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SEPARATION OF RYE SECALINS BY A-PAGE

Petrovičová Lenka*, Balážová Želmíra, Vivodík Martin, Gálová Zdenka

Department of Biochemistry and Biotechnology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Nitra, Slovak Republic

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The aim of our study was to evaluate the electrophoretic profiles of secalins of fifteen genotypes of rye (*Secale cereale* L.), which were obtained by polyacrylamide gel electrophoresis in the presence at pH 3.1 (A-PAGE). Electrophoretic separation of storage proteins was conducted according to the methodology recommended by an international organization ISTA, with some own modifications. Fractions from the preparative separation were pooled in such a way that no components from one pool were present in the others. Doc-It LS software was used to detect and to calculate variability of genotypes within individual rye species.Preparative electrophoresis at low pH allowed a simple separation of γ75k-secalins, ω-secalins and γ40k-secalins from the crude material under non-denaturing conditions. The content of γ75k-secalins varied in analyzed collection of rye from 22.86% (variety Čerkascanka tetra) to 53.93% (genotype Valtické) with an average 39.27%. The proportion of ω-secalins in our samples was in an average 39.27%. The largest percent representation of ω-secalins was proved in variety České (58.68%) and the lowest part of this subunits was detected in variety Radomske (25.83%). Our results showed that average representation of γ40k-secalins was 22.05%, with the highest content in variety Vígľašské (32.44%) overleaf with the lowest part of this fraction was detected in Valtické (8.28%). The variety of wheat Chinese spring and Marquis were used as standards. Storage proteins consist of three fractions, which are the main part of grain proteins and are used as a marker not only for genetic variability investigation, but also for characterization of genotypes. These pooled fractions could be used as starting material for single polypeptide purification.

Keywords: cereals; *Secale cereale*; electrophoresis; A-PAGE; secalins

Introduction

Rye (*Secale cereale* L) is a traditional cereal in Central, Eastern and Northern Europe, where it is used for the production of bread and crispbread and as fodder (Hansen et al., 2003). The cereal proteins contribute to the nutritional value of the diet and they are integral and fundamental part of food components. Nutritionally, they are the good source of energy and its amino acids are essential for growth and maintenance (Horszwald et al., 2009). Functionally, they affect the physicochemical and sensory properties of various foods. Recently published data indicate that rye and rye-based products (including breads) are a good source of lignans, phytosterols and phenolic compounds that are biologically active and posses antioxidant properties (mainly they are good free-radical scavengers, reducing agents, potential complexers of prooxidant metals and quenchers of the singlet oxygen formation) (Zielinski, 2002; Michalska et al., 2007; Horszwald et al., 2009).

***Corresponding author:** Lenka Petrovičová, Department of Biochemistry and Biotechnology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Nitra, Slovak Republic, ✉ petrovicovalenka22@gmail.com

Genetic diversity has played a vital role in the success of crop improvement. Knowledge of genetic diversity has been successfully used for efficient germplasm management and utilization, genetic fingerprinting and genotype selection (FAO, 1998; Engles et al., 2002). However, there are many methods for estimating genetic diversity since they provide a simple way of quantifying genetic variation while assessing genotype performance under normal growing environments.

However, morphological traits are limited in number, modified by the environment and may be controlled by epistatic and pleiotropic gene effects (Fufa et al., 2005). The gliadin protein markers, as primary product of gene expression, are not affected by the plant growth environment and can reveal small changes (e.g. mutations) in accessible to visual examinations (Alieva et al., 2012). The objective of our work was to evaluate the electrophoretic profile secalins of rye by A-PAGE.

Materials and methodology

We analyzed five Czechoslovak, five Czech and five SUN (Union of Soviet Socialist Republics) genotypes of *Secale cereale*, which we obtained from the Gene Bank of Slovak Republic in Piešťany and Gene Bank of Czech Republic in Prague (Table 1). Secalins fractions were isolated from rye 2-chlorethanol extract. Storage proteins were extracted from the endosperm of mechanical homogenized grains. 10 µl of each sample was loaded into polyacrylamide gel. Electrophoretic separation of secalins was conducted by the standard reference electrophoretic method by ISTA (Draper, 1987). Separation was performed using analytical electrophoresis at pH 3.1 (A-PAGE). Electrophoresis was carried out at constant electric current 10 mA, 500V, 50W for approximately 14 hours.

Table 1 List of 15 rye cultivar, their country of origin and Taxone used for A-PAGE analyses

Genotype	Country of origin	Taxone
Valtické	Czechoslovakia	*S. cereale* L. subsp. *cereale* var. *cereale*
Keřkovské	Czechoslovakia	*S. cereale* L. subsp. *cereale* var. *cereale*
České	Czechoslovakia	*S. cereale* L. subsp. *cereale* var. *cereale*
Vígľašské	Czechoslovakia	*S. cereale* L. subsp. *cereale* var. *cereale*
Breno	Czechoslovakia	*S. cereale* L. subsp. *cereale* var. *cereale*
Aventino	Czech Republic	*S. cereale* L.
Selgo	Czech Republic	*S. cereale* L. subsp. *tetraploidum* KOBYL
Radomske	Czech Republic	*S. cereale* L.
České normální	Czech Republic	*S. cereale* L.
Křmne žito	Czech Republic	*S. cereale* L.
Tetra start	Union of Soviet Socialist Republics	*S. cereale* L. subsp. *tetraploidum* KOBYL
Čerkascanka tetra	Union of Soviet Socialist Republics	*S. cereale* L. subsp. *tetraploidum* KOBYL
Voschod 1	Union of Soviet Socialist Republics	*S. cereale* L. subsp. *cereale* var. *cereale*
Golubka	Union of Soviet Socialist Republics	*S. cereale* L. subsp. *cereale* var. *cereale*
Mnogokoloskaja	Union of Soviet Socialist Republics	*S. cereale* L. subsp. *cereale* var. *cereale*

Union of Soviet Socialist Republics – SUN

Electrophoreograms were coloured in the mixture containing trichloroacetic acid and Comassie Brilliant Blue R-250. Electrophoretic profiles were visualized in photo device with a 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 five grain of each variety (*Secale cereale*). The variety of wheat Chinese spring and Marquis were used as standards. The DocIt-LS software was used for statistical interpretation of the electrophoreograms.

Results and discussion

Rye is important for breeding purposes and for gene introgression in other cereal species like wheat, as a source of favorable 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 and this can be attributed to significant in the content and structure of starch and proteins, which are influences by harvest year and genotype (Hansen et al., 2003, 2004; Ribeiro et al., 2012).

Table 2 Content of protein electrophoretic subfractions in rye genotypes

Genotype	Country of origin	γ75k-secalins	ω-secalins	γ40k-secalins
Valtické	CSK	53.93	37.78	8.28
Keřkovské	CSK	39.39	43.43	17.18
České	CSK	29.60	58.68	17.71
Vígľašské	CSK	40.33	27.22	32.44
Breno	CSK	52.68	26.27	21.50
Aventino	CZ	41.46	38.79	19.74
Selgo	CZ	44.35	30.14	25.50
Radomske	CZ	53.62	25.83	20.54
České normální	CZ	25.83	47.17	27.0
Kŕmne žito	CZ	37.93	40.15	21.91
Tetra start	SUN	35.96	41.39	22.63
Čerkascanka tetra	SUN	22.86	51.56	25.57
Voschod 1	SUN	38.22	39.65	22.13
Golubka	SUN	30.67	42.58	26.74
Mnogokoloskaja	SUN	39.64	38.42	21,93
Min		22.86	25.83	8.28
Max		53.93	58.68	32.44
X		39.10	39.27	22.05
SE, %		10.13	10.17	6.49
VK, %		25.92	25.91	29.43

CSK – Czechoslovakia; CZ – Czech Republic; SUN – Union of Soviet Socialist Republics; min. – minimum; max. – maximum; *x* – note: average; σ (%)– standard deviation; VK (%)– coefficient of variation

The 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 fifteen genotypes of rye γ75k-secalins, ω-secalins and γ40k-secalins were identified by electrophoretic spectrum. The content of γ75k-secalins varied from 22.86% (variety Čerkascanka tetra) to 53.93% (genotype Valtické) with an average 39.10%. The proportion of ω-secalins in samples was in an average 39.27%. The largest amount of the subunits was observed in variety České (58.68%) and the lowest one was detected in variety Radomske (25.83%). Our results showed that average representation of γ40k-secalins was 22.05%, with the highest content in variety Vígľašské (32.44%) overleaf with the lowest part of this fraction was detected in Valtické (8.28%) (Table 2).

In report Rumbo et al. (2002), the optimization of a preparative electrophoretic method to fractionate secalins is described. Separation was performed in preparative 7% polyacrylamide gels of 4 cm length at pH 3.1. Preparative electrophoresis at low pH allowed a simple separation of γ- and ω-secalins from crude material under nondenaturing conditions.

Conclusions

The aim of the present work was to describe profiles of storage proteins in fifteen genotypes of *Secale cereale* by A-PAGE. The methodology by ISTA is a suitable method for differentiation of rye genotypes, while storage proteins were separated very well into to γ75k-secalins, ω-secalins and γ40k-secalins. In conclusion, it was shown that preparative electrophoresis at pH 3.1 is a useful methodology for the preparation of purified secalins components and for a study of breadmaking quality of rye.

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