Agriculture (Poľnohospodárstvo), 55, 2009 (1): 2-9

HISTORICAL VARIETIES OF SPRING BARLEY (*HORDEUM VULGARE* L.) AND THEIR USE AS GENETIC RESOURCES OF MALTING QUALITY

HISTORICKÉ ODRŮDY JEČMENE JARNÍHO (*HORDEUM VULGARE* L.) A JEJICH VYUŽITÍ JAKO GENETICKÝCH ZDROJŮ SLADOVNICKÉ KVALITY

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PSOTA, V. – BRADOVÁ, J.: Historical varieties of spring barley (*Hordeum vulgare* L.) and their use as genetic resources of malting quality. Agriculture (Poľnohospodárstvo), vol. *55*, 2009, N. 1, pp. 2–9.

Genetic markers of prolamin proteins – hordeins were used for the evaluation of genetic resources of old barley varieties. In the studied set, newer varieties of spring barley had markedly lower hordein polymorphism than the older ones. The selected set of varieties reflects the breeding progress of the malting quality of spring barley, first of all starch modification and proteolytic modification. Amylolytic modification of the historical varieties corresponds to the current requirements. An acceptable level of cytolytic modification was observed only in the varieties Slovenský 802 and Čelechovický Hanácký; the other studied varieties exhibited weak activity of cytolytic enzymes. The variety Rubín had the best wort quality with respect to current requirements. The historical varieties or hordein lines detected in them can be used as donors of significant agronomic and qualitative traits of barley for the construction of new varieties in the breeding process.

Key words: barley, variety, hordein, electrophoresis, polymorphism, malting quality

Application of new technologies and use of genetic diversity for improving the biological potential of agricultural plants are principal contributions to the development of plant production. Innovation of varieties is one of the crucial factors for the enhancement of crop productivity. Implementation of biotechnological methods enables to increase selection efficiency and fasten the breeding process; it enables to extend genetic diversity of varieties and achieve new breeding goals.

Over the last decade the structure of Czech agriculture went through important changes that brought the increased requirements for specific quality of production and widening of the range of grown species and varieties of crops. Regional and old bred varieties with some characteristic traits that modern varieties often lack can be important initial materials for breeding improvements of grain quality in spring barley varieties and can thus contribute to the enlargement of varietal diversity and improvement of some traits. Considerable advance has also been made in the area of research of quality; new varieties are defined with a number of new quality parameters that, obviously, were not

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followed in old varieties. Typically, many modern varieties of spring barley have relatively narrow genetic basis and to a certain extent they are similar. Landraces that were many times created by a combination of natural selection and selection made by farmers, possess some valuable traits; these can significantly contribute to current breeding (Keller et al. 1991).

Protein genetic markers can be used for the evaluation of plant genetic resources. Protein genetic markers in barley include prolamin proteins – hordeins, a prerequisite of hordein use as genetic markers is given by their polymorphism, relatively simple determination using electrophoresis, specificity of hordein patterns in individual barley genotypes (lines, varieties), high heritability of hordein patterns and possibility of genetic interpretation of electrophoretic hordein patterns in a form of sets of allelic hordein blocks. Therefore, they are suitable for distinguishing and identification of the individual barley genotypes (lines, varieties). Electrophoresis of hordeins can be used for the description of the genetic structure of the varieties, i.e. for the determination whether the varieties are one-line or multi-line populations. Protein lines classified on the basis of the characteristics of storage proteins of one heterogeneous variety (population) can differ significantly in a number of important traits, therefore, the given methods can be used as the selection method. Signal protein genes can also be used for the identification of homozygotes or heterozygotes on the level of the individual analyzed grains. These genes can also mark some important agricultural characteristics. Protein genetic markers represent suitable tools for marking some agricultural parameters and characteristics of barley (Černý and Šašek 1998).

MATERIAL AND METHODS

The seed of historical barley varieties was obtained from various suppliers (Table 1). Electrophoretic hordein patterns were obtained using the method of vertical electrophoresis in columns of starch gel (ČSN 461085-1 1998). Alellic blocks of hordein zones *Hrd-A, Hrd-B, Hrd-F, Hrd-D, Hrd-E, Hrd-C,* and *Hrd-G* were singled out in the electrophoretic hordein patterns according to the catalogue of allelic hordein blocks (Bradová and Sýkorová 2006).

Samples (0.5 kg) were malted in a micro-malting plant. Laboratory malting was performed with the following method, traditionally used in the RIBM (Research Institute of Brewing and Malting). This method is principally the same as the method of Mebak (1997). Temperature of water and temperature of air in air rests in the course of steeping was 14.5°C. Steeping length: 1st day – 5 hours; 2nd day – 4 hours. The 3rd day water content in germinating grain was adjusted by steeping or spraying to 45.5%. Temperature during germination was 14.5°C. Total steeping and germination time was 144 hours. Kilning was conducted on a one-floor electrically heated kiln. Total kilning time was 22 hours, pre-kilning temperature 55°C, kilning temperature was 80°C for 4 hours. Technological parameters were determined pursuant to the methods of EBC (1998), Mebak (1997) and Basařová et al. (1992). Barley malting quality was assessed according to the malting quality index (Psota and Kosař 2005).

RESULTS AND DISCUSSION

Hordein polymorphism and frequency of hordein allelic blocks

Compared to the current varieties, historical varieties of spring barley are characterized by much higher hordein polymorphism (Bradová and Sýkorová 2005; Bradová and Sýkorová 2006; Bradová et al. 2001). In the set of 20 spring barley varieties, 42 hordein patterns (Table 2) were determined using the electrophoretic analysis of hordeins, i.e. on average ca 2 hordein patterns per each the variety analyzed. The set had totally 26 types of electrophoretic hordein pattern. Table 3 shows that the highest allelic variability was recorded at hordein locus *Hrd B,* where 14 hordein allelic blocks were identified. Lower allelic variability was determined at the locus *Hrd A,* with 11 allelic hordein blocks identified, the lowest diversity was found in the locus *Hrd F* (4 alellic hordein blocks). Hordein loci *Hrd C*, *Hrd D*, and *Hrd E* exhibited very low polymorphism. According to Černý and Šašek (1998) they are composed of only one gene in a dominant or recessive ("null allele") state.

In the given set of spring barley varieties, the following hordein blocks occurred most frequently: *HRD A2* (64.2%), *HRD B19* (28.6%), *HRD F1* (52.4%) (Table 3).

Opavský Kneifel, Valtický, Čelechovický Hanácký, Ekonom, Perun, Chlumecký, Dregerův Imperial, Branišovický C, Novun, and Rubín were hordein homogeneous one-line varieties, i.e. each line was characterized by only one type of the hordein electrophoretic pattern. Polymorphism in the electrophoretic pattern of hordeins was recorded in the varieties Norimbergský (6 hordein lines), Dobrovický staročeský, Slovenský 802, Stupický plnozrnný, Hanácký jubilejní, and Spartan (3 hordein lines), Slovenský dunajský trh, Hanácký Kargyn, Bohatýr, and Semčický hospodárský (2 hordein lines), (Table 4, Fig. 1). Electrophoresis detected minimal distribution of heterozygote grains (ca 1–2 grains) in the varieties Ekonom, Norimbergský, and Branišovický C.

Verification of barley variety origin using protein genetic markers

Hordein genetic markers can be used to assess, by checking the electrophoretic patterns, whether the polymorphic varieties are of sister character, i.e. whether they come from the same crossing, parental combination (Table 1, 4). At the same time the electrophoresis of hordein

T a b l e 2

Hordein polymorphism of historical varieties of spring barley

Number of varieties	20
Number of identified *Hrd* - lines	42
Mean number of *Hrd* - lines per variety	2.1
Number of polymophic varieties	11
Number of homogeneous varieties	9

T a b l e 4

Examples of the varieties with different hordein polymorphism

Variety	Hordein line	Hordein allelic blocks of loci		
		Hrd A	*Hrd B*	*Hrd F*
Opavský Kneifel	A	2	17	3
Valtický	A	2	19	1
Slovenský 802	A	2	47	1
	B	2	19	1
	C	2	17	3
	D	2	21	0
Ekonom	A	2	19	1

T a b l e 3

Diversity of hordein allelic blocks of historical varieties of spring barley

HRD allelic blocks, alleles											
Hrd locus A			*Hrd* locus B			*Hrd* locus F			Minority *Hrd* loci C, D, E		
Labelling	Number	%	Labelling	Number	%	Labelling	Number	%	Min.Hrd allele	Sn	%
1	2	4.8	1	3	7.0	0	2	4.8	C	3	7.0
2	27	64.2	6	2	4.8	1	22	52.4	D	1	2.4
4	2	4.8	8	1	2.4	2	6	14.3	E	7	16.7
12	2	4.8	9	1	2.4	3	12	28.6			
18	1	2.4	16	4	9.6						
21	1	2.4	17	3	7.0						
23	1	2.4	19	12	28.6						
24	1	2.4	21	2	4.8						
32	3	7.0	25	2	4.8						
34	1	2.4	29	3	7.0						
N2	1	2.4	45	1	2.4						
			47	5	12.0						
			53	1	2.4						
			64	2	4.8						

genetic markers allows to compare the electrophoretic patterns of the above mentioned markers of the parental varieties and their hybrids. If a hybrid variety is distinguished by the presence of a foreign protein allele, foreign allelic block, not occurring in the parental varieties, then the declared origin of this hybrid variety is not correct, legitimate. The variety Ekonom is an example of a legitimate origin, it comes from the declared crossing of parental varieties (Valtický x Kenia) and has a hordein pattern identical with the variety Valtický. Situation in the case of the polymorphic variety Slovenský 802, the origin of which is declared as a selec-

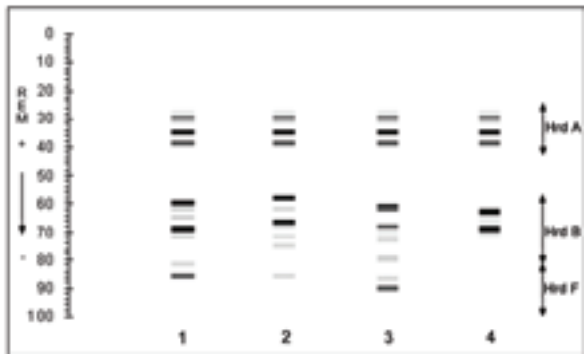

Fig. 1. An example of genetic structure of spring barley variety Slovenský 802. Relative electrophoretic mobility (REM). Loci encoding hordeins (*Hrd A*, *Hrd B*, *Hrd F*). Hordein line "A" (1), "B" (2), "C" (3) and "D" (4).

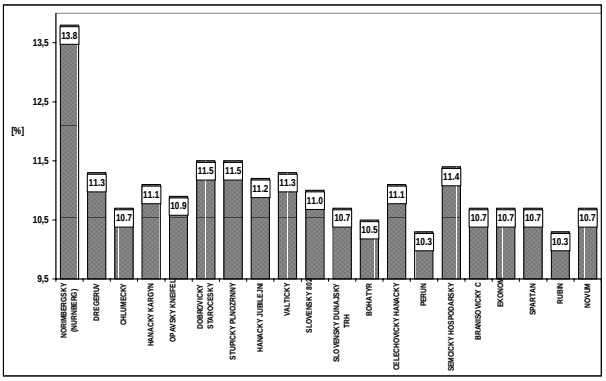

Fig. 2. Protein of grain barley

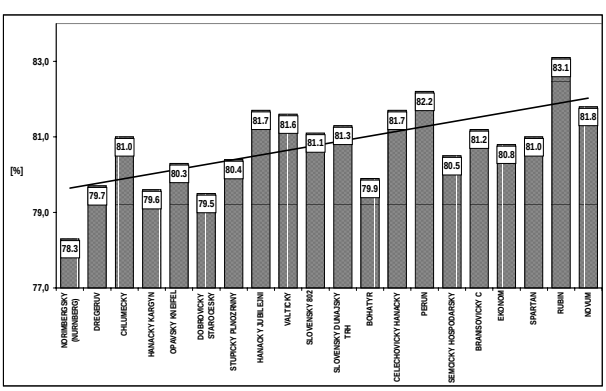

Fig. 3. Extract of malt

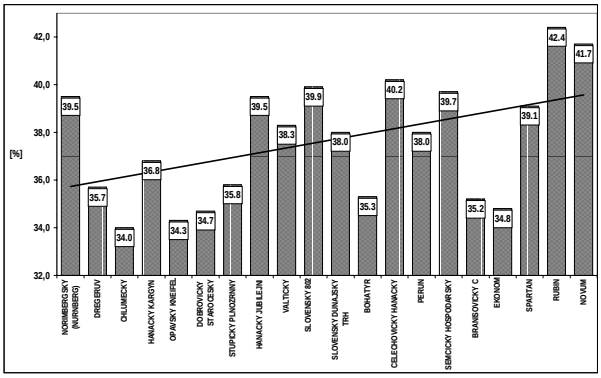

Fig. 4. Relative extract at 45°C

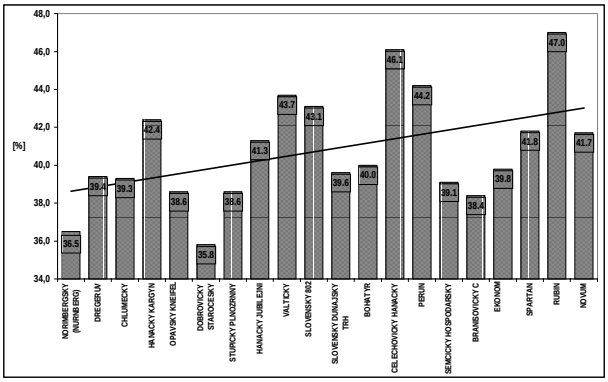

Fig. 5. Kolbach index

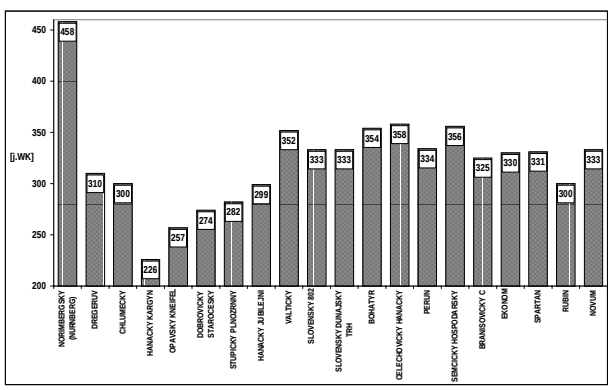

Fig. 6. Diastatic power

tion from the variety Opavský Kneifel, is rather more complicated. Slovenský 802 is characterized by four hordein lines, of which only one hordein line (line "C") corresponds to the hordein electrophoretic pattern of Opavský Kneifl.

Quality analyses

Quality of the historical varieties studied was assessed according to the current requirements (Fig. 2–10). The varieties were malted under the same conditions as the varieties within the registration procedure today.

With the exception of the variety Norimbergský (13.8%), content of nitrogenous substances of the samples of the other studied varieties was on the optimum level (10.2–11.0%) or close to it (Fig. 2).

Extract content in malt is a very important technological and economic parameter. It is evident from Figure 3 that this parameter has gradually been improved. It increased from the values of ca 79% (e.g. Dregerův Imperial) to values over 82% (Perun) or even higher than 83.1% (Rubín).

Proteolytic modification given by the values of relative extract at 45°C (Fig. 4) and Kolbach index (Fig. 5) has been improved as well and after some time varieties with the required levels of these parameters began to appear more frequently.

Diastatic power (Fig. 6) characterizing the level of amylolytic modification was not a principal problem in the studied historical varieties.

Situation with cytolytic modification was more complicated. Regular monitoring of the level of cytolytic modification started only at the beginning of the 1990s. Nevertheless, level of friability (Fig. 7) and b-glucan content in wort (Fig. 8) in some of the studied varieties was acceptable for today's malsters (Valtický, Slovenský 802, Perun, Čelechovický Hanácký).

Quality of wort evaluated indirectly using

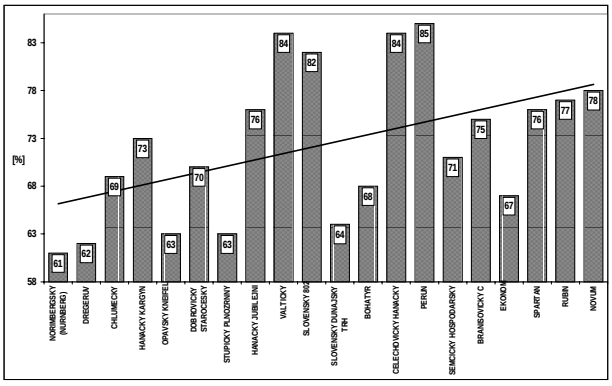

Fig. 7. Friability

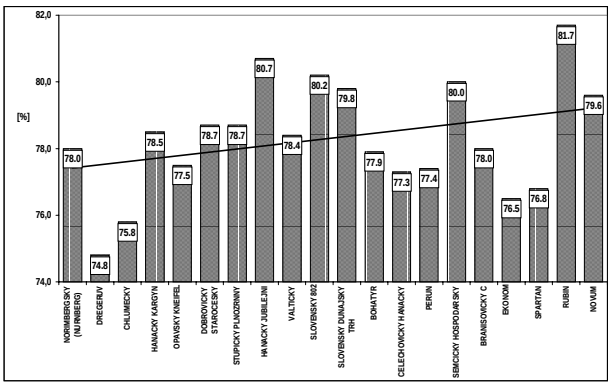

Fig. 9. Final attenuation of laboratory wort

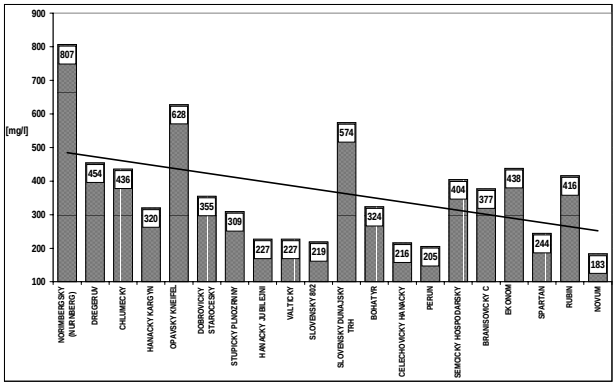

Fig. 8. b-glucan content of wort

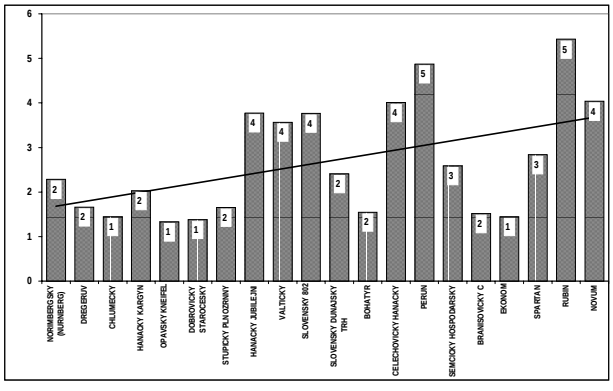

Fig. 10. Malting quality index

apparent final attenuation (Fig. 9) was in most of the historical varieties insufficient from today's point of view. The varieties with acceptable level of this parameter are as follows: Hanácký jubilejní, Slovenský dunajský trh, Slovenský 802, Semčický hospodárský, and Novum. Wort quality of the variety Rubín was on the level of the best current varieties (Psota and Horáková 2007).

Malting quality index (Fig. 10) which is used for the intervarietal comparison of quality of spring barley varieties, proves breeding progress even in this small set of historical varieties. The best results in terms of current malting requirements were determined in the varieties Perun and Rubín.

CONCLUSION

The younger varieties of spring barley were characterized by markedly lower hordein polymorphism than the older varieties in the studied set.

The studied varieties showed breeding progress in malting quality of spring barley, mainly in starch modification and proteolytic modification. Amylolytic modification of the historical varieties corresponded to the current requirements. Acceptable level of cytolytic modification was found only in the varieties Slovenský 802 and Čelechovický Hanácký; the other varieties studied, showed only weak activity of cytolytic enzymes. Wort quality in terms of today's requirements was the best in the variety Rubín.

The historical varieties or hordein lines detected in them can be applied purposefully as donors of significant agronomic and qualitative traits of barley for the construction of new varieties in the breeding process.

Acknowledgements. The results were obtained during solution of the projects of MSM6019369701 "Research of malting and brewing raw materials and technologies" and MZE0002700602 "Study and utilization of biodiversity, genetic mechanism and new methods with the aim to improve the biological potential of cultivars and sustainable development of agriculture".

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SOUHRN

V souboru 20 odrůd jarního ječmene bylo elektroforetickou analýzou hordeinů zjištěno celkem 42 hordeinových spekter. Soubor se vyznačoval celkem 26 typy elektroforetického hordeinového spektra. Nejvyšší alelickou variabilitou se vyznačoval hordeinový lokus *Hrd B* (14 hordeinových alelických bloků). Nižší alelická variabilita byla zjištěna na lokuse *Hrd A* (11 hordeinových alelických bloků) a nejnižší diverzitou se vyznačoval lokus *Hrd F* (4 hordeinové alelické bloky). Nízkým polymorfismem se vyznačují hordeinové lokusy *Hrd C*, *Hrd D* a *Hrd E*. Nejčastěji se vyskytovaly hordeinové bloky *HRD A2* (64,2 %), *HRD B19* (28,6 %), *HRD F1*(52,4 %). Polymorfismus v eletroforetické skladbě hordeinů prokázaly odrůdy Norimberský (6 hordeinových linií), Dobrovický staročeský, Slovenský 802, Stupický plnozrnný, Hanácký jubilejní a Spartan (3 hordeinové linie), Slovenský dunajský trh, Hanácký Kargyn, Bohatýr a Semčický hospodářský (2 hordeinové linie). U odrůd Ekonom, Norimberský a Branišovský C byla pomocí elektroforézy zjištěna heterozygotní zrna. Ostatní odrůdy byly hordeinově homogenní. Odrůda Ekonom byla vyšlechtěna křížením odrůd (Valtický x Kenia) a vyznačuje se identickým hordeinovým spektrem právě k odrůdě Valtický. U polymorfní odrůdy Slovenský 802, která vznikla výběrem z odrůdy Opavský Kneifel, je situace složitější. Slovenský 802 je charakterizován 4 hordeinovými liniemi, z čehož pouze linie „C“ odpovídá hordeinovému spektru Opavského Kneifelu. Kromě vzorku odrůdy Norimberský (13,8 %) měly vzorky ostatních odrůd obsah dusíkatých látek na optimální úrovni (10,2–11,0 %) nebo se obsah dusíkatých látek této úrovni blížil. Obsah extraktu se šlechtěním dařilo postupně zvyšovat. Z hodnot asi 79 % (Dregerův Imperiál) vzrostl až na hodnoty vyšší než 82 % (Perun, Rubín). Proteolytické rozluštění se zlepšovalo a postupně se začaly objevovat odrůdy, u nichž bylo na dnes požadované úrovni. Diastatická mohutnost nebyla pro sledované, historické odrůdy problémem. Horší to bylo s úrovní cytolytického rozluštění, přesto mají odrůdy Valtický, Slovenský 802, Perun, Čelechovický Hanácký úroveň friability a obsahu b-glukanů ve sladině na dnes požadované úrovni. Pouze odrůdy Hanácký jubilejní, Slovenský dunajský trh, Slovenský 802, Semčický hospodářský, Novum a Rubín vykazovaly dosažitelný stupeň prokvašení na dnes akceptovatelné úrovni. Historické odrůdy se mohou uplatnit jako donory významných hospodářských vlastností ječmene při konstrukci nových odrůd.

Klíčová slova: ječmen, odrůda, hordein, elektroforéza, polymorfismus, sladovnická kvalita