Detection of the varietal purity in sample of harvested wheat and triticale grains by prolamin marker

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ABSTRACT

In 1997 and 1998 we used samples of harvested grain to verify the possibility of distinguishing 14 winter wheat genotypes and six triticale genotypes and detecting the impurity on the basis of the detection of polymorphism of prolamin kernel proteins using the methods of the PAGE ISTA. On the basis of the identity index two sister prolamin lines with different percentage of participation, which was based on the weather conditions of the year of harvest, were discovered in seven wheat genotypes (Astella, Brea, Hana, Ilona, Siria, Sofia and Šárka) and two triticale genotypes (Tornádo and KM 779). A foreign genotype was detected in the Hana and Astella varieties. The identity index of the impurity to the Astella and Hana variety (i.e. *ii* = 0.28 and *ii* = 0.20, respectively) was considerably lower. In an unknown genotype (impurity) we detected the gliadin block *Gld 1B3*, which is the genetic marker of rye translocation T1BL.1RS, the *Sr31* gene of resistance to black rust, higher cold resistance and the marker of poor baking quality (presence of secalin genes). The results proved the potential practical application of the method of electrophoretic detection of polymorphism of prolamin proteins as markers of impurities of foreign genotypes in a seed sample.

Keywords: winter wheat; winter triticale; prolamin proteins; electrophoresis; admixture

In breeding work it is important to make sure to use initial seed stock that has the required properties, but also to use material of corresponding varietal (genotypic) purity. This also applies to farmers who produce and sell seeds in the following year and, last but not least, it concerns the processing industry where admixtures of an undesirable genotype have a negative effect and result in poorer quality of the end product.

Both wheat (*Triticum aestivum* L.) and triticale (*Xtriticosecale* Wittmack) are self-pollinating crops with a low share of cross-pollination (4–5%). This means that most of the varieties are of the line type or they are a mixture of isogenic lines (Chloupek 2000). A number of methods can be used to detect the impurities in a sample of harvested seeds. In our case we used the polymorphism of reserve kernel proteins, i.e. the alcohol-soluble fraction – prolamins (gliadins, secalins). High polymorphism was used by many authors (Šašek and Sýkorová 1989, Metakovsky 1991, Černý et al. 1992) and is characteristic of these proteins. Compared to other variability markers they offer a number of advantages.

Compared to other biochemical markers, the isoenzymes, they are not so dependent on conditions of the external environment and they are independent on the ontogenetic stage of the plant. Other markers, which are undergoing considerable development at the present time, are monitoring on the level of DNA polymorphism. However, compared to the detection of polymorphism of prolamin proteins these methods are much more expensive due to the used chemicals and technology.

In terms of the above aspects, polymorphism of reserve proteins is more suitable for the detection of varietal purity in kernel samples. The objective of the present study was to detect the varietal purity from a sample of harvested seed stock using electrophoretic detection of the polymorphism of prolamin proteins of wheat and triticale.

MATERIAL AND METHODS

Harvested kernels of six genotypes of winter hexaploid triticale ($2n = 6x = 42$, genome AABBRR) – Chrono, Kolor, Presto, Tornádo, Modus and KM 779, were used as experimental material. In hexaploid wheat ($2n = 6x = 42$, genome AABBDD) we evaluated 14 winter genotypes – Hana, Astella, Ilona, Sofia, Šárka, Barbara, Livia, Vlada, Rexia, Saskia, Siria, Brea, Euro 90.07 and Euro 94.02 (certified seed only).

Triticale and wheat seeds harvested on experimental plots of the Agricultural Research Institute Kroměříž in 1997 and 1998 were used for electrophoretic analyses using the vertical polyacrylamide electrophoresis of the firm OMNI BIO according to the method of PAGE ISTA (Bednář et al. 1999, ISTA 1999). From each genotype we analysed 105 randomly selected seeds, each one sepa-

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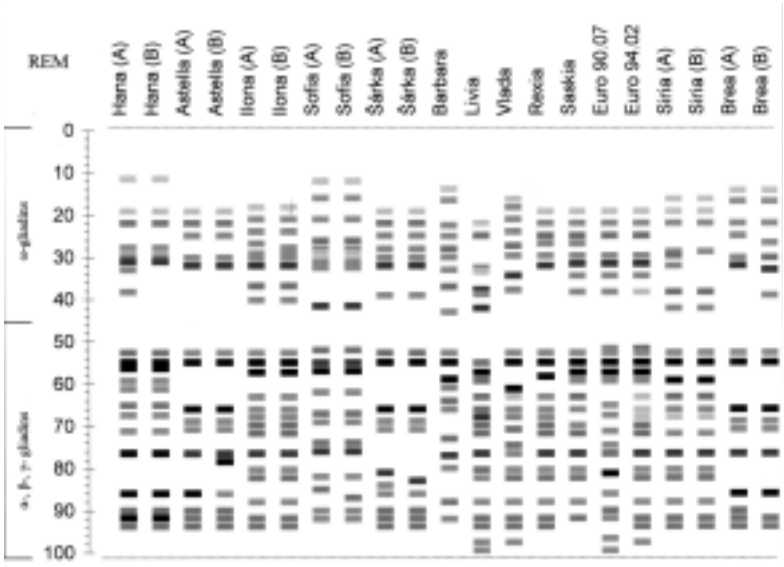

Figure 1. Prolamin spectra of analysed genotypes of winter wheat

rately (one seed = one electrophoretic pathway). The resulting electrophoreograms were qualitatively interpreted using REM (relative electrophoretic mobility) where the reference band was the protein variant with a 55 REM mobility, which can be found in all D-genome hexaploid wheat. Quantitative interpretation was based on the intensity of the colouring of protein variants in the resulting electrophoretic spectrum. If heterogeneous spectra appeared, the identity index (*ii*) for the determination of related electrophoretic spectra was estimated (Hadačová et al. 1980). These spectra were plotted using the Žížala programme in MS Excel by REM and the intensity of the colouring of protein variants. Genetic interpretation of the results was based on Metakovsky et al. (1986).

Table 1. Participation of prolamin lines in the analysed wheat and triticale genotypes

Genotypes	Years	Representation (%)		Index identity of line A and B
		line A	line B	
Astella	1997	87	13	0.94
	1998	86	14	
Brea	1997	75	25	0.78
	1998	75	25	
Hana	1997	52	48	0.90
	1998	51	49	
Ilona	1997	80	20	0.92
	1998	80	20	
Siria	1997	68	32	0.95
	1998	69	31	
Sofia	1997	78	22	0.91
	1998	78	22	
Šárka	1997	80	20	0.88
	1998	80	20	
KM 779	1997	80	20	0.69
	1998	80	20	
Tornádo	1997	80	20	0.88
	1998	80	20	

RESULTS AND DISCUSSION

Černý and Šašek (1996) indicated that the polymorphism of prolamin seed proteins is high compared to the other important fraction of wheat protein – glutenins. Using electrophoretic separation of gliadins Metakovsky and Branlard (1998) characterised and differentiated French varieties approved and registered in the past 25–50 years; Metakovsky et al. (2000) detected the variability in 100 varieties registered in Spain in the past 40 years.

By means of gliadin polymorphism, in our experiment we were able to distinguish all 14 genotypes of winter wheat and six genotypes of winter triticale; prolamin uniform electrophoretic spectra were detected in genotypes of Barbara, Livia, Rexia, Saskia, Vlada, Euro 90.07, Euro 94.02 wheat in 1997 and 1998. On the other hand, heterogeneous spectra consisting of two prolamin lines were discovered in genotypes of Ilona, Sofia, Šárka, Astella, Brea, Hana and Siria wheat in both years. The gliadin protein variant with REM 55 appeared in all the analysed genotypes (Figure 1). In the Hana, Ilona, Siria and Brea varieties we detected differences in gliadins with a higher molecular weight – ω-gliadins, which make up a part of the electrophoretic spectrum with REM ranging between 0 and 42. In contrast, the differences in the area belonging to α-, β-, γ-gliadins with a lower molecular weight in the REM area ranging between 50 and 100 were found in the Astella, Sofia and Šárka varieties. In

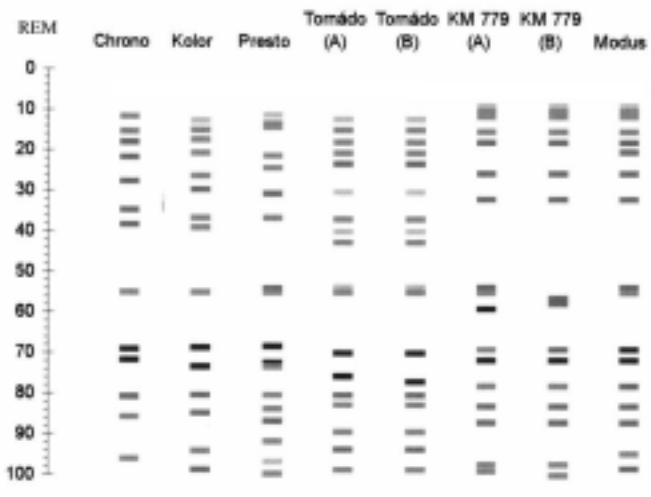

Figure 2. Prolamin spectra of analysed genotypes of winter triticale

winter triticale varieties Chrono, Kolor, Presto and Modus single-line spectra were detected in both experimental years, while two prolamin spectra were detected in the Tornádo and KM 779 genotypes (Figure 2).

In heterogeneous genotypes the identity index ranged from 0.69 (KM 779) to 0.95 (Siria) proving that it is a sister prolamin line (Table 1). By means of starch electrophoresis Šašek et al. (1998) reached the same conclusions in the Astella, Brea, Hana, Ilona, Sofia and Šárka varieties. The participation of the respective lines expressed in percents was genotype specific. The representation of lines in the Hana, Astella and Siria varieties was virtually balanced in both years. There were only minor differences (±1%) in participation between the respective years.

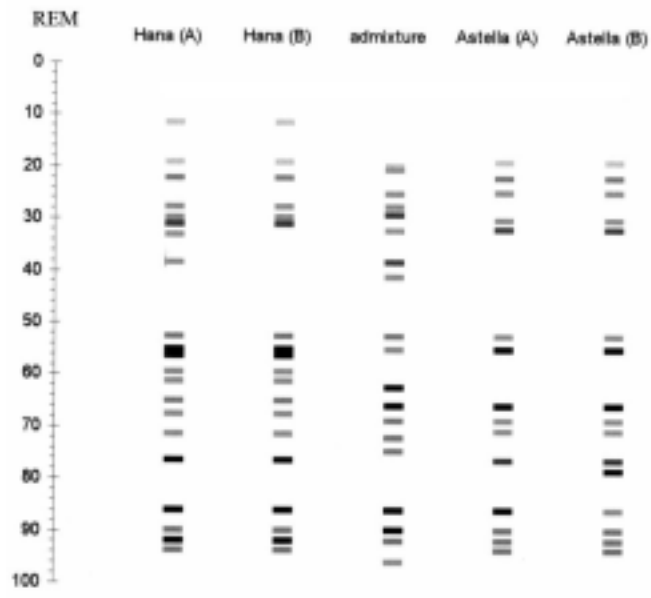

Figure 3. Comparison of prolamin spectra of the Hana and Astella varieties with admixture

The percentage participation of sister gliadin lines in the Brea, Ilona, Sofia and Šárka, Tornádo and KM 779 genotypes was the same in 1997 and in 1998. The representation of the individual sister prolamin lines is dependent on external conditions.

Černý et al. (1993) determined the varietal authenticity and varietal purity of common wheat seeds using electrophoresis in starch gel. ISTA recommends using polymorphism of reserve proteins in *Triticum* (ISTA 1999) as a method of determination of the varietal purity but does not mention the specified limit values. In contrast to triticale, we detected foreign genotypes in two wheat genotypes. The percentage representation of foreign genotypes in the Hana variety was 13.3% in 1997 and 1.9% in 1998, and was lower in the Astella variety, i.e. 0.95% and 1.9% in 1997 and 1998, respectively. The foreign genotype had one electrophoretic spectrum and markedly low identity index compared to the analysed varieties. In the case of Astella and Hana the identity index was $ii = 0.28$ and $ii = 0.20$, respectively (Figure 3).

Characteristic of the foreign genotype was a gliadin cluster genetically determined by the *Gld 1B3* allele. According to Černý and Šašek (1996) this gliadin block detects the occurrence of the rye translocation T1BL.1RS in wheat, which is the marker of higher cold resistance, poorer baking quality (secalin genes in the wheat genome) and the *Sr31* gene of resistance to black rust. The high percentage of foreign genotypes in the Hana variety in 1997 could have been caused by insufficient purity of the used seed sample or a foreign genotype introduced during harvest. Based on consultations with Ing. Petr Martinek, CSc., from the Agricultural Research Institute who provided the samples for analyses, we are inclined towards the first alternative.

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ABSTRAKT

Detekce odrůdové čistoty ve vzorku sklizených obilek pšenice a tritikale pomocí prolaminového markeru

Byla ověřena možnost rozlišení 14 genotypů ozimé pšenice a šesti genotypů tritikale a zjištění příměsi na základě detekce polymorfismu prolaminových bílkovin zrna pomocí metodiky PAGE ISTA ve vzorcích sklizených obilek v letech 1997 a 1998. U sedmi genotypů pšenice (Astella, Brea, Hana, Ilona, Siria, Sofia a Šárka) a dvou genotypů tritikale (Tornádo a KM 779) byl detekován a na základě indexu identity zjištěn výskyt dvou sesterských prolaminových linií s různým procentuálním zastoupením v závislosti na roku sklizně. U odrůd Hana a Astella byl detekován výskyt cizího genotypu. Příměs měla výrazně nízký index identity k odrůdě Astella (*ii* = 0,28) a Hana (*ii* = 0,20). U neznámého genotypu (příměsi) byl detekován gliadinový blok *Gld 1B3*, který je genetickým markerem žitné translokace T1BL.1RS, genu *Sr31* rezistence ke rzi travní, vyšší zimovzdornosti a markerem špatné pekařské jakosti (přítomnost sekalinových genů). Výsledky ukazují možnost praktického využití metody elektroforetické detekce polymorfismu prolaminových bílkovin jako markerů příměsi cizího genotypu ve vzorku osiva.

Klíčová slova: ozimá pšenice; ozimé tritikale; prolaminy; elektroforéza; příměs

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