Use of Prolamin Polymorphism to Describe Genetic Variation in a Collection of Barley Genetic Resources

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Abstract: The polymorphism of prolamin storage proteins was studied in seed samples of 20 historical cultivars of spring barley (*Hordeum vulgare* L.) of Czech and Slovak origin, using polyacrylamide gel electrophoresis (PAGE). Only two samples were uniform. Most heterogeneity of prolamin patterns was observed in the oldest accessions. By means of a prolamin identity index it was possible to distinguish sister lines from admixtures within the seed samples. The obtained spectra will be used as additional descriptors for the spring barley core collection of the Collection of Genetic Resources of the Agricultural Research Institute Kroměříž, Ltd.

Keywords: spring barley; *Hordeum vulgare* L.; hordeins; PAGE-ISTA; genetic resources

Within genetic resources often genetic variability is present, which is important to know before a resource is utilised in breeding. Barley (*Hordeum vulgare* L.) is a self-pollinated species with an outcrossing rate of up to 5%, depending on genotype and environment. Most grown varieties are therefore pure lines or mixtures of closely related lines. Many methods have been used to describe genetic diversity within and among cultivars, reviewed recently by Koch (1998). Prolamins, i.e. alcohol soluble storage proteins, have been frequently used to study genetic diversity in many species, since they are highly polymorphic and environmentally stable. The analysis of prolamins by PAGE, was therefore internationally recommended by the International Seed Testing Association (ISTA) for the verification of species and cultivars (ISTA 1999). Prolamins have been recently used to study genetic diversity in barley by Molina-Cano *et al*. (2001, 2002), Dudin *et al*. (1998), Vapa and Radovic (1998) and Lookhart *et al*. (1999). Although a number of methods is now available for studying polymorphism at DNA level, the analysis of prolamins is still justified by lower costs of equipment and chemicals. We used therefore prolamin patterns to describe the genetic diversity of the investigated accessions.

MATERIAL AND METHODS

Twenty spring barley accessions of Czech and Slovak origin from the collection of genetic resources of the Agricultural Research Institute Kroměříž, Ltd., were analysed (Table 1).

Vertical PAGE, as recommended by ISTA (1999), was performed with 30 random seeds of most of the accessions and with 60 seeds of the nine oldest accessions. The relative electrophoretic mobility (REM) of the protein bands and their coloration intensity was evaluated. One seed of the bread wheat variety Astella was analysed together with each accession sample, producing a reference band with REM of 55%. In heterogeneous samples an identity index (*ii*) was calculated for patterns deviating from the prevailing pattern, according to (Hadačová *et al*. 1980). Prolamin patterns with $ii > 0.6$, were considered as sister patterns and lower values as admixtures of other genotypes in the analysed sample. The patterns were visualised in Figures 1–3 using an Excel macro.

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Table 1. Evaluated spring barley accessions

Variety name	Accession number[1)]	On-farm use	In collection since	Analysed seeds
Dobrovický staročeský	03C0600032	earlier	1957	60
Dregerův Imperial	03C0600065	earlier	1955	60
Chlumecký	03C0600023	1902	1958	60
Hanácký Kargyn	03C0600013	1919	1952	60
Proskovcův hanácký	03C0600015	1919	1958	60
Ratbořský	03C0600017	1925	1958	60
Selecta Hanak 1	03C0600033	1926	1952	60
Stupický hanácký	03C0600010	1926	1952	60
Tepelský 421	03C0600025	1930	1958	60
Hodonínský Kvas	03C0600029	1937	1952	30
Stupický plnozrnný	03C0600007	1937	1958	30
Hanácký Jubilejní	03C0600002	1938	1957	30
Olešenský	03C0600044	1938	1952	30
Triumf	03C0600011	1938	1952	30
Valtický	03C0600019	1938	1958	30
Opavský Kneifl	03C0600005	1939	1958	30
Židlochovický Gloria	03C0600037	1940	1952	30
Bučianský	03C0600001	1946	1957	30
Slovenský dunajský trh	03C0600008	1946	1959	30
Bohatýr	03C0600012	1948	1958	30

[1)] Accession number in the EVIGEZ database of the Gene Bank

RESULTS AND DISCUSSION

All 20 spring barley accessions could be distinguished by their prolamin patterns. Only two accessions, Hanácký Kargyn and Slovenský dunajský trh, were uniform in their prolamin pattern and are possibly pure lines, since uniformity of 30 seeds significantly excludes heterogeneity above approximately 10%*). The other accessions were heterogeneous. We detected from two to five different prolamin patterns per accession, with different percentages. The patterns detected in the analysed samples are summarised in Figures 1 to 3. The patterns within accessions were given letters from A through E according to their frequency in the analysed sample. The percentages of the patterns and the identity indices in relation to the predominant pattern in each sample are summarised in Table 2.

From the nine samples with two prolamin patterns (Figure 2), the B patterns had a high identity index in 6 samples, indicating them as sister lines of patterns A. In three samples, of Bohatýr, Olešenský and Triumf, the identity index was 0.52, 0.48 and 0.17, respectively, indicating admixtures, most obvious in Triumf. Černý and Šašek (1998) indicated an *ii* of 0.6 as the lowest acceptable value for sister lines

From the eight samples with three prolamin patterns (Figure 3), one sample (Opavský Kneifl) contained only sister lines and another sample (Hodonínský Kvas) sister lines and an admixture. In the remaining samples only admixtures to the A pattern were present.

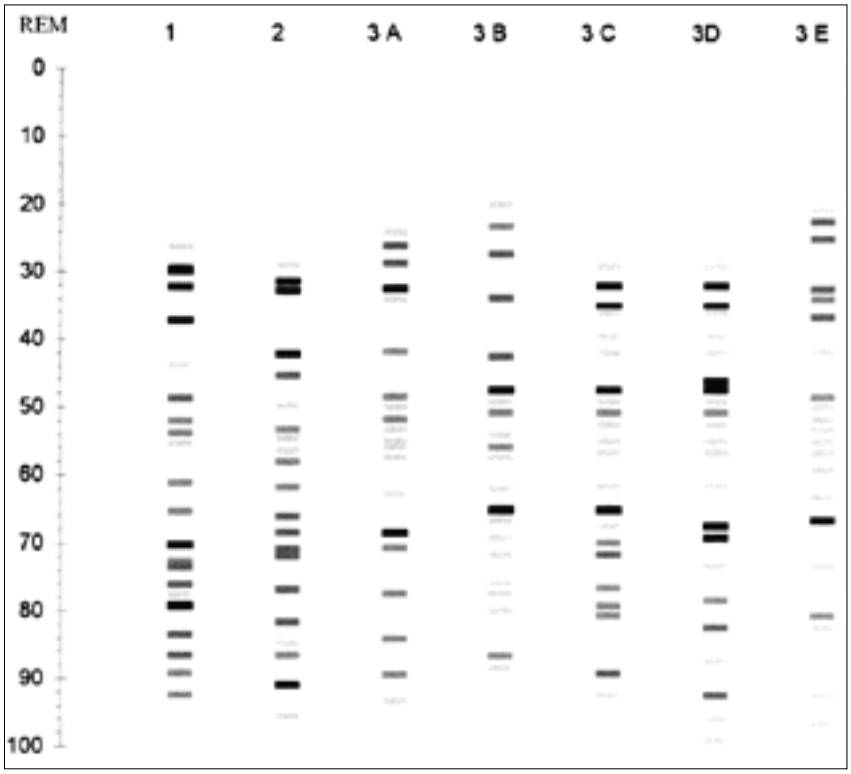
Some of the admixtures were more close to each other than to the prevailing pattern A. This was the case with the patterns C relative to B in the sample of Selecta Hanak 1, where *ii* was 0.27, and in the sample of Bučianský, where *ii* of D relative to C was 0.48, compared with 0.16 relative to A.

The presence of sister lines with slightly different prolamin spectra was reported for instance in

*) because of $(1 - f)^n = p$, where f = frequency of heterogeneity, p = detection probability and n = number of observations. With 30 seeds and f =10% we obtain $(1 - 0.1)^{30} = 0.05$

Table 2. Frequency and relationship of prolamin patterns within seed samples of spring barley genetic resources

Genotype	Pattern	Percentage	Identity index
Two prolamin paterns			
Bohatýr	A	82	
	B	18	0.52
Dobrovický staročeský	A	68	
	B	32	0.92
Chlumecký	A	83	
	B	17	0.66
Olešenský	A	93	
	B	7	0.48
Ratbořský	A	77	
	B	23	0.69
Tepelský 421	A	68	
	B	32	0.92
Triumf	A	81	
	B	19	0.17
Valtický	A	67	
	B	33	0.91
Židlochovický Gloria	A	82	
	B	18	0.92
Five prolamin patterns			
Bučianský	A	41	
	B	32	0.08
	C	14	0.17
	D	9	0.16
	E	4	0.31
Three prolamin paterns			
Dregerův Imperial	A	51	
	B	40	0.41
	C	9	0.33
Hanácký Jubilejní	A	73	
	B	16	0.24
	C	11	0.15
Hodonínský Kvas	A	81	
	B	14	0.65
	C	5	0.28
Opavský Kneifl	A	48	
	B	34	0.81
	C	18	0.71
Proskovcův hanácký	A	79	
	B	16	0.45
	C	5	0.50
Selecta Hanak 1	A	74	
	B	14	0.21
	C	12	0.19
Stupický hanácký	A	77	
	B	14	0.37
	C	9	0.13



1 – Slovenský dunajský trh, 2 – Hanácký Kargyn, 3 – Bučianský

Figure 1. Prolamin patterns in seed samples of spring barley accessions

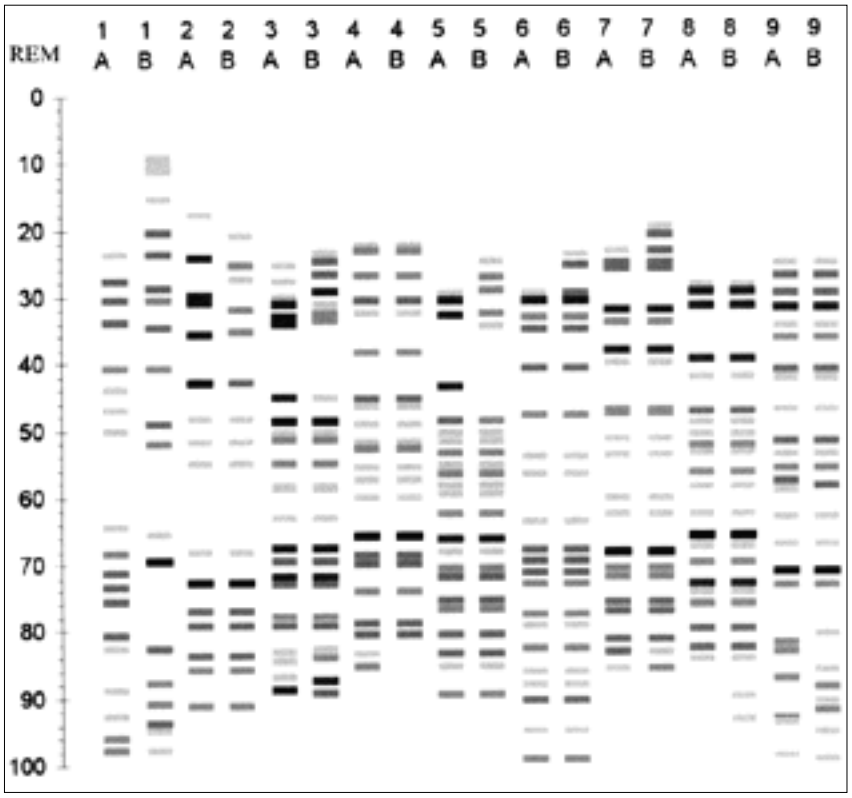
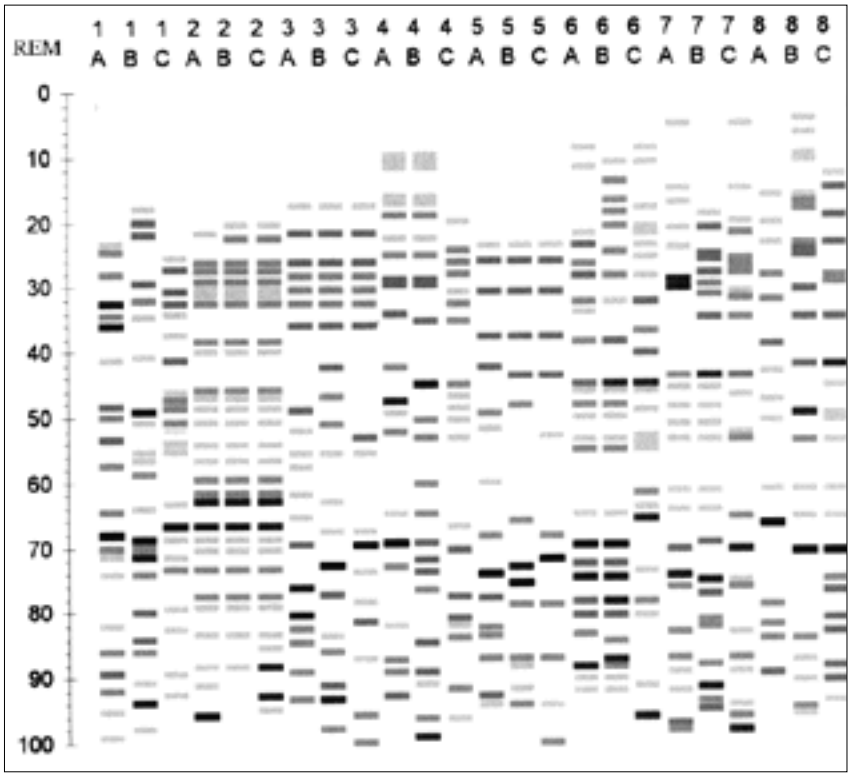


Figure 2. Hordein patterns in dimorphic seed samples of spring barley accessions

1 – Triumf, 2 – Bohatýr, 3 – Ratbořský, 4 – Valtický, 5 – Chlumecký, 6 – Tepelský 421, 7 – Židlochovický Gloria, 8 – Dobrovický staročeský, 9 – Olešenský

wheat (Šašek *et a*l. 1998), barley (Černý *et al*. 1999; Pomortsev 2001) and triticale (Vyhnánek & Bednář 2000). The detection of sister lines is limited by the number of analysed seeds, since approximately a three times higher number of seeds than the reciprocal frequency of a deviating type is required to capture it with a probability of 5%*). Environmental conditions may also affect the frequency and thus the detection of sister lines, since natural selection might prefer some sister line. Maintenance breeding



1 – Hanácký Jubilejní, 2 – Opavský Kneifl, 3 – Stupický plnozrnný, 4 – Stupický hanácký, 5 – Proskovcův hanácký, 6 – Hodonínský Kvas, 7 – Dregerův Imperial, 8 – Selecta Hanak 1

Figure 3. Prolamin patterns in trimorphic seed samples of spring barley accessions

can also change the frequency and number of sister lines. Šašek *et al*. (1998) described a case, where a dimorphic variety changed to a monomorphic. The frequency of sister lines in a cultivar can influence its agronomic performance, since their interactions with environment might be different. Langer *et al*. (1994) have found in the spring barley cultivars Rubín, Perun, Kredit and Profit differences in lodging, leaf rust resistance, grain fractions, 1000-grain weight and extract content between sister lines. Molina-Cano *et al*. (2001, 2002) observed similar effects in the variety Triumph. It is likely, that also admixtures of less related genotypes affect the agronomic performance.

Černý and Šašek (1998), analysing gliadins and HMW-glutenins in wheat landraces, have found polymorphism in 40% of the examined accessions, i.e. two to four protein lines. Pomortsev (2001), analysing hordeins in 147 Ethiopian barleys by starch gel electrophoresis, has found from one to six different hordein patterns per accession.

An efficient exploitation of polymorphic genetic resources requires separation and evaluation of lines with different protein pattern. A possible approach consists in the analysis of prolamins in the endosperm of individual seeds, selection of protein patterns and regeneration of plants from the corresponding embryos cultivated *in vitro*.

Conclusion

This paper describes the polymorphism of prolamin storage proteins in seed samples of 20 historical spring barley accessions from the collection of genetic resources of the Agricultural Research Institute Kroměříž, Ltd. The obtained data will be used for the creation of a catalogue of hordein patterns for the core collection of spring barley genetic resources as additional descriptors and for the description of possible heterogeneity of the accessions. This might improve the efficiency of exploitation of the analysed resources in breeding.

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Abstrakt

Vyhnánek T., Bednář J., Helánová S., Nedomová L., Milotová J. (2003): **Využití polymorfismu prolaminů k popisu genetické variability v kolekci genetických zdrojů ječmene.** Czech J. Genet. Plant Breed., **39**: 45–50.

U 20 genetických zdrojů jarního ječmene (*Hordeum vulgare* L.) českého a slovenského původu byl studován polymorfismus zásobních prolaminových bílkovin zrna pomocí polyakrylamidové gelové elektroforézy (PAGE). Větší heterogenita prolaminů byla detekována u starších genetických zdrojů. Byl vypočten index identity prolaminových bílkovin pro určení sesterských hordeinových linií ve vzorku zrna. Vyhodnocená prolaminová spektra budou využita k doplnění popisných údajů core kolekce ječmene jarního v kolekci genetických zdrojů Zemědělského výzkumného ústavu Kroměříž, s. r. o.

Klíčová slova: jarní ječmen; *Hordeum vulgare* L.; hordeiny, PAGE-ISTA; genetické zdroje

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