Genetic diversity of European cultivars of common wheat (*Triticum aestivum* L.) based on RAPD and protein markers

Genetická diverzita európskych odrôd pšenice (*Triticum aestivum* L.) založená na RAPD a proteínových markeroch

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Abstract

The knowledge of genetic diversity of wheat genotypes based on DNA and protein polymorphism is very important for breeding programs (MAS). The objective of this study was to assess the genetic variability among twenty – four European cultivars of common wheat using SDS – PAGE and RAPD techniques. Protein electrophoreses showed 31 polymorphic bands with 63.2% polymorphism. Genetic similarity based on Jaccard´s coefficient ranged from 0.067 to 0.933. Also twelve different Glu – 1 encoded allelic variants were identified among these 24 genotypes resulting from combination of 3 alleles of Glu – 1A, 7 of Glu – 1B and 2 of Glu – 1D loci. One novel allelic variant 6.5+7.5 at the Glu – 1B locus was identified. RAPD analysis showed that the number of polymorphic amplicons was 56 out of a total of 63 amplicons, thus revealing a level of 37.56% polymorphism. Maximum level of polymorphism (55.8%) was observed for the primer OPA – 03 and minimum one for OPD – 08 (13.8%). Genetic similarity based on Jaccard´s coefficient ranged from 0.396 to 0.805. As a result of this investigation, might be expected that the SDS – PAGE using protein markers and RAPD – PCR using DNA markers would be a useful tool for assessment of genetic diversity among wheat genotypes.

Key words: genetic divergence, RAPD – PCR, SDS – PAGE, *Triticum aestivum* L.

Abstrakt

Znalosť genetickej diverzity genotypov pšenice na báze DNA a bielkovinového polymorfizmus je pre šlachtiteľské programy (MAS) veľmi dôležitá. Cieľom tejto štúdie bolo detegovať genetickú diverzitu 24 európskych genotypov pšenice letnej formy ozimnej použitím SDS – PAGE a RAPD techník. SDS – PAGE zásobných proteínov preukázal 31 polymorfných znakov s 63,2% polymorfizmom. Identifikovaných bolo 12 alelických variánt na lokuse Glu – 1, a to 3 alely na lokuse Glu – 1A, 7 na lokuse Glu – 1B and 2 na Glu – 1D lokuse. Na lokuse Glu – 1B bola identifikovaná nova alela 6,5+7,5. Genetická podobnosť na základe Jaccardovho koeficientu sa pohybovala v rozmedzí od 0,067 do 0,933. RAPD analýza ukázala 56 polymorfných z celkového počtu 63 amplikónov (37,56% polymorfizmus). Maximálna úroveň polymorfizmu (55,8%) bola pozorovaná primerom OPA – 03 a minimálna primerom OPD - 08 (13,8%). Genetická podobnosť na základe Jaccardovho koeficientu sa pohybovala v rozmedzí od 0,396 do 0,805. Výsledky dokumentujú, že SDS – PAGE a RAPD – PCR techniky sú užitočným nástrojom pre posúdenie genetickej diverzity genotypov pšenice letnej.

Kľúčové slová: genetická diverzita, RAPD – PCR, SDS – PAGE *Triticum aestivum* L.

Introduction

One of the key factors for the improvement of plant crops including wheat, as the most important cereals in the world due to its multifaceted use and nutritive value, is genetic diversity. Evaluation of genetic diversity using molecular markers is a cornerstone for understanding genome structure, the characterization and maintenance of genetic variation on plant germplasm, identifying genes underlying important traits and devising optimal breeding strategies for crop improvement (Hayden et al., 2010 cited in Aida et al., 2012).

Therefore, in recent years the exploitation of molecular markers has received much attention for the evaluation of genetic diversity. Molecular markers are useful tools for estimating genetic diversity as these are not influences by environment, are abundant and do not require previous pedigree information (Bohn et al., 1999). Electrophoretic pattern of storage proteins have been widely used to identify and characterize different plant crops because of its rapidity and accurate description. Nowadays it is possible to assess identity and agronomic properties of protein patterns of each cultivar using of appropriate and refined techniques. Among the biochemical markers, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS – PAGE) of storage proteins has been successfully used due to its simplicity and effectiveness for estimating genetic diversity (Ahmed et al., 2010). Nowadays, this method is considered as a low cost, high reproducible and rapid approach, because of that it became accepted valuable tool.

Among the different DNA markers, random amplified polymorphic (RAPD) markers have been frequently used due to simplicity, efficiency and rapidity. It may be used

for analysis of many plant samples using small quantities, pedigree analysis (Gallois et al., 1998), molecular mapping (Reiter et al., 1992) or identification of genotypes associated with genes of interest and genetic studies. The RAPD fingerprint has been used to differentiate between closely related genotypes of wheat species due to its stability at high temperatures and conserved structure within all tissues (Shehata, 2004).

The aim of the present study was to provide molecular patterns for the identification and characterization of the phylogenetic relationships among twenty – four European cultivars of common wheat, included the genotype with probably novel high – molecular – weight glutenin subunits (HMW – GS) identified by SDS – PAGE (Kuťka Hlozáková et al., 2015). This will be helpful in future for genomic mapping studies leading to obtain improved crop productivity with fresh genetic background.

Material and methods

Plant material

Twenty – four genotypes of hexaploid wheat (*Triticum aestivum* L.) grain originating from five different geographical areas (Slovakia – 6 genotypes, Czech Republic – 6, Hungary – 3, Germany – 2, France – 6) of Europe were obtained from the collection of genetic wheat resources of the Gene Bank of Slovak Republic in Piešťany.

Protein extraction and SDS – PAGE

For the isolation of seed storage proteins were used endosperm of intact, dry and mature single seeds. Seed homogenization of one hundred individual grains from each genotype was carried out by grinding. Glutenins were extracted by standard referee method ISTA and were performed by discontinuous PAGE based on ISTA methodology (Wrigley, 1992) using the electrophoretic unit Protean II (BioRad). Protein fractions were stained by Coomassie Brilliant Blue R – 250. The nomenclature of Payne and Lawrence (1983)was used to identification of the separate gluten subunits.

Genomic DNA Isolation

DNA of 24 genotypes of wheat was extracted from the endosperm of intact, dry and mature single seeds using the Gene JET Plant Genomic DNA Purification Mini Kit.

RAPD Analysis

Amplification of RAPD fragments was performed according to Cifri and Yagdi (2012) using decamer arbitrary primers (Operon technologies Inc, USA; SIGMAD, USA). Amplifications were performed in a 25 µl reaction volume containing 5 µl DNA (100 ng), 12.5 µl Master Mix (Genei, Bangalore, India), and 1 µl of 10 pmol of primer. Amplification was performed in a programmed thermocycler (Biometra, Germany) with initial denaturation at 94 °C for 3 min, 40 cycles of denaturation at 94 °C for 30 sec, primer annealing at 38 °C for 1 min, extension at 72 °C for 2 min, and final extension at 72 °C for 10 min. Amplified products were separated in 1.2 % agarose in

1× TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system Grab-It 1D for Windows.

Data analysis

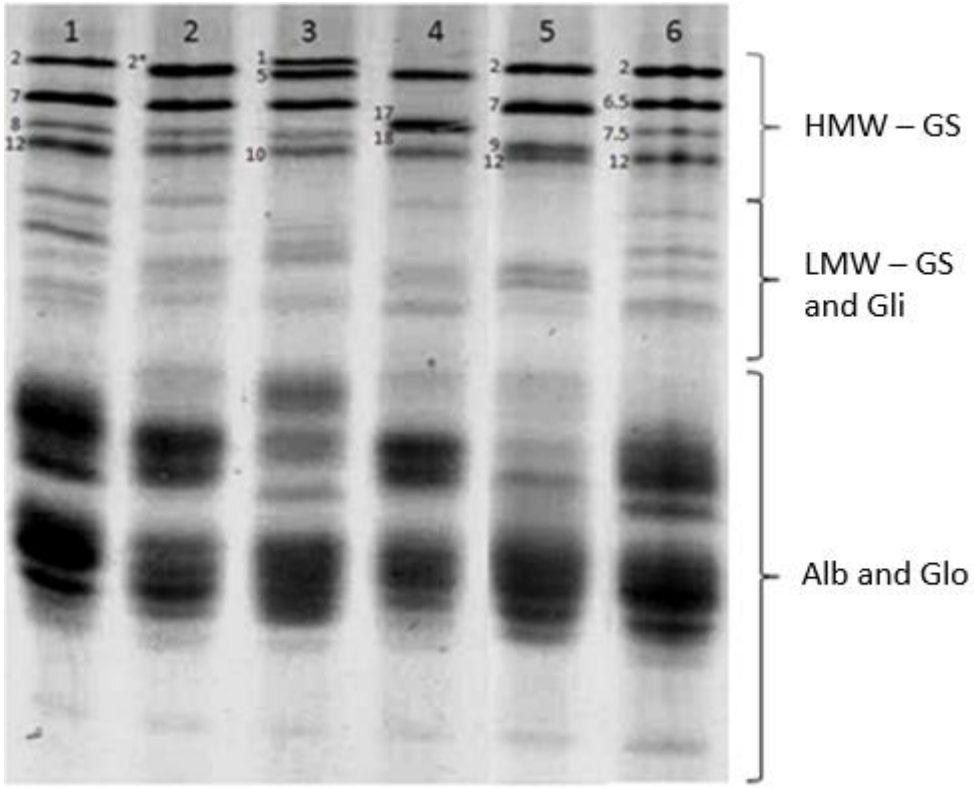
The SDS – PAGE and RAPD bands were scored in a binary form as presence (1) or absence (0), each of which was treated as an independent character regardless of its intensity. SPSS professional statistics version 17 software package was used to calculate Jaccard´s coefficient (Aida et al., 2007). A dendrogram based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) was also constructed with the SPSS software (version 17).

Results and discussion

SDS – PAGE

The characterization of cultivars based on molecular genetic markers are regarded as an important requirement for the modern seed industry (Masood et al., 2004). The efficiency of genetic gain by selection can be improved if the patterns of genetic diversity within a population of breeding lines are known. Genetic similarity or distance estimates among genotypes are helpful in the selection of parents to be used in the breeding programs (van Becelaere et al., 2005).

For describing the genetic structure of crop germplasm, protein electrophoresis is a useful method (Ciaffi et al., 1993). Storage proteins in endosperm of wheat grain represent over 80% of the whole quantity of proteins in grain and are soluble in 70% ethanol. Information about the extent and nature of genetic diversity within a crop species is essential for an effective breeding program. SDS – PAGE showing the protein banding patterns of wheat lines are given in Figure 1. The protein patterns of the genotypes were inspected visually and compared with each other. A total of 31 bands were scored among the twenty – four wheat lines giving 63.2% polymorphism. Maximum polymorphism (95.8%) was observed in HMW – GS proteins due to identification of novel HMW – GS pair on chromosome 1B (6.5+7.5) in French genotype Bagou. Minimum level of polymorphism (25%) was found in albumin and globulin proteins. Because it is difficult to identify LMW – GS variation using total protein extracts by SDS – PAGE because LMW – GS have molecular weights close to that of the monomeric gliadin storage proteins (Ahmed et al., 2010).



HMW – GS – high molecular glutenin subunits, LMW – GS low molecular glutenin subunits, Gli – gliadins, Alb – albumins, Glo – globulins

HMW – GS – vysokomolekulárne glutenínové podjednotky, LMW – GS – nízkomolekulárne glutenínové podjednotky, Gli – gliadíny, Alb – albumíny, Glo – globulíny

1 – Chinese Spring (marker), 2 – Ilona (SVK), 3 – GK David (HUN), 4 – Bonpain (FRA), 5 - Iris (SVK), 6 – Bagou (FRA)

Figure 1 SDS – PAGE profiles of chosen wheat genotypes

Obrázok 1 SDS – PAGE vybraných genotypov pšenice

The HMW – GS of wheat proteins are quantitatively minor, but functionally an important group of gluten proteins in the process of bread making. Twelve different Glu – 1 encoded allelic variants were identified among these 24 genotypes resulting from combination of 3 alleles of Glu – 1A, 7 of Glu – 1B and 2 of Glu – 1D loci (Table 1). Also one novel allelic variant (6,5+7,5) at the Glu – 1B locus was identified. All three allelic variants were detected at the Glu – 1A, the most frequent allele was Glu – 1A0 (null allele) in 20 lines (83%), Glu – 1A1 were found in 3 lines (13%) and Glu – 1A2* was found in one line (3%). High polymorphism of glutenin proteins was observed at the locus Glu – 1B, where alleles 20 and 7 and allelic pairs 6+8, 7+8, 7+9 and 17+18 were observed. For the Glu – 1B locus, the allele Glu – 1B 7+9 was the most frequent (46%). The new allele at the Glu – 1B was found in France cultivar Bagou. The existence of two alleles at the locus Glu – 1D was revealed; in fact 79% of them showed the subunit pairs 5+10 correlated with good bread – making properties. Based on HMW – GS variations Glu – score was calculated. Data showed

that glu – score varied within an interval from four to nine. The most frequent value of Glu – score was 8 (39%), which means middle bread making quality.

Table 1 Complete review of specific electrophoretic protein profiles within 24 wheat cultivars

Tabuľka 1 Kompletný prehľad elektroforetických profilov špecifických proteínov 24 genotypov pšenice

Genotype	Origin	Glu-A1	Glu-B1	Glu-D1	Glu-score
Altos	DEU	0	17+18	5+10	8
Bagou	FRA	0	6.5+7.5	2+12	-
Balthazar	FRA	1	6+8	2+12	6
Banquet	CZE	0	7+8	5+10	8
Boka	CZE	0	7+9	5+10	7
Bonita	SVK	0	7+9	5+10	7
Bonpain	FRA	0	17+18	5+10	8
Bruneta	CZE	0	7+9	5+10	7
Ebi	CZE	0	7+8	5+10	8
Elephant	FRA	0	7	2+12	4
GK Garaboly	HUN	2*	7+9	5+10	9
GK Rába	HUN	0	7+9	5+10	7
Hana	CZE	0	7+8	5+10	8
Ibis	DEU	0	7+9	5+10	8
Insegrain	FRA	0	7+8	5+10	8
Iris	SVK	0	7+9	2+12	5
Ilona (K)	SVK	2*	7+9	5+10	9
Kalif	FRA	0	20	5+10	6
Malyska	SVK	0	6+8	5+10	6
MV Optima	HUN	2*	7+9	5+10	9
Samanta	CZE	0	7+8	5+10	8
Simila	CZE	0	7+8	5+10	8
Stanislava	SVK	0	7+9	5+10	7
Verita	SVK	0	7+9	2+12	5

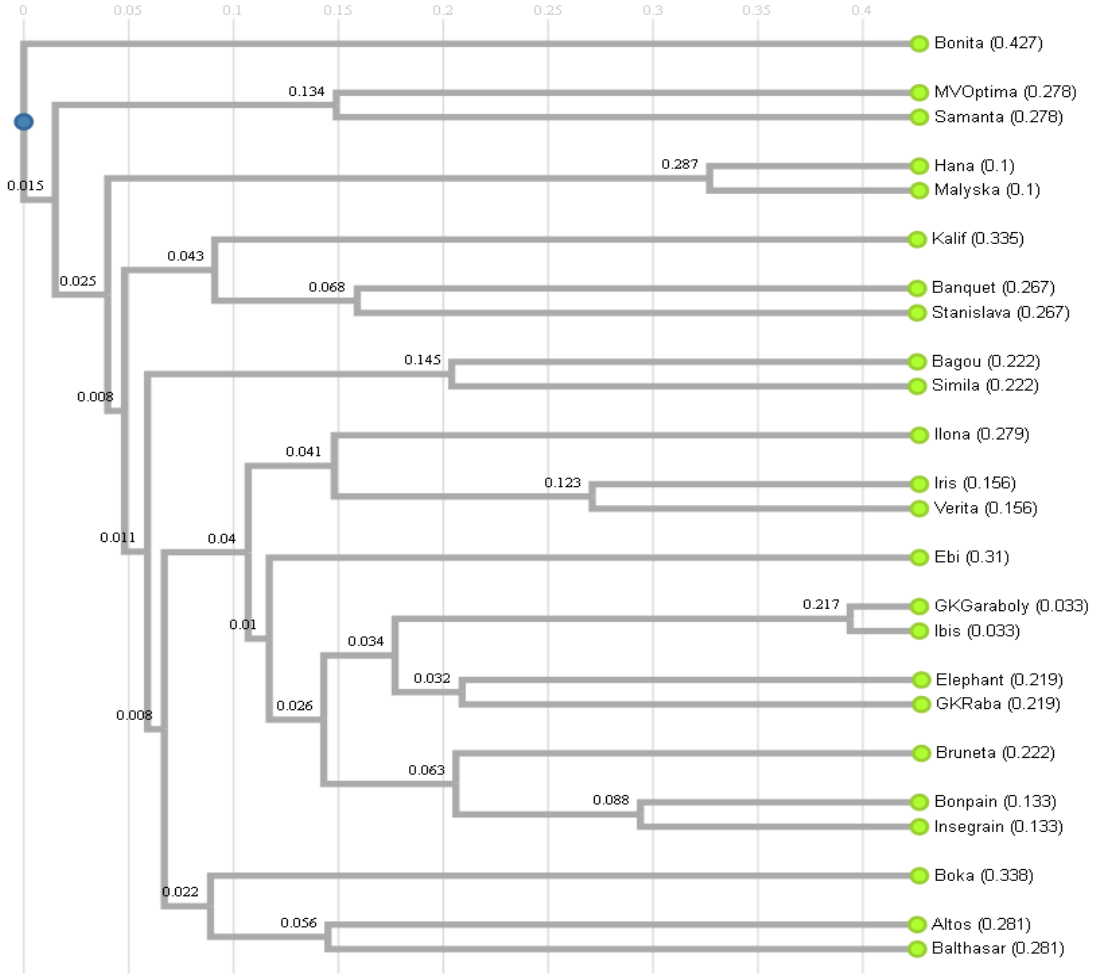


Figure 2 Dendrogram of 24 wheat genotypes prepared based on SDS – PAGE data

Obrázok 2 Dendrogram 24 genotypov pšenice pripravený na základe SDS – PAGE dát

Genetic similarity based on Jaccard´s coefficient ranged from 0.067 (MV Optima and Kalif) to 0.933 (GK Garaboly and Ibis). A dendrogram based on UPGMA analysis (Figure 2) separated unique genotype Bonita (cluster I) from other 23 genotypes (cluster II), which also showed the lowest similarity indexes. The second cluster included three subclusters, two of them at 0.04 coefficient level and the third one at 0.149 coefficient level. The third subcluster contained only two genotypes (MV Optima and Samanta). These results show that protein patterns obtained by SDS – PAGE may be used as a sufficient tool for differentiation of the studied cultivars by comparing the amount and quality of protein bands.

RAPD – PCR: Different methods are available for analysis of genetic diversity among germplast accessions. Molecular methods, such as RAPD – PCR analysis, offer a valuable opportunity to characterize genetic variation and structure in plant population (Ayana and Bekele, 2000). In this study, DNA was successfully extracted

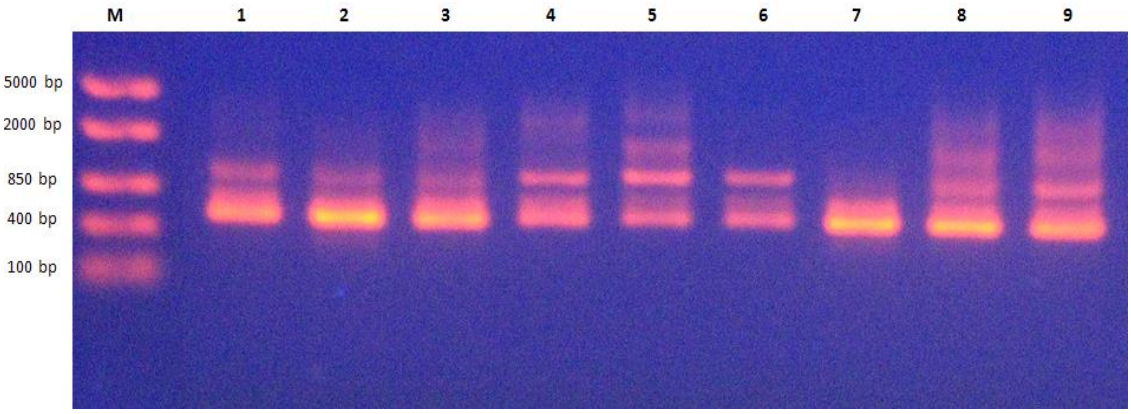
from all samples. PCR amplification of DNA using 12 decamer primers (Table 2) for RAPD analysis produced 63 DNA fragments, 56 of which were polymorphic. The number of fragments for these primers ranged between 2 and 9 with size ranging from 300 bp to 3000 bp. An electrophorogram showing patterns from some genotypes is given in Figure 3. The average number of polymorphic bands per primer was 4.67 and the average of polymorphism degree was 37.56%. Maximum level of polymorphism (55.8%) was observed for the primer OPA – 03 and minimum one for OPD – 08 (13.8%).

Table 2 Banding patterns and amplifications of 24 wheat lines using selected 12 RAPD primers

Tabuľka 2 Základné informácie o vybraných 12 RAPD primeroch a získaných produktoch u 24 línii pšenice

No.	Primer code	Primer sequence (5´- 3´)	No. of amplified bands	No. of polymorphic bands	Degree of polymorphism (%)
1	OPA – 02	TGCCGAGCTG	5	5	33.3
2	OPA – 03	AGTCAGCCAC	5	5	55.8
3	OPA – 13	CAGCACCCAC	4	3	30.2
4	OPB – 08	GTCCACACGG	3	2	47.2
5	OPD – 02	GGACCCAACC	3	3	30.5
6	OPD – 07	TTGGCACGGG	6	4	34.0
7	OPD – 08	GTGTGCCCCA	3	2	13.8
8	OPE – 07	AGATGCAGCC	5	5	48.3
9	RLZ 6	GTGATCGCAG	10	9	41.7
10	RLZ 7	GTCCACACGG	5	4	32.5
11	RLZ 8	GTCCCGACGA	7	7	40.5
12	SIGMA –D – P	TGGACCGGTG	7	7	44.0
		Total	63	56	-
		Average	5.25	4.67	37.56

Genetic similarity based on Jaccard´s coefficient ranged from 0.396 (Bagou and Bruneta) to 0.805 (Bagou and Ilona). A dendrogram based on UPGMA analysis (Figure 4) also separated unique genotype Bonita (cluster I) from other 23 genotypes (cluster II) as well as using SDS – PAGE analysis. The second cluster included two subclusters at 0.008 coefficient level. The first subcluster comprised from ten genotypes included Bagou and one separated genotype Iris. The second subcluster contained eleven genotypes and one separated genotype Ebi.



Lane M – Thermo Scientific FastRuler Middle Range DNA Ladder, 1 – Banquet (CZE), 2 – Kalif (FRA), 3 – Bonpain (FRA), 4 – Verita (SVK), 5 – Hana (CZE), 6 – MV Optima (HUN), 7 – Balthasar (FRA), 8 – Bagou (FRA), 9 – Ilona (SVK)

Figure 3 PCR amplification products of nine wheat genotypes with RLZ 7 primer:

Obrázok 3 PCR amplifikované produkty deviatich genotypov pšenice použitím primeru RLZ 7

The two genetic marker systems used in this study were compared using various parameters like percentage polymorphism, similarity indexes and clustering of the genotypes. The total number of polymorphic bands detected by 12 RAPD primers (63) was much higher than SDS – PAGE (31) however the results in this study suggested that SDS – PAGE markers were superior to RAPD markers for assessing genetic diversity. The possible reason for the higher polymorphism of storage proteins obtained for SDS – PAGE in the present study could be due to relatively high genetic diversity of HMW – GS.

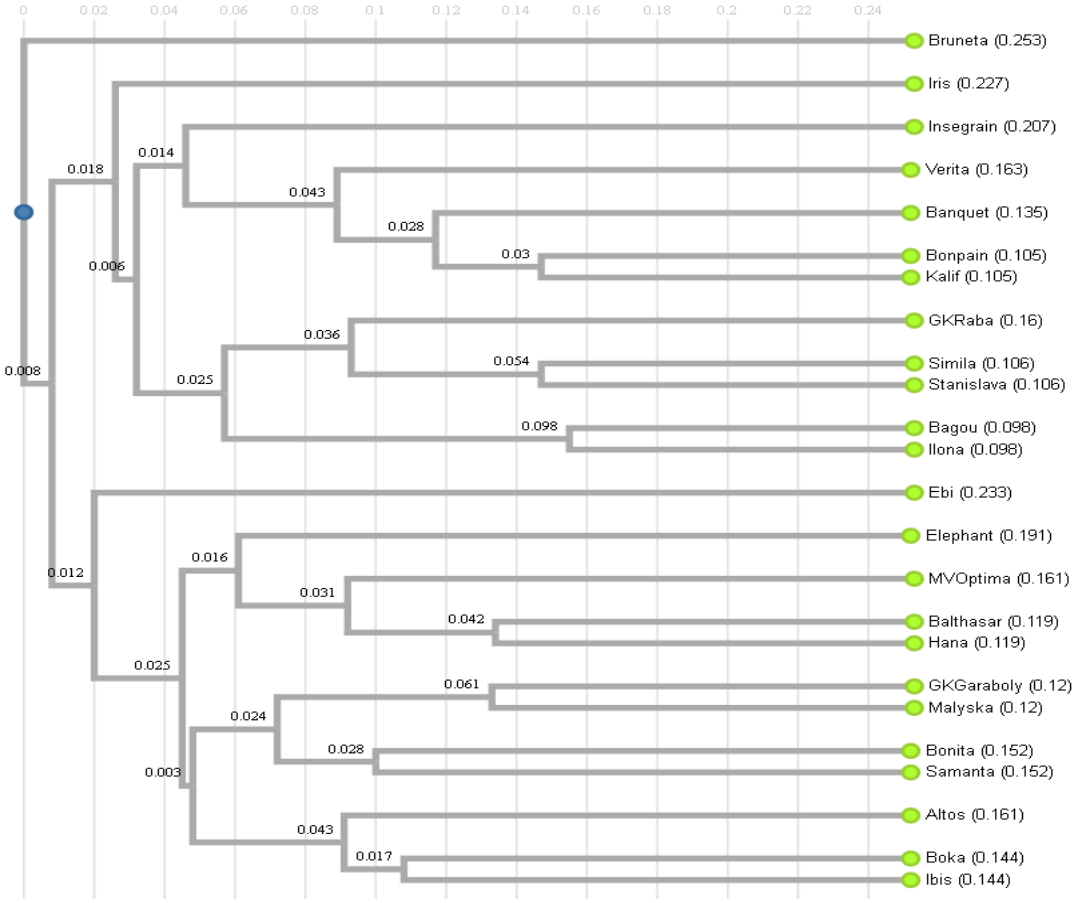


Figure 4 Dendrogram of 24 wheat genotypes prepared based on RAPD – PCR data

Obrázok 4 Dendrogram 24 genotypov pšenice pripravený na základe RAPD – PCR dát

Different kinds of electrophoretic methods based on seeds storage protein patterns have been used for the identification and characterization of crop cultivars (Chňapek et al., 2013, 2014; Gregová et al., 2000; Kuťka Hlozáková et al., 2015; Oslovičová et al., 2010;). Some previous studies based on protein profile demonstrated that numbers of cultivars are indistinguishable (Marchylo and LaBerge, 1981; Shewry et al., 1978), because of that Ahmed et al. (2010), Fouda et al. (2011) and Shehata (2004) used also DNA marker such as RAPD to differentiate wheat genotypes. Further Tahir (2014) used these methods to differentiate barley varieties and for assessment of genetic diversity in cowpea. Several of them reported higher percentage of diversity using RAPD – PCR technique than using SDS – PAGE. However, Ahmed et al. (2010) reported higher percentage of diversity using seed storage protein, which consists with these results.

Conclusion

Seed storage protein as well as RAPD molecular markers can be useful for the establishment of phylogenetic relationships among a collection of wheat genotypes. They will not be only helpful in the development of wheat cultivars with wider genetic base but will also generate patterns of them.

Collection of analyzed European wheat genotypes reported various technological quality based on Glu – score, some of which can be a good source of genes of interest for MAS breeding.

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