



PROTEIN POLYMORPHISM IN GENETIC RESOURCES OF RYE USING SDS-PAGE

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ABSTRACT

The aim of this work was to evaluate protein's profile in 18 genotypes of rye (*Secale cereale* L.) on the basis electrophoretic spectrum of storage proteins in SDS PAGE. Content of HMW was 7.43 %, LMW 68.69 % and the content of residual albumins and globulins were 23.86 %. In samples of rye, there were detected multiline, whereby in collection of 18 rye genotypes (*Secale cereale* L.) we detected in five genotypes 5 lines, in further five genotypes 7 lines, in four varieties were 8 lines, in three genotypes were 6 lines and in one genotype was 4 lines.

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

INTRODUCTION

Rye is a relatively young crop and belongs to the family *Poaceae* - formerly *Gramineae* (Bushuk, 2001; Kole, 2011). It is cultivated in temperate zone of six continents, mainly in dry and colder areas at higher altitudes (Kole, 2011). Rye is our traditional cereal

utilized for food, forage, technical (ethanol) and pharmaceutical industry (ergot). Rye flour is an essential ingredient of bread, gingerbread and pasta.

Rye (*Secale cereale* L.) and wheat (*Triticum aestivum* L.) are closely related by taxonomy and their endosperm contains homologous storage proteins. The content of nutritionally important proteins (albumins, globulins) in rye is on average 55%, while in wheat around 25-30 %. In contrast to wheat, rye is unable to form gluten when flour is mixed with water, whereas content of prolamins and glutelins in the grain rye is only around 45 %, while in the grain wheat is 75 - 80 % (**Hulín et al., 2008**). The storage proteins of rye (secalins) have been studied less intensively than those of wheat (gliadins and glutenins) (**Gellrich et al., 2003**).

Storage proteins of cereal seeds are very important from the point of view germination and growth, they represent more than 50 % of the total protein content in the mature cereal seeds (**Tatham and Shewry, 2008; Cunsolo et al., 2011**). Prolamins in the endosperm storage proteins of cereal grains (mainly wheat, rye and barley), make up 30-50 % of the total protein content (**Xu and Messing, 2009; Yalcin, 2010**). The wheat prolamins are called gliadins, hordeins in barley, secalins in rye and avenins in oat (**Weber et al., 2009, Van Eckert et al., 2010**).

Storage proteins are the most used type of plant proteins for identification and differentiation of genotypes. One of the main ways studies of protein polymorphism are electrophoretic and chromatographic methods (**Jomová et al., 2009, Gálová et al., 2011**).

The aim of our work was to detect genetic variability in a collection of rye genotypes on the basis of protein polymorphism using SDS-PAGE.

MATERIAL AND METHODS

In this work, we analyzed 18 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 Praha (tab. 1). Samples come from Central European countries, e.g. from Czechoslovakia, Czech Republic and Poland.

Storage proteins were extracted from individual grains by the standard reference electrophoretic method by ISTA in the presence of sodium dodecyl sulfate (SDS-PAGE) (**Wrigley, 1992**). Electrophoreograms were coloured in the mixture containing trichloroacetic acid and Comassie Brilliant Blue R250. Electrophoretic profiles were visualized in photo device with black and white camera with a filter and lenses. Gels can be evaluated using

documentation and evaluation system Doc-It LS Image analysis UVP. We analysed fifteen-grain for each variety (*Secale cereale* L.). We used high molecular marker M-3788 (MW 36 000 – 205 000) and low molecular marker M-3913 (MW 6500-66 000) as standarts.

RESULTS AND DISCUSSION

Rye is an important crop due to its nutrient efficiency, winter hardiness, and tolerance to other environmental stresses such as drought. It is also a valuable genetic resource for wheat and triticale breeding programs. A better knowledge of genetic diversity and relationships between the existing rye inbred lines would be useful in the development of breeding programs that efficiently utilize available rye germplasm (**Bolibok et al., 2005**).

Nutritional and technological quality of cereals is evaluated primarily in terms of protein content. Nutritional quality is characterized by a high content of essential amino acids, while technological quality depends not only on the amount of proteins and their functional composition, but also particularly the proportion of individual fractions of storage proteins that related to gluten quality and its impact on the final product Rye gluten can not be washed from dough. Rye proteins are not unable to form a solid frame - dough compose from soluble pentosans with seelling-up proteins and starch grains (**Palenčárová, 2010**).

Storage proteins of analyzed rye genotypes were separated by SDS-PAGE into three different groups, namely high molecular weight subunits (HMW), low molecular weight subunits (LMW-GS) and the residual albumins and globulins. Content HMW was detected in the range of 5.41 % to 9.68 % with an average 7.43 %. The largest percent representation of HMW subunits we proved in Czechoslovak variety Židlochovický Panis (9.68 %) and the lowest part of HMW subunits was detected in Czech variety Aventino (5.41 %). Number of bands of high molecular weight subunits was from 1 to 4, whereby the molecular weight was in the range 145,000 to 102,000 Da.

Petr et al. (2008) used SDS-PAGE for detection of storage proteins and they detected similar proportion of high molecular subunits 6.3 %. Comparable results of HMW subunits in rye (*Secale cereale* L.) detected also **Palenčárová (2010)**. The average content of HMW subunits was 7,98 % whereas they detected HMW subunits from 5.41 % to 9.48 %.

In the second part of polyacrylamid gel, low molecular subunits were separated, which variability ranged from 59.31 to 76.65 % and molecular weight was from 95,000 to 28,000 Da.

Table 1 Contents of protein electrophoretic subfractions in rye genotypes

Variety	Country of Origin	HMW (%)	LMW + prolamins (%)	alb + glo (%)
Židlochovický Panis	CZK	9.68	69.87	20.43
Nalžovské	CZK	9.18	70.1	20.69
Dobrovické	CZK	8.37	71.6	20.03
Víglášske	CZK	7.0	70.9	22.09
Ratbořské	CZK	7.48	70.1	22.42
Laznické	CZK	7.96	65.26	26.78
Breno	CZK	5.72	64.05	30.23
Dobřenické krmné	CZK	7.06	65.8	27.13
Aventino	CZ	5.41	59.31	35.28
Selgo	CZ	6.46	63.6	29.93
Warko	PL	8.12	76.65	15.23
Dankowskie Zlote	PL	8	71.56	20.44
Zduno	PL	7.9	69.52	22.57
Motto	PL	5.83	68.5	25.65
Pancerne	PL	8.27	75.78	15.94
Wojcieszycie	PL	6.78	66.7	26.52
Universalne	PL	7.89	68.15	23.95
Dankowskie Nowe	PL	6.67	69.01	24.31
X		7.43	68.69	23.86
Max		9.68	76.65	35.28
Min		5.41	59.31	15.23
σ (%)		1.26	4.75	5.63
V (%)		17.07	6.924	23.60

CZK – Czechoslovakia; CZ - Czech Republic; PL - Poland; HMW (%) - high molecular weight glutenin subunits (HMW-GS); LMW (%) - low molecular weight glutenin subunits (LMW-GS); alb + glo (%) – albumins and globulins; x - average; σ (%) - Standard deviation; V (%) - coefficient of variation

In our samples percent content of LMW subunits and prolamins was 68.69 %. The highest representation of LMW, we detected in the Polish variety Warko (76.65 %), on the other hand the lowest part LMW subunits we found, in Czech variety Aventino (59.31 %). The number of bands of low molecular weight subunits and prolamins was from 12 to 17.

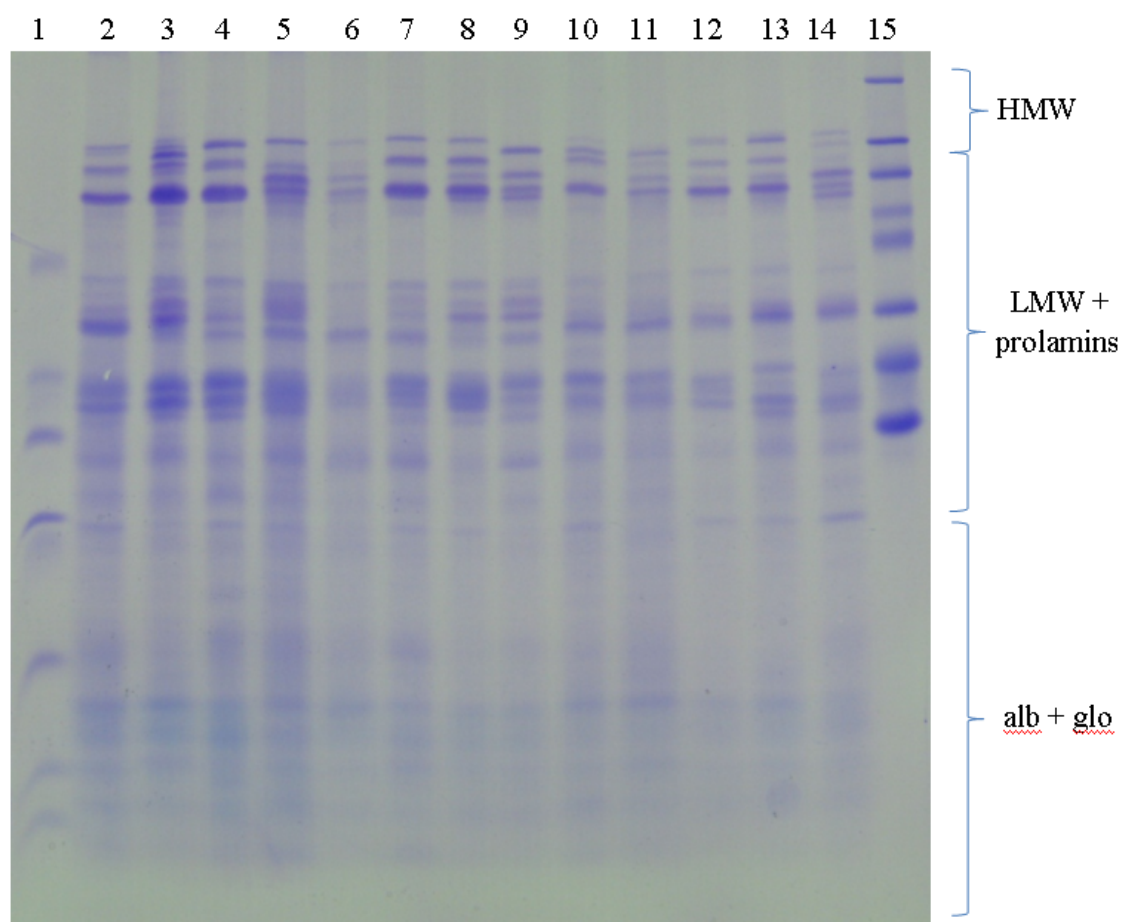
Petr et al. (2008) used electrophoretic analysis of storage proteins (SDS-PAGE) in the different collection of rye part LMW and prolamins in average 68.1 %, what is in accordance

with our results. Also **Michalík et al. (2006)** and **Palenčárová (2010)** analysed contents of LMW subunits and prolamins in rye (74 % and 62.30 %).

In the collection of rye (table 1) the average contents of albumins and globulins reached 23.86 %. Our results revealed that the the highest representation of albumins and globulins had Czech variety Aventino (35.28 %) and the lowest proportion was found in the Polish variety Wargo (15.23 %). Number of bands of albumins and globulins was from 6 to 10, whereby the molecular weight ranged from 29,000 to 7,000 Da. Nutritional quality of grain is affected by many others factors e.g. representation of albumins and globulins, that have the best composition of amino acids, because of the highest amount of essential amino acids (**Chňápek et al., 2010**).

Others authors **Gálová et al. (2003)**, **Petr et al. (2003)**, **Palenčárová and Gálová (2009)** state, that contents of the individual fractions of proteins complex and their amount is very variable and varies according to conditions of cultivation genetic traits and also in the process of ripening grain.

In samples of rye we have recorded multiline varieties by comparing of high molecular weight subunits, low molecular weight subunits, albumins and globulins. We have detected in rye genotypes Dobřenické krmné, Aventino, Selgo and Universalne 8 lines, in genotypes Nalžovské, Víglášské, Pancerne, Wojcieszycie and Dankowskie Nowe 7 lines, in genotypes Židlochovický Panis, Dobrovické and Zduno 6 lines, in genotypes Laznické, Breno, Warko, Dankowskie Zlote and Motto 5 lines, and in genotype Ratbořské 4 lines. Our results also confirmed detections of other authors, who found out multiline of rye varieties (**Shewry et al., 1983; Kubiczek et al., 1993**).



HMW -high molecular weight glutenin subunits (HMW-GS); LMW - low molecular weight glutenin subunits (LMW-GS) and prolamins; alb. + glo. - albumins and globulins. 1 - low molecular marker M-3913 (MW 6500-66 000), 15 - high molecular marker M-3788 (MW 36 000 - 205 000), 2 - 13 genotyp Woicieszyckie.

Figure 1 Electrophoretic spectrum of storage proteins in SDS-PAGE

CONCLUSION

The aim of our work was to evaluate storage protein profiles in 18 genotypes of rye (*Secale cereale* L.) by SDS PAGE. On the basis of electrophoretic spectrum of high molecular weight subunits, low molecular weight subunits and residual albumins and globulins, we found multiline in analyzed groups rye, and the number of lines was from 4 (Ratbořské) to 8 (Dobřenické krmné, Aventino, Selgo and Universalne). Content of HMW subunits was from 1 to 4, LMW subunits from 12 to 17 and residual albumins and globulins ranged from 6 to 10.

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