Polymorphism of prolamin proteins of the grain of triticale varieties certified in the Czech Republic

T. Vyhnánek, J. Bednář

Mendel University of Agriculture and Forestry in Brno, Czech Republic

ABSTRACT

Genetic diversity was detected in 11 varieties of triticale registered in the Czech Republic by means of polymorphism of prolamin proteins using the PAGE ISTA method. The polymorphism of prolamin proteins allowed the differentiation of the individual triticale varieties in 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 rye translocation T1BL.1RS, gene *Sr31* with resistance to black rust, higher cold resistance and lower baking quality of the wheat. 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.

Keywords: triticale; X*Triticosecale* Wittmack.; prolamin proteins; electrophoresis; admixture

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 in the detection of polymorphism in storage proteins of triticale grain, i.e. the alcohol-soluble fraction – prolamins (gliadins and secalins). Prolamin proteins are characterised by high polymorphism 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 et al. 2000, Vyhnánek and Bednář 2003). By 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. There 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 in 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 prolamin storage proteins in the grain of 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 (Table 1). Mixed samples of certified seeds from the 2002 and 2003 harvests were obtained from Ing. František Beneš of 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

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Table 1. Analysed triticale varieties

Variety		Year of registration	Property
Spring	Gabo	1999	Hodowla Roslin Strzelce, Sp. z o.o., Poland
Winter	Disco	1997	DANKO Hodowla Roslin, Sp. z o.o., Poland
	Lamberto	2003	DANKO Hodowla Roslin, Sp. z o.o., Poland
	Kitaro	2003	DANKO Hodowla Roslin, Sp. z o.o., Poland
	Kolor	1996	SELGEN, a.s., Czech Republic
	Marko	2001	Hodowla Roslin Strzelce, Sp. z o.o., Poland
	Modus	1998	NORDSAAT Saatzuchtgesellschaft mbH, Germany
	Presto	1990	DANKO Hodowla Roslin, Sp. z o.o., Poland
	Sekundo	2000	Hodowla Roslin Szelejewo Sp. z o.o., Poland
	Ticino	2003	Pflanzenzucht Saka GbR, Germany
	Tricolor	2002	Florimond Desprez, France

the PAGE ISTA method (ISTA 1999, Vyhnánek and Bednář 2003). From each genotype we analysed 105 randomly selected seeds, each one separately (one grain = one electrophoresis path). The resulting 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.

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 polymor-

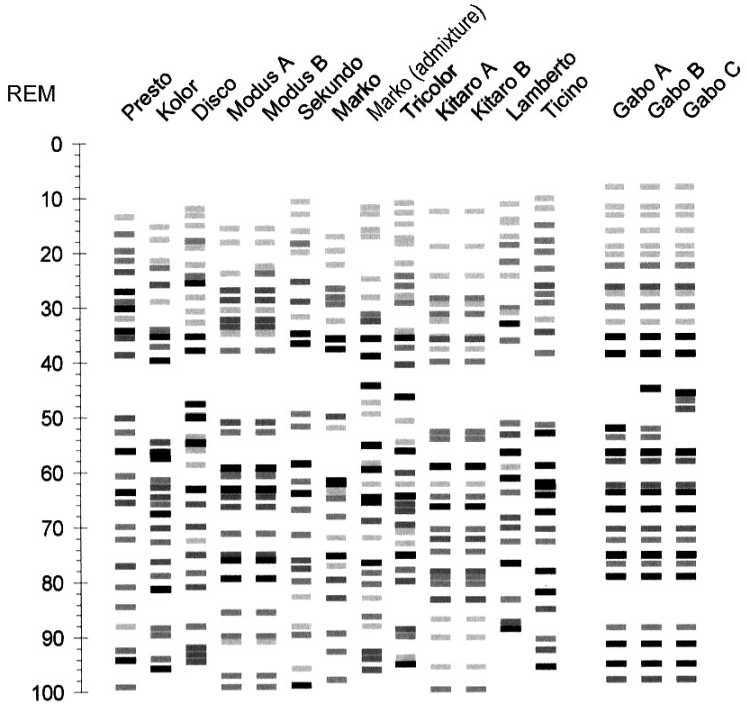

Figure 1. Prolamin spectrums of triticale varieties

Table 2. 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

phism of prolamin grain proteins enabled us to differentiate all the 11 analysed triticale varieties (Figure 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 triticale winter varieties (Presto, Kolor, Disco, Sekundo, Marko, Tricolor, Lamberto and Ticino) and it is the case of a one-line variety (Figure 1). Two prolamin spectrums with different percentages of participation dependent on the year were detected in the remaining genotypes of winter triticale (Table 2). 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. Basing on Dice's coefficients of similarity and the dendrogram it is evident that this is a case of a sister prolamin line (Table 3, Figure 2). On the basis of Dice's coefficient of similarity (0.38), in

Table 3. 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	L16
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

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

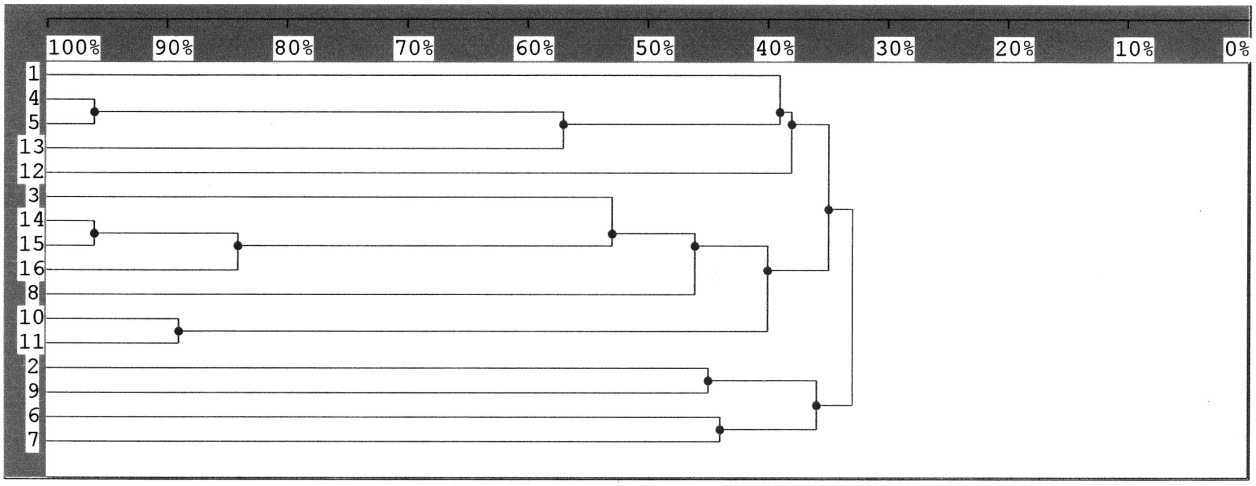

Figure 2. Dendrogram of similarity of prolamin spectrums of triticale using the Bio 1D++ software (Jaccard's coefficient, *P* = 99%)

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

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 rye translocation T1BL.1RS, gene *Sr31* with resistance against black rust, higher cold resistance and lower baking quality of wheat. 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.

Electrophoresis spectrums of prolamins 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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ABSTRAKT

Polymorfismus prolaminových bílkovin obilky registrovaných odrůd tritikale v ČR

Byla provedena detekce genetické diverzity u 11 odrůd tritikale, které jsou registrovány v ČR, pomocí polymorfismu prolaminových bílkovin obilky metodou PAGE ISTA. Jednotlivé odrůdy tritikale ze sklizně 2002 a 2003 se podařilo pomocí polymorfismu prolaminových bílkovin obilky navzájem rozlišit. Na základě výpočtu Diceho podobnostních koeficientů byly zjištěny vedle uniformních genotypů genotypy s výskytem sesterských prolaminových spekter s rozdílným procentuálním zastoupením v jednotlivých letech. Uniformní spektrum bylo detekováno u odrůd Disco, Kolor, Lamberto, Marko, Presto, Sekundo, Ticino a Tricolor; dimorfní byly odrůdy Kitaro a Modus. Odrůda Gabo vykazovala v roce 2003 výskyt tří sesterských prolaminových linií a v roce 2004 jen dvou. U odrůdy Marko byla v roce 2003 detekována příměs cizího genotypu s výskytem 5 %. Pro příměs neznámého genotypu byl charakteristický gliadinový blok *Gld 1B3*, který je markerem žitné translokace T1BL.1RS, genu *Sr31* rezistence ke rzi travní, vyšší zimovzdornosti a nižší pekařské kvality u pšenice. Prolaminové bílkoviny obilky tritikale lze vhodně využít pro detekci genetické diverzity a stanovení odrůdové pravosti a čistoty ve vzorku osiva u odrůd tritikale registrovaných v ČR.

Klíčová slova: tritikale; *XTriticosecale* Wittmack.; prolaminové bílkoviny; elektroforéza; příměs

Corresponding author:

Ing. Tomáš Vyhnánek, Ph.D., Mendelova zemědělská a lesnická univerzita v Brně, Zemědělská 1, 613 00 Brno, Česká republika
phone: + 420 545 133 185, fax: + 420 545 133 025, e-mail: vyhnanek@mendelu.cz