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Polymorphism of grain storage proteins in triticale varieties registred in the Czech Republic*

Polimorfizm białek zapasowych ziarna w odmianach pszenżyta wpisanych do rejestru w Republice Czech

Analizowano polimorfizm białek zapasowych w 11 odmianach pszenżyta w Republice Czech, wykorzystując metodę PAGE ISTA (ISTA, 1999) i SDS PAGE (Laemmli, 1970). Ocena polimorfizmu białek zapasowych pozwoliła na zróżnicowanie indywidualnych odmian pszenżyta ze zbiorów uzyskanych w roku 2002 i 2003. Opierając się na współczynniku podobieństw (według Dice) wykryto, równolegle z genotypami, genotypy ze spektrum prolamin siostrzanych ze zróżnicowanym procentem uczestnictwa w poszczególnych latach. Jednolite spektrum zostało wykryte w następujących odmianach: Disco, Kolor, Lamberto, Marko, Presto, Sekundo, Ticino i Tricolor, natomiast odmiany Kitaro i Modus były formami dwuliniowymi. W 2003 roku trzy linie prolamin siostrzanych pojawiły się w odmianie Gabo, a w roku 2004 tylko dwie. W 2003 roku 5% domieszki obcego genotypu wykryto w odmianie Marko. Typowy w przypadku nieznanego genotypu był gliadin blok *Gld 1B3*, który jest markerem translokacji pszenno-żytniej T1BL.1RS. Prolaminy białek z ziarna pszenżyta są odpowiednie do wykrycia różnorodności genetycznej i oszacowania odmianowej autentyczności, a także czystości w próbkach ziaren pszenżyta zarejestrowanych w Republice Czech. W odmianach pszenżyta: Presto, Sekundo, Tricolor, Ticino, Gabo, wykryto allele *Glu* 1*A*2* i *Glu 1B 7+8*. W odmianach Kitaro, Marko i Disco stwierdzono allele *Glu* 1*A*1, *Glu* 1*B 7+8*. Heterogeniczny skład 1/2* w locus *Glu* 1*A* został wykryty w odmianach Kitaro i Sekundo (dwa ziarna w 2003 roku). W odmianach Kolor i Lamberto wykryto podjednostki HMW takie jak: *Glu 1 A0* i *Glu 1 B 7+8*. Jedynie w odmianie Modus stwierdzono allele *Glu* 1*A0* i *Glu 1B 6+8*.

Słowa kluczowe: białko zapasowe, elektroforeza, odmiana, pszenżyto

Genetic diversity was detected in 11 varieties of triticale registered in the Czech Republic by means of polymorphism of storage proteins using the PAGE ISTA method (ISTA, 1999) and SDS PAGE method (Laemmli, 1970). The polymorphism of storage proteins allowed the differentiation of the

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individual triticale varieties of the 2002 and 2003 harvests. On the basis of Dice's calculations of coefficients of similarity we discovered, in parallel with the uniform genotypes, genotypes with sister prolamin spectrums with a different percentage of participation in the respective years. A uniform spectrum was detected in the following varieties: Disco, Kolor, Lamberto, Marko, Presto, Sekundo, Ticino and Tricolor; Kitaro and Modus were dimorphous varieties. In 2003 three sister prolamin lines appeared in the variety Gabo, and in 2004 only two. In 2003 a 5% admixture of a foreign genotype was detected in the variety Marko. Typical of the unknown genotype was the gliadin block *Gld 1B3*, which is the marker of wheat-rye translocation T1BL.1RS. The prolamin proteins of triticale grain are suitable for the detection of the genetic diversity and for the assessment of varietal authenticity and purity in seed samples of triticale varieties registered in the Czech Republic. The individual glutenin electrophoreograms were interpreted qualitatively by means of a catalogue of glutenin alleles for wheat (Payne & Lawrence, 1983). In the triticale varieties Presto, Sekundo, Tricolor, Ticino and Gabo the alleles *Glu 1A2** and *Glu 1B 7+8* were detected. In the varieties Kitaro, Marko and Disco the alleles *Glu 1A1* and *Glu 1B 7+8* were found. The heterogeneous constitution *1/2** on locus *Glu 1A* was detected in varieties Kitaro and Sekundo (two grains in 2003). In the varieties Kolor and Lamberto the *Glu 1A0* and *Glu 1B 7+8* of HMW glutenin subunits were found. Only in the variety Modus the alleles *Glu 1A0* and *Glu 1B 6+8* were found.

Key words: triticale, X *Triticosecale* Wittmack, storage proteins, electrophoresis

INTRODUCTION

Triticale (X *Triticosecale* Wittmack.) is an autogamous plant with a low share of cross-pollination (4–5%). This means that the majority of varieties are of the line type or a mixture of isogenic lines (Chloupek, 2000). A number of methods are now available for the detection of the genetic variability (diversity), e.g. morphological characteristics; analysis of pedigrees; biochemical markers, particularly proteins and their various isoenzyme variants; molecular (DNA) markers etc. The author's department has gained important experience in the area of detection of polymorphism of storage proteins of triticale grain, i.e. the alcohol-soluble fraction — prolamins (gliadins and secalins). Prolamin proteins are characterised by high polymorphism in comparison with HMW glutenin subunits and many authors detected them in a number of crops, e.g. wheat (*T. monococcum, T. spelta* and *T. aestivum*), barley (*H. vulgare*) and triticale (Šašek and Černý, 1996; Šašek *et al.*, 2000; Vyhnánek and Bednář, 2003). In comparison with other markers of genetic variability they have many advantages. There are not so dependent on environmental conditions as isoenzymes, and they are not dependent on the ontogenetic stage of the plant (Koch, 1998). Other markers of genetic variability, the importance of which is continually increasing, are DNA markers. These methods, however, are extremely costly in terms of material and instrumentation compared to the detection of polymorphism of prolamin proteins. Considering these aspects the polymorphism of storage proteins of grain is very suitable for the detection of the genetic variability of cereals.

The objective of the present study was to detect the genetic variability by means of spectrums of grain storage proteins (prolamins and HMW glutenin subunits) in triticale varieties registered in the Czech Republic.

MATERIAL AND METHODS

The polymorphism of storage proteins of grain was analysed in 10 winter forms and one spring form of triticale varieties (X *Triticosecale* Wittmack., 2n = 6x = 42, AABBRR) registered in the Czech Republic. Mixed samples of certified seeds from the 2002 and 2003 harvests were obtained from the Central Control and Testing Agricultural Institute, testing station in Hradec nad Svitavou.

Electrophoresis analysis of prolamin proteins by means of vertical polyacrylamid electrophoresis of the firm Biometra was conducted according to the PAGE ISTA method (ISTA, 1999) and electrophoresis analysis of HMW glutenin subunits was conducted according to the SDS PAGE (Laemmli, 1970). From each genotype we analysed 105 (prolamins) and 44 (glutenins) randomly selected seeds, each one separately (one grain = one electrophoresis path). The resulting prolamin electrophoreographs were qualitatively interpreted using REM (relative electrophoresis mobility), where a protein variant with electrophoresis mobility was the reference band REM = 55. Quantitative evaluations were based on the intensity of colouring of the protein variants in the resulting electrophoresis spectrum. These prolamin spectrums were graphically processed using macro Žížala in MS Excel. The Bio 1D++ software (Vilber Lourmat, France) was used for statistical interpretation of the electrophoreographs, i.e. by calculation of Dice's coefficients of similarity and elaboration of a dendrogram. The individual glutenin electrophoreograms were interpreted qualitatively by means of a catalogue of glutenin alleles for wheat (Payne and Lawrence, 1983).

RESULTS AND DISCUSSION

Metakovsky and Branlard (1998) used prolamin proteins of wheat grain to explore the genetic diversity and to differentiate the French wheat varieties. Prolamin proteins were also used to detect the variability in 100 wheat varieties registered in Spain in the past 40 years (Metakovsky *et al.* 2000). Our present results in the detection of polymorphism of prolamin grain proteins enabled to differentiate all the 11 analysed triticale varieties (Fig. 1). The effect of the weather conditions of the respective year on the use of electrophoresis spectrums for verification of the varieties was not confirmed.

The occurrence of varieties with a uniform prolamin spectrum and genotypes with two and more sister prolamin lines dependent on the harvest year was discovered for example in wheat (Černý and Šašek, 1996), barley (Černý and Šašek, 1998, Šašek *et al.* 2000) and triticale (Vyhnánek and Bednář, 2000). A uniform electrophoresis prolamin spectrum was discovered in 8 tritical winter varieties (Presto, Kolor, Disco, Sekundo, Marko, Tricolor, Lamberto and Ticino) and it is the case of a one-line variety (Fig. 1). Two prolamin spectrums with different percentage of participation dependent on the year were detected in the remaining genotypes of winter triticale (Tab. 1). In our case the difference did not exceed ± 6%. In both years the electrophoresis spectrums were identical and were not influenced by the weather conditions of the year. In both years two prolamin spectrums were detected in the varieties Modus and Kitaro.

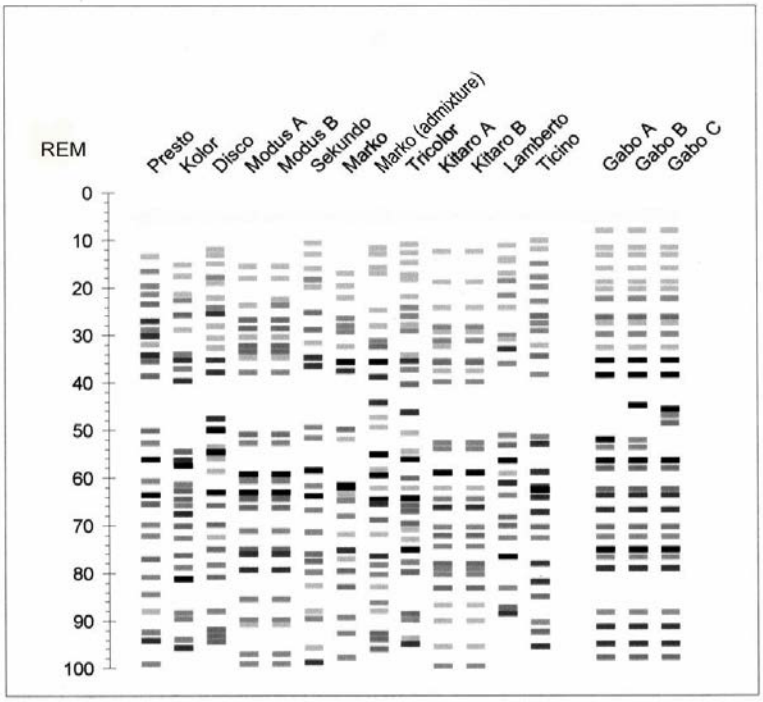


Fig. 1. Prolamin spectrums of triticale varieties

Table 1

Participation of sister prolamin lines (in %)

Variety	Line	Year of harvest	
		2002	2003
Modus	A	73	70
	B	27	30
Kitaro	A	64	70
	B	36	30
Gabo	A	73	75
	B	23	25
	C	4	0

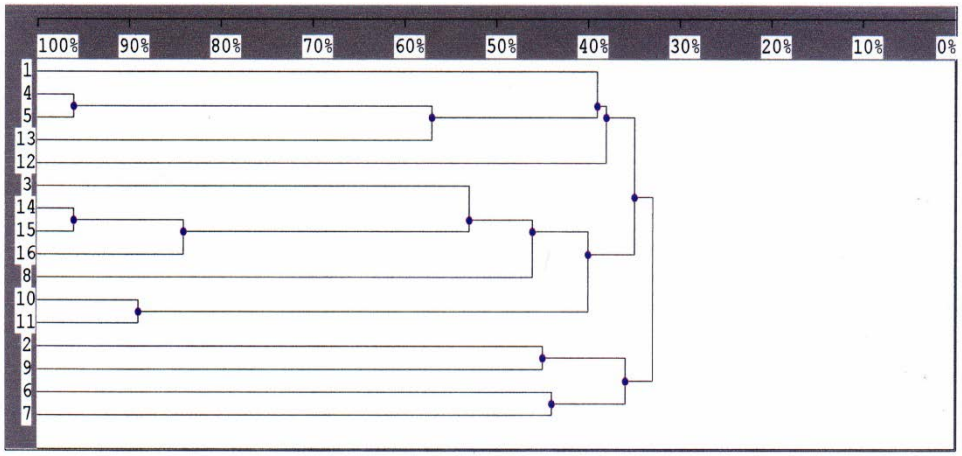
Based on Dice's coefficients of similarity and the dendrogram it is evident that this is a case of a sister prolamin line (Tab. 2, Fig. 2). On the basis of Dice's coefficient of similarity (0.38), in the variety Marko, where two prolamin spectrums were also discovered in the 2002 harvest, we can assume that it is an admixture of a foreign genotype in the seed sample. Typical of the admixture of the unknown genotype was the gliadin block *Gld 1B3*, which is the marker of wheat-rye translocation T1BL.1RS.

Table 2

Matrix of Dice's coefficients of similarity of prolamin spectrums of triticale using the Bio 1D++ software (P = 99%)

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	LL16
L1	1.00															
L2	0.28	1.00														
L3	0.41	0.37	1.00													
L4	0.33	0.36	0.37	1.00												
L5	0.39	0.35	0.36	0.96	1.00											
L6	0.26	0.38	0.30	0.34	0.33	1.00										
L7	0.39	0.34	0.29	0.33	0.44	0.44	1.00									
L8	0.31	0.30	0.47	0.24	0.23	0.32	0.38	1.00								
L9	0.43	0.45	0.42	0.32	0.34	0.28	0.33	0.27	1.00							
L10	0.38	0.38	0.41	0.41	0.43	0.43	0.39	0.40	0.40	1.00						
L11	0.34	0.37	0.40	0.36	0.38	0.38	0.34	0.36	0.39	0.90	1.00					
L12	0.39	0.28	0.35	0.23	0.22	0.29	0.31	0.28	0.30	0.32	0.28	1.00				
L13	0.42	0.34	0.35	0.58	0.61	0.36	0.39	0.26	0.40	0.32	0.28	0.35	1.00			
L14	0.40	0.43	0.54	0.32	0.34	0.41	0.49	0.42	0.41	0.44	0.36	0.33	0.44	1.00		
L15	0.39	0.45	0.56	0.38	0.37	0.40	0.51	0.41	0.43	0.39	0.32	0.36	0.43	0.97	1.00	
L16	0.33	0.45	0.52	0.31	0.33	0.37	0.47	0.44	0.43	0.36	0.29	0.29	0.39	0.84	0.82	1.00

Explanations: L1 — Presto, L2 — Kolor, L3 — Disco, L4 — Modus A, L5 — Modus B, L6 — Sekundo, L7 — Marko, L8 — admixture of variety Marko, L9 — Tricolor, L10 — Kitaro A, L11 — Kitaro B, L12 — Lamberto, L13 — Ticino, L14 — Gabo A, L15 — Gabo B, L16 — Gabo C



Explanations: 1 — Presto, 2 — Kolor, 3 — Disco, 4 — Modus A, 5 — Modus B, 6 — Sekundo, 7 — Marko, 8 — admixture of variety Marko, 9 — Tricolor, 10 — Kitaro A, 11 — Kitaro B, 12 — Lamberto, 13 — Ticino, 14 — Gabo A, 15 — Gabo B, 16 — Gabo C

Fig. 2. Dendrogram of similarity of prolamin spectrums of triticale using the Bio 1D++ software (Jaccard's coefficient, P = 99%)

This admixture was detected in the first year only and with all probability it is a genotype of hexaploid wheat. In the 2002 harvest three sister prolamin spectrums were detected in the spring triticale variety Gabo and two prolamin spectrums in the 2003 harvest (absence of line C). Based on Dice's coefficients of similarity and the dendrogram, the prolamin spectrum most similar to Disco is the prolamin spectrum of the variety Gabo, the only spring triticale variety registered in the Czech Republic.

When detecting polymorphism of storage proteins of triticale grain it was discovered that subunits of glutenin with high molecular weight had a lower level of polymorphism compared to the gliadin fraction (Vyhnánek & Bednář, 2000). On chromosome 1A we detected three alleles in homozygous state *0* (27%), *1* (27%), *2** (45%) (Tab. 3).

Table 3

Genetic interpretation of HMW glutenin subunits of triticale

Variety	Chromosome		Glu score (Šašek *et al*., 2000)
	1A	1B	
Gabo	*2**	*7+8*	6
Disco	*1*	*7+8*	6
Lamberto	*0*	*7+8*	4
Kitaro[+]	*1* and *1/2**	*7+8*	6/6
Kolor	*0*	*7+8*	4
Marko	*1*	*7+8*	6
Modus	*0*	*6+8*	2
Presto	*2**	*7+8*	6
Sekundo[+]	*2** and *1/2**	*7+8*	6/6
Ticino	*2**	*7+8*	6
Tricolor	*2**	*7+8*	6

[+] Heterogeneous constitution — two grains in 2003

In 2003 heterozygous constitution *1/2** was discovered in two grains of varieties Kitaro and Sekundo. On chromosome 1B of triticale the cluster *Glu 1B 7+8* (91%) was dominant. The glutenin block *Glu 1B 6+8* (9%) was detected only in the variety Modus. Amiour *et al.* (2002) obtained the same results with varieties Ticino and Tricolor. An average to low level of the Glu score was calculated where the highest value in points was 6 (Gabo, Disco, Kitaro, Marko, Presto, Sekundo, Ticino and Tricolor), 4 (Lamberto and Kolor) and the lowest 2 (Modus). The information capacity of the Glu score in triticale is considerably affected by using point values of the Glu score for wheat (*Triticum aestivum* L.), i.e. by the absence of the D genome of wheat and presence of the genome R of rye in triticale. That is why it is important to discover the correlation between the technological quality of triticale and the genetic constitution of polymorphism of glutenins with VMH. Starovičová *et al.* (2003) drew the same conclusions with hard wheat (*T. durum*, genom AABB).

Electrophoresis spectrums of storage proteins were identical in both years and were not affected by the weather conditions of the year, confirming the results of a number of authors.

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