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GENETIC DIVERSITY OF CZECHOSLOVAK ORIGIN RYE VARIETIES (*SECALE CEREALE* L.) BASED ON PROTEIN POLYMORPHISM

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ABSTRACT

The aim of our study was to evaluate the electrophoretic profiles of storage proteins of seven genotypes of rye (*Secale cereale* L.), which were obtained by polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE). Electrophoretic separation of storage proteins was conducted according to the methodology recommended by an international organization ISTA, with some own modifications. Results showed, that all analyzed materials were multiline and there were from 4 (Valtické) to 9 (Keřkovské and Zenit) lines. Content of high molecular weight glutelin subunits varied from 6.67 % (variety České) to 10.86 % (variety Valtické). The highest proportion of storage proteins with low molecular weight glutelin subunits and prolamins was detected in Keřkovské (69.89 %) and the lowest one in variety Tešovské (61.96 %). The highest percentage of albumins and globulins associated with high nutritional value of grain was detected in variety Tešovské (31.02%) and lowest one in variety Valtické (20.97 %). The Jacquard coefficient of similarity and UPGMA algorithm was used for construction of dendrogram of similarity.

Keywords: rye, SDS-PAGE, polymorphism of proteins, HMW, LMW, genetic variability

INTRODUCTION

Rye (*Secale cereale* L.) is mainly a European cereal with 75% of the global production growing in Russia, Belarus, Poland, Germany and Ukraine. It has the best overwintering ability and the highest tolerance to drought, salt and aluminium stress of all small-grain cereals. Harvest is used for bread making, feed and in growing demands for ethanol and biomethane production as a renewable energy source **(Carena, 2009)**. The genus *Secale* L. (Triticeae; Poaceae), the tertiary gene pool of wheat, has shown great potential to be a valuable genetic resource to increase the genetic variability and to introduce desirable genes/characters for wheat improvement **(Shang *et al.*, 2005; Jiang *et al.*, 2010)**.

Storage proteins account for about 50 % of the total protein in mature cereal grains and have important impacts on their functional properties in food processing **(Shewry and Halford, 2002)**. Cereal storage proteins are characterized by high polymorphism. Their utilization as markers suitable for identification and differentiation of genotypes variability in comparison to other markers of genetic variability has many advantages. Methods based on storage proteins analysis are not extremely costly in terms of material and instrumentation compared to the detection of polymorphism by DNA markers **(Vyhnánek *et al.*, 2005; Chňapek *et al.*, 2013)**.

Progress has been achieved in biochemical extraction and characterization of the prolamin fractions (secalins) in cultivated rye **(Field *et al.*, 1982; Tantam and Shewry 1991; Gellrich *et al.*, 2003)**. SDS-PAGE analysis revealed that the prolamin fractions of rye contained four storage protein types (i.e., HMW, γ–75k, γ–40k and ω-secalins) **(Shewry *et al.*, 1983; Shang *et al.*, 2005)**. HMW secalins are homologous with HMW subnits of wheat glutenin and ω-secalins are homologous with the ω 1,2 type of wheat gliadins **(Köhler and Wieser 2000; Gellrich *et al.*, 2003).** SDS-PAGE showed two types of γ-secalins with molecular weights of 40 000 (γ-40k) and 75 000 (γ-75k) **(Kasarda *et al.*, 1983)**.

MATERIAL AND METHODS

We analyzed seven Czechoslovak genotypes of rye (*Secale cereale* L.), which we obtained from the Gene Bank of Slovak Republic in Piešťany and Gene Bank of Czech Republic in Prag (table 1). Genotypes of rye were breeding in Czechoslovakia.

Electrophoretic separation of glutelins in SDS-PAGE was conducted by the standard reference electrophoretic method by ISTA in the presence of sodium dodecyl sulfate (SDS-PAGE) **(Wrigley, 1992)**. Storage proteins were extracted from the endosperm of mechanical homogenized grains. 10 µl of each sample was loaded into polyacrylamide gel. Electrophoresis was carried out at constant electric current 10mA, 500V, 50W for approximately 14 hours.

Electrophoreograms were coloured in the mixture containing trichloroacetic acid and Comassie Brilliant Blue R-250. Electrophoretic profiles were visualized in photo device with black and white camera with a filter and lenses. Gels were evaluated using documentation and evaluation system Doc-It LS Image analysis UVP. We analysed fifteen grain of each variety (*Secale cereale* L.). The variety of wheat Chinese spring and Marquis were used as standards. The DocIt-LS software was used for statistical interpretation of the eletrophoreograms, i.e. by calculation of Jacquard coefficient of similarity and elaboration of a dendrogram.

RESULTS AND DISCUSSION

Rye is important for breeding purposes and for gene introgression in other cereal species like wheat, as a source of favourable agronomic traits. Features such as nutrient efficiency, tolerance of diseases, allowing a reduced usage of pesticides and fertilizers during production.

Rye proteins are important for rye bread-making quality, namely in the dough-mixing step. Some rye varieties show highly different bread-making quality and this can be attributed to significant in the content and structure of starch and proteins, which are influenced by harvest year and genotype **(Hansen *et al.*, 2003, 2004; Ribeiro *et al.*, 2012).**

Nutritional and technological quality of grain is a complex variable related to the chemical composition of grain especially with the percentage representation of individual protein fraction which determines the direction of using grains (rye – baking process, pharmaceutical use) **(Chňapek *et al.*, 2010)**.

Within seven Czechoslovak genotypes, high molecular weight glutelin subunits (HMW-GS), low molecular weight glutelin subunits (LMW-GS) and prolamins, the residual albumins and globulins were identified by electrophoretic spectrum (Figure 1).

The content of HMW-GS varied in analyzed collection of rye from 6.67 % (variety České) to 10.86 % (variety Valtické) with the average 8.73 % (Table 1). Number of HMW-GS bands was from 1 to 5. Research conducted by **Michalík *et***

***al.* (2006)**, also evaluated the presence of HMW subunits in rye collection. In his study states 7.58 % share of the subunits.

The proportion of LMW-GS subunits and prolamins in our samples was in average 66.78 %. The largest amount of LMW-GS subunits and prolamins was observed in variety Keřkovské (69.89 %) and the lowest one was detected in variety Tešovské (61.96 %). The number of bands of low molecular weight subunits and prolamins varied from 11 to 16 (Table 1). These results are in accordance with other authors **Palenčárová and Gálová (2009)** and **Petr (2008)**, who confirmed our results.

Table 1 Contents of protein electrophoretic subfractions in rye genotypes

Variety	Country of Origin	Multiline	HMW-GS (%)	LMW-GS + prolamins (%)	alb + glo (%)
Valtické	**CSK**	**4**	**10.86**	**68.17**	**20.97**
Tešovské	**CSK**	**8**	**7.02**	**61.96**	**31.02**
Keřkovské	**CSK**	**9**	**8.69**	**69.89**	**21.42**
Zenit	**CSK**	**9**	**8.80**	**68.82**	**22.38**
Chlumecké	**CSK**	**8**	**8.67**	**67.01**	**24.32**
České	**CSK**	**6**	**6.67**	**64.12**	**29.20**
Albedo	**CSK**	**8**	**10.39**	**67.52**	**22.08**
x		***7.42***	***8.72***	***66.78***	***24.48***
σ (%)		***1.93***	***1.55***	***2.78***	***4.01***
V (%)		***26.00***	***17.79***	***4.17***	***16.41***

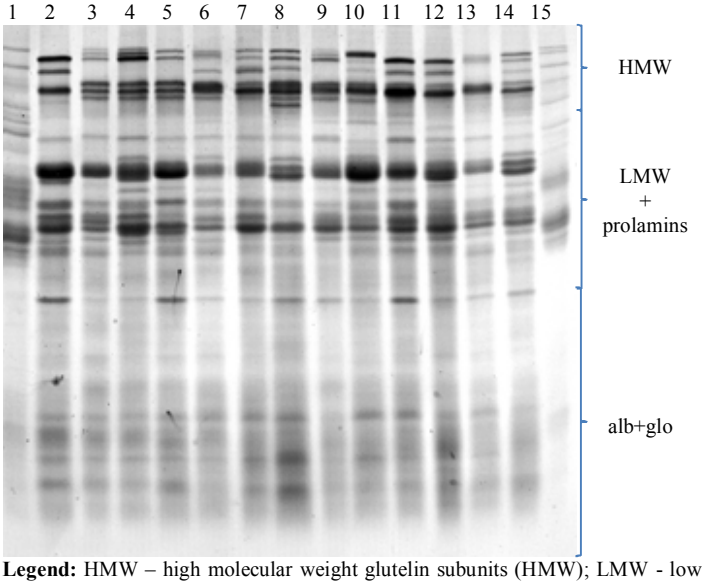
Legend: CSK – Czechoslovakia; **HMW-GS (%) -** high molecular weight glutelin subunits; LMW-GS (%) - low molecular weight glutelin subunits; alb + glo (%) – albumins and globulins; **x - average;** σ (%) - standard deviation; **V (%) -** *coefficient of variation*

Most of studies dealt with the prolamins, that normally represent more than half of the kernel proteins and are deficient in certain essential amino acids, especially lysine. The soluble proteins, albumins and globulins, which may represent up to one fourth of the kernel proteins and close to one half of the total lysine, have received less attention, in spite of their nutritional relevance (**Singh and Skerritt, 2001**).

Our results showed that an average representation of residual albumins and globulins was 24.48 %. The highest content of albumins and globulins and thus the highest nutritional value were observed in variety Tešovské (31.02 %) overleaf in variety Valtické (20.97 %) the lowest part of this fraction was detected (Table 1). Number of bands of residual albumins and globulins in our analyzed group was from 6 to 10. Several researchers have addressed the storage proteins of rye issue such as **Shewry *et al.* (2002), Gellrich *et al.* (2003), Shang *et al.* (2005), Horszwald *et al.* (2009)**.

The multiline was detected by comparing of high molecular weight subunits, low molecular weight subunits, albumins and globulins in analyzed Czechoslovak collection of rye. We recorded 4 lines in genotype Valtické, 6 lines in genotype České, 8 lines in Albedo, Tešovské and Chlumecké, 9 lines in genotypes Keřkovské and Zenit.

Figure 1 Electrophoretic spectrum of rye storage proteins in SDS-PAGE



Legend: HMW – high molecular weight glutelin subunits (HMW); LMW - low molecular weight glutelin subunits (LMW) and prolamins; alb + glo - albumins and globulins; 1- wheat Marquis; 15 – wheat Chinese Spring; 2-14 variety Chlumecké

Application of UPGMA algorithm in dendrogram construction the collection of rye genotypes was separated into two main clusters with dissimilarity 0.904. Each of main clusters was divited into two subclusters with dissimilarity 0.882 and 0.885 respectively. Cluster with the largest number of genotypes was divided into several subclusters with dissimilarity from 0.839 to 0.200. Into clusters with dissimilarity from 0.885 to 0.333 was classifiable smaller part of observed genotypes. Determination of the most different genotypes within clusters is very difficult from the point of view of multiline character of rye (Figure 2). This finding is in accordance with the results of other authors **(Konzun *et al.*, 2001; Chňapek *et al.*, 2013)**.

CONCLUSION

The aim of the present work was to describe profiles of storage proteins in seven genotypes of rye (*Secale cereale* L.) by SDS PAGE. The methodology by ISTA is a suitable method for differentiation of rye genotypes, while storage proteins were separated very well into to individual high molecular weight glutelin subunits, low molecular weight glutelin subunits, prolamins, albumins and globulins. Representation of HMW-GS bands was from 1 to 5, LMW-GS bands from 11 to 16 and residual albumins and globulins ranged from 6 to 10.

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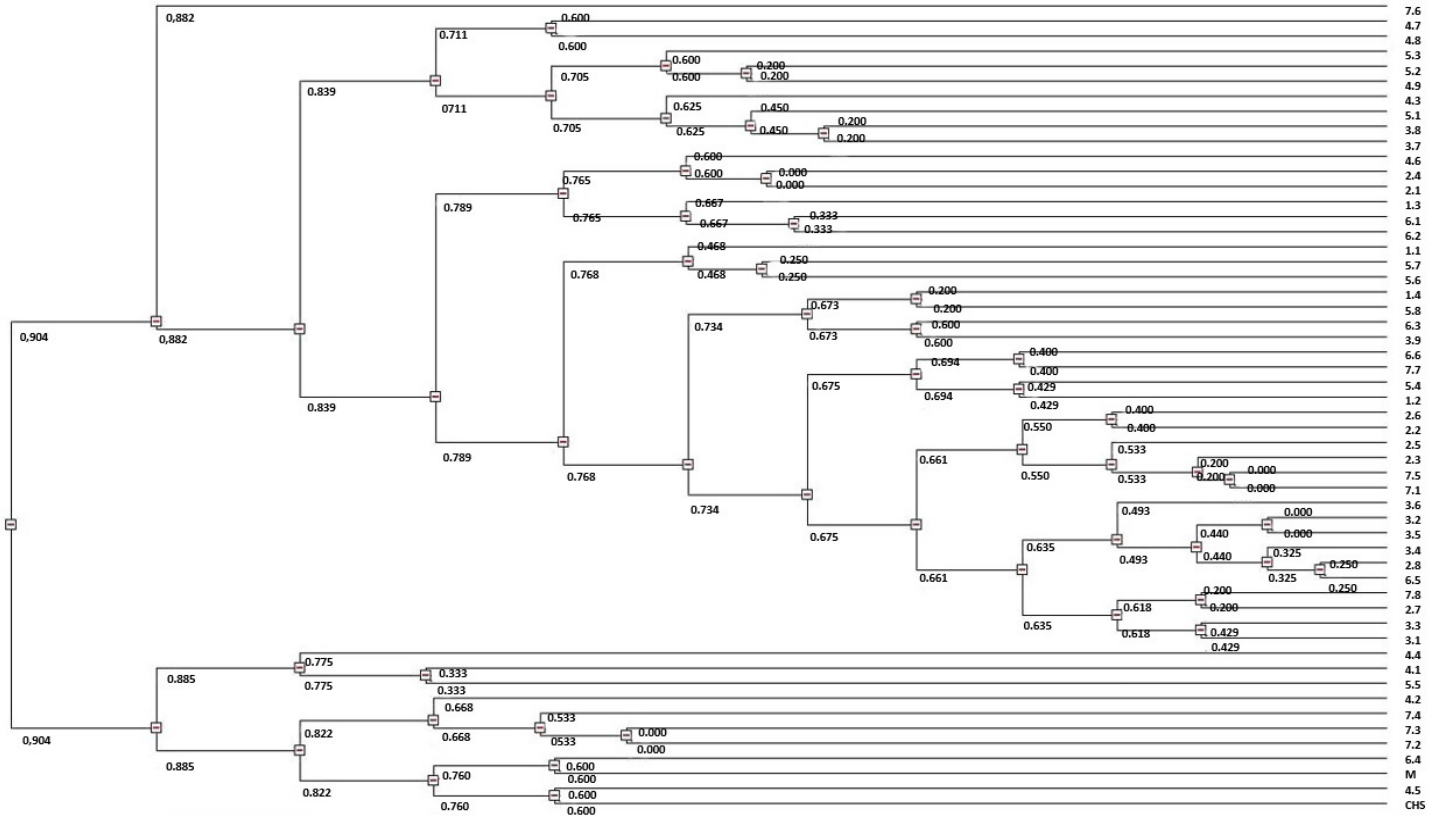
Figure 2 Dendrogram of rye storage protein polymorphism



Legend: 1.1 - 1.4 Valtické; 2.1 - 2.8 Tešovské; 3. 1. - 3.9 Keřkovské; 4.1 - 4.9 Zenit; 5.1 - 5.8 Chlumecké; 6.1 - 6.6 České; 7.1. - 7.8. Albedo; CHS - Chinese spring; M - Marquis

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