding lines of Pakistan through SDS-PAGE. *Pakistan Journal of Botany*, 45 (3), 871-876.
- Michalík, I. (2002) Unifikovaná metóda diskontinuálnej frakcionácie bielkovinového komplexu zrna obilnín. *Poľnohospodárstvo*, 48 (7), 333-341.
- Muhammad, R.W., Qayyum, A., Ahmad, M.Q., Hamza, A., Yousaf, M., Ahmad, B., Younas, M., Malik, W., Liagat, S., Noor, E. (2017) Characterization of maize genotypes for genetic diversity on the basis of inter simple sequence repeats. *Genetics and Molecular Research*, 16 (1). DOI: <https://dx.doi.org/10.4238/gmr16019438>
- Shewry, P.R. (2007) Improving the protein content and composition of cereal grain. *Journal of Cereal Science*, 46 (3), 239-250. DOI: <https://dx.doi.org/10.1016/j.jcs.2007.06.006>
- Socha, P., Raždíková, A., Urmínská, D. (2010) Optimization of celiac disease active proteins estimation in cereals and pseudocereals. *Potravinárstvo Slovak Journal of Food Sciences*, 4 (SI), 497-508.
- Uher, A., Andrejiová, A., Bernáth, S., Černý, I., Kóňa, J., Mezey, J., Paulen, O., Valšíková-Frey, M. (2012). *Poľné a záhradné plodiny*. 2nd edition. Nitra: Slovak University of Agriculture.
- Vivodík, M., Gálová, Z., Balážová, Ž., Petrovičová, L., Kuřka Hložáková, T. (2016) Genetic variation and relationships of old maize genotypes (*Zea mays* L.) detected using SDS-page. *Potravinárstvo Slovak Journal of Food Sciences*, 10 (1), 532-536. DOI: <https://dx.doi.org/10.5219/661>
- Wrigley, C.W. (1992) Identification of cereal varieties by gel electrophoresis of the grain proteins. *Seed Analysis*. Berlin: Heilderberg, Springer-Verlag. DOI: https://dx.doi.org/10.1007/978-3-662-01639-8_2
- Wu, Y., Messing, J. (2014) Proteome balancing of the maize seed for higher nutritional value. *Frontiers in Plant Science*, 5. DOI: <https://dx.doi.org/10.3389/fpls.2014.00240>
- Zeb, A., Zahir, A., Ahmad, T., Abdumanon, A. (2006) Physiochemical characteristics of wheat varieties growing in the same and different ecological regions of Pakistan. *Pakistan Journal of Biological Sciences*, 9 (9), 1823-1828.

Zhao, W., Canaran, P., Jurkuta, R., Fulton, T., Glaubitz, J., Buckler, E., Doebley, J., Gaut, B., Goodman, M., Holland, J., Kresovich, S., McMullen, M., Stein, L., Ware, D. (2006) Panzea: a database and resource for molecular and functional diversity in the maize genome. *Nucleic Acids Research*, 34, D752–D757. DOI: <https://dx.doi.org/10.1093/nar/gkj011>