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Breeding of waxy barleys using molecular markers[1]

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SUMMARY – Low-amylose starch is characteristic of specific physico-chemical properties and in barley (*Hordeum vulgare* L.) it is connected with high contents of beta-glucans, non-starch polysaccharides with health benefit. A lower proportion of amylose of endosperm starch in grain of initial barley *waxy* mutants is caused by different expression of granule-bound starch synthase I (GBSS I, E.C. 2.4.1.11) encoded at the *Waxy* loci. DNA sequence analysis of the insertion/deletion polymorphism, analyzed by PCR reaction at the 5´leader sequence of the *waxy* gene, was employed as a molecular marker of the presence of endosperm *waxy* character in hybrids. Use of various *waxy* mutants and modern *waxy* cultivars for hybridization with productive parental partners induced high variability of the yield, vegetative and morphological parameters. The effect of different genetic background on amylose content and other examined agronomic traits of the low-amylose barley lines for food use is discussed.

Introduction

Barley grain, due to its chemical composition, is a suitable raw material for nutrition of both healthy populations and consumers endangered by chronic diseases. The proportion of barley for direct consumption is not high (2 to 5%) in the Czech Republic and other European countries. However, an increasing interest in this cereal can be envisaged due to greater attention which is paid to healthy nutrition. Therefore, breeding activities should be aimed at the development of new varieties with the required grain chemical composition, acceptable organoleptic properties and satisfactory grain yield.

Starch and non-starch polysaccharides ((1→3,1→4)-β-D-glucans and pentosans) are important nutritional components of barley grain. The polysaccharide content and composition affect the physico-chemical characteristics, and therefore the possible end-use of barley. Starch technological quality depends on the proportions of the two main starch polymers, amylose and amylopectin. In commonly grown barley varieties, the contents of amylose and amylopectin in starch are ca. 25-30% and 74-79%, respectively. However, there are some types that contain starches with high (>35%, high-amylose) or, conversely, low proportions of amylose (0-10%, zero- and low-amylose). Endosperms with lower amylose content have a bright light colour and are flourier and softer, and are therefore designated as "waxy". Low-amylose barleys tend to be associated with increased levels of (1→3,1→4)-β-D-glucans. These non-starch polysaccharides have higher extractability (Izydorczyk *et al*. 2000, Swanston 1997), and have been shown to have numerous health benefits (e.g. in lowering blood cholesterol, reducing elevated levels of glucose and insulin in diabetes, providing weight control).

Amylose synthesis in endosperm of barley grain is catalyzed by the GBSSI enzyme (granule-bound starch synthase I, E.C. 2.4.1.11) encoded by the *waxy* gene that is localized on chromosome 7HS (Rohde *et al*., 1988). The *Waxy* starch phenotype is due to mutations at this locus (Domon *et al*., 2002, Patron *et al*., 2002). Lines with decreased amylose content, in which deletion of part of the promoter and 5'-untranslated region of the gene alters the spatial and/or temporal expression of GBSSI in the endosperm, and zero-amylose genotypes (from chemically mutagenized populations of wild-type barley) have been detected. Varieties of hulless spring barley with low/zero-amylose starch have been registered in Canada and the USA (Bhatty and Rossnagel, 1997, Washington *et al*., 2000)

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and more *waxy* barleys have been produced in Japan, Korea and Australia. Nevertheless, they cannot be grown under Czech soil and climatic conditions because of their low resistance to lodging and leaf diseases, and low productivity (Ehrenbergerova *et al*., 2003).

Domon *et al*. (2002) detected insertion/deletion polymorphisms in the *Waxy* locus using PCR to amplify the 5´leader sequence of the *waxy* gene from a selection of different barley genetic resources. They suggested appropriate molecular markers (designated p-197 and p+606) to detect the presence of three different *Waxy* alleles (*waxy*, *Waxy* and novel *Waxy*) affecting grain endosperm starch. The variability in the *Waxy* locus will provide an effective genetic marker for selecting the *waxy* allele at the seedling stage in a breeding program for *waxy* barley varieties. Simultaneous use of verified molecular markers (DNA-marker-assisted selection) accelerates the breeding process of low-amylose materials (Polakova *et al*., 2004).

Materials and methods

Plant material

Spring barley materials with the *waxy* gene from the USA (C.W. Newman, Montana University), and Canadian registered *waxy* varieties of hulless spring barley maintained in the Collection of Spring Barley at the Agricultural Research Institute Kromeriz, Ltd. and our own hybrid materials derived from crosses of *waxy* donors with hulled productive varieties and new breeding material of malting barley (see Table 1) were studied. The experimental material was grown in the field after oilseed rape as a preceding crop in 2004 and 2005. Depending on available amount of grain, they were planted in a breeding nursery (0.82 cm long rows, 3.75 x 7.5 cm spacing, hand sowing) or in plots of the 10 m^2 area (yield trials, in four replications). In the breeding nursery, both the parental varieties, progeny of earlier hybrid combinations to detect the *Waxy* allele, and the plants selected from segregating materials with the *waxy* phenotype (verified previously), were grown.

Agronomic data from field evaluation (emerging, heading and ripening date, height in cm, lodging and disease resistance on a scale 9-1) in the yield trials were collected according to a Descriptor List of the Genus *Hordeum* L. (Lekes *et al*., 1986). After harvesting, the grain yield (in $t.ha^{-1}$) and 1000 kernel weight (in g) were calculated. Manually harvested randomly selected plants from breeding nurseries were taken for assessment of the following yield parameters: number of tillers and of productive tillers (NT, NPT), number of kernels per plant and ear (NKP, NKE), weight of kernel per plant and ear (WKP, WKE), 1000 kernel weight (TKW), and harvest index (HI, for parents only).

Molecular methods and chemical analyses

DNA Extraction: DNA was isolated from 14 days old plants using the DNeasy Plant Mini Kit (Qiagen) with some modifications. About 50 mg of fresh leaves were directly transferred into the tubes containing mixture of the AP1 Buffer, RnaseA, and a carbide bead. Plant cells were pulverized by a Qiagen-Mixer Mill MM 300. All of the other steps followed the Qiagen Handbook. DNA concentrations and qualities were determined using spectrophotometry and electrophoresis.

Molecular Markers Analyses: PCR products were generated using the verified primer pair p-197 (5'-CAAACAGACGACAAGCGGAGAA-3´, forward) and p+606 (5'-TAGAAAAAGAAAACATCAAGCA-3´, reverse). The PCR reaction was performed in 20 µl volume as described by Domon *et al.* (2002) and Polakova *et al*. (2004). The length polymorphism of PCR products was evaluated after electrophoretic separation in 1.5% agarose gel and visualisation with ethidium bromide on a UV transilluminator.

Chemical Analyses: beta-glucan content (BG) was assessed using a mixed-linkage beta-glucan assay procedure (McCleary method, McCleary and Glennie-Holmes, 1985) and the proportions of amylose and amylopectin with an amylose/amylopectin assay kit (a modification of a Con A method developed by Yun and Matheson, 1990), both from Megazyme.

Results were analyzed by standard statistical methods using STATISTICA 7.0 for Windows (StatSoft, Inc.).

Results and discussion

Since 2000, a simple colorimetric iodine test (Seguchi *et al*., 2000) has been employed to select progenies developed by crossing with *waxy* donors. Verification of the proportions of amylose and amylopectin proportions using chemical methods is possible in later generations after crossing but only when a sufficient amount of grain is available. Variability in the *Waxy* locus detected with the ins/del markers enabled the parental varieties, homozygous and heterozygous lines to be differentiated (Fig. 1). Thus, DNA-MAS selection can be also used to test hybrid materials that also segregate for other characteristics. Hybrids developed by crossing NoD7 and Nordus with *waxy* CDC Candle, HB803 and Merlin and selected using DNA-MAS (Polakova *et al*., 2004) have become the basis for new breeding materials, and further crosses with other productive malting varieties (Barke, Madeira, Prestige) have been carried out. Assessment of the yield components of the individual parental plants (Table 1) as well as other evaluations of a total of 1,343 reselected plants from segregating progenies (results not shown) confirmed that the lowest variability (except in plant height) was found in grain weight (TKW), in contrast with the parameter of productive tillering (NPT) and other derived components. In accordance with our previous findings (Vaculova and Machova-Polakova 2004), the newly developed hybrids also differed according to individual alleles of the *waxy* gene. Furthermore, within lines with the *waxy* allele, the hulless lines surpassed the covered ones in NPT, NKP and WKP. However, average values of the characteristics and the variability depended on the selected parents, e.g. the variety Nordus contributed to higher average grain yield per unit area more as a male than as a female parent. There were differences in yield components among individual parents. Although most of these were not significant due to high variability, the total compensatory effect of the main yield components was confirmed. It is a pity that higher values for important yield components (such as NPT or NKE) were found in materials with the longest stems and the lowest levels of resistance to fungal diseases (data not shown) because these agronomic traits are critical factors of yield productivity under our soil and climatic conditions. Nevertheless, Jeung *et al*. (1998) reported that barleys with the *waxy* allele did not have reduced yield characters. The results of evaluation in yield trials (Table 2) demonstrate different yielding ability of the new materials with *waxy* endosperm starch. Chemical analyses showed that low-amylose genotypes were selected, and in agreement with the results of Swanston (1997), different levels of beta-glucans were present in these materials. The new lines are valuable initial donors for further breeding work aimed at developing barley varieties for specialty food and industrial uses.

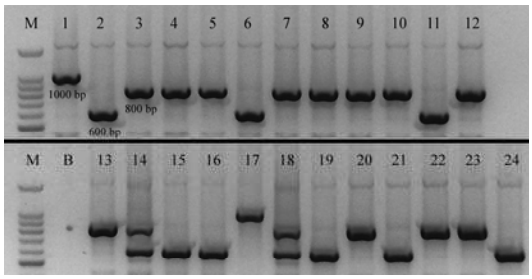

Fig. 1. Example of the length polymorphisms in the *Waxy* locus of the parental and hybrid materials. Lanes: M=100-bp ladder; B=blank; 1=breeding line No94609D7 (shortly NoD7); 3=variety Nordus (both covered spring barleys with normal type of starch, bred by NORDSAAT Saatzuchtgesellschaft mbH, Germany); 2=variety CDC Candle (a waxy hulless variety, bred at the CDC Saskatoon, Canada); 4,5,7,8,9,10,12,13,20,22 and 23=homozygous hybrid materials with 800 bp (Waxy); 6,11,15,16,19,21 and 24=homozygous hybrid materials with 600 bp (waxy); and 17=homozygous hybrid material with 1000 bp (novel Waxy) fragments. Lanes 14 and 18 demonstrate the co-dominant character of the hybrid with heterozygous (Waxy/waxy) character of endosperm starch.

Table 1. Results of the assessment of the yield parameters and their variability in the individual plants of the selected hybrid parents

Variety	Grain type	N	Height, cm††			NPT			NKP			NKE		
			mean	s_x	CV%	mean	s_x	CV%	mean	s_x	CV%	mean	s_x	CV%
NoD7†	cov-N	37	89.1	1.3	8.8	8.2	0.6	46.9	180.1	14.2	48.1	21.9	0.6	16.6
Nordus	cov	24	96.5	0.9	4.4	7.5	0.6	36.3	166.3	14.5	42.7	21.7	0.7	16.9
HB803	nud-w	66	88.6	0.8	7.5	4.4	0.2	40.7	92.0	5.0	43.9	21.1	0.4	15.2
Wapana	cov-w	50	102.3	1.0	6.7	7.8	0.4	40.3	169.6	10.2	42.4	21.7	0.4	13.2
Washonubet	nud-w	61	97.3	1.0	8.0	6.7	0.4	44.2	155.0	10.2	51.3	22.8	0.5	18.3
Wabet	cov-w	44	101.9	1.1	7.0	7.5	0.4	37.8	187.5	12.0	42.6	24.6	0.5	13.8
Wanubet	nud-w	48	102.2	0.8	5.6	7.3	0.4	41.8	169.9	11.6	47.4	22.7	0.4	13.5
Merlin	nud-w	33	66.2	0.7	6.4	5.5	0.3	35.2	103.7	7.7	42.4	18.7	0.6	17.3
CDC Candle	nud-w	33	104.1	0.9	4.8	7.2	0.4	34.8	180.1	13.0	41.3	24.8	0.7	15.9
Variety	Grain type	N	WKP, g			WKE, g			TKW, g			HI		
			mean	s_x	CV%	mean	s_x	CV%	mean	s_x	CV%	mean	s_x	CV%
NoD7†	cov-N	37	6.6	0.5	48.9	0.81	0.03	22.8	36.9	0.7	11.4	47.7	1.6	20.2
Nordus	cov	24	6.8	0.6	42.8	0.89	0.03	18.7	41.2	1.0	12.0	49.0	1.4	14.2
HB803	nud-w	66	3.8	0.2	45.6	0.88	0.02	22.1	41.6	0.7	13.6	48.3	1.0	16.3
Wapana	cov-w	50	7.3	0.5	45.2	0.93	0.02	17.2	42.8	0.6	10.4	49.1	1.1	15.9
Washonubet	nud-w	61	4.7	0.3	50.9	0.71	0.02	24.1	30.7	0.5	13.8	41.3	0.9	17.7
Wabet	cov-w	44	6.3	0.4	43.5	0.82	0.02	19.6	33.1	0.6	11.6	42.6	0.9	14.6
Wanubet	nud-w	48	5.8	0.4	50.1	0.76	0.02	18.4	33.6	0.4	8.5	45.4	0.8	12.3
Merlin	nud-w	33	3.5	0.3	48.5	0,62	0.02	23.0	32.7	0.6	10.2	43.1	1.6	21.3
CDC Candle	nud-w	33	5.7	0.4	43.1	0.79	0.03	20.3	31.7	0.4	6.6	51.6	1.0	10.8

†See Fig.1; ††See Materials and methods; CV = Coefficient of variability.

Table 2. Results of the field performance and nutritional quality analyses of new hybrids and varieties with *waxy* starch character (2004-2005)

Variety, Line	Grain type††	Yield, % std†††	TKW, g	Height, cm	Amylose, %	Amylo-pectin, %	Beta-glucan, %
Nordus x CDC Candle	nud-w	81.81	32.4	85	6	94	6.06
Nordus x CDC Candle	nud-w	55.43	35.4	105	10	90	6.52
Nordus x CDC Candle	nud-w	66.19	38.5	100	8	92	6.92
Nordus x CDC Candle	cov-w	102.27	41.3	110	12	88	5.90
Nordus x CDC Candle	nud-w	73.33	37.6	112	9	91	6.21
Wanubet x KM1057	nud-w	57.56	41.4	93	9	91	9.18
(Wabet x Wsnb) x Wsnb†	cov-w	85.71	39.6	81.5	11	89	8.03
(Wabet x Wsnb) x Wsnb	cov-w	85.66	40.7	84.5	21	79	7.78
Merlin x NoD22	nud-w	66.30	39.9	84	21	79	6.09
Wanubet x KM1057	nud-w	63.44	32.7	76	11	89	6.42
HB 803 x NoD22	nud-w	70.26	46.7	98	9	91	7.33
NoD7 x Merlin	nud-w	66.25	40.4	88	7	93	6.45
NoD7 x Merlin	nud-w	66.91	37.1	74	9	91	6.23
NoD7 x HB803	nud-w	66.91	39.2	81	9	91	7.11
Nordus x CDC Candle	nud-w	53.21	36.4	75	9	91	6.21
NoD7 x CDC Candle	nud-w	67.39	38.0	85	10	90	6.94
Nordus x CDC Candle	nud-w	60.00	30.6	90	8	92	6.21
NoD7 x CDC Candle	nud-w	70.55	38.1	106	7	93	6.21
Candle	nud-w	75.81	36.0	126	8	92	6.45
HB803	nud-w	54.61	44.5	97	11	89	7.46
Merlin	nud-w	57.61	40.0	77	5	95	5.72
Alamo	nud-w	62.92	44.2	105	8	92	7.41
Annabell, std†	cov	98.75	44.3	89	25	75	4.72
Tolar, std	cov	101.25	48.5	95	21	79	5.35

†std = standard variety; Wsnb = Washonubet, Nord 92 KOO15D22 = shortly NoD22; ††nud-w = hulless grain, *waxy* starch, cov-w = covered grain, *waxy* starch; †††see Materials and methods.

References

Bhatty, R.S. and Rossnagel, B.G. (1997). *Cereal Chemistry,* 74: 190-191.

Domon, E., Fuijita, M. and Ishikawa, N. (2002). *Theoretical and Applied Genetics,* 104: 132-138.

Izydorczyk, M.S., Storsley, J., Labossiere, D., MacGregor, A.W. and Rossnagel, B.G. (2000). *Journal of Agricultural and Food Chemistry,* 48: 982-989.

Ehrenbergerova, J., Vaculova, K., Psota, V., Havlova P. and Šerhantova, V. (2003). *Plant Soil Environment,* 49: 443-450.

Jeung, J., Park, K.G. and Blake, T. (1998). *Barley Newsletter,* 42.

Lekeš, J. *et al*. (1986). *Klasifikátor genus Hordeum* L., 46 pp.

McCleary, B.V. and Glennie-Holmes, M. (1985). *J. Inst. Brew.,* 91: 285-295.

Patron, N.J., Smith, A.M., Fahy, B.F., Hylton, C.H., Naldrett, M.J., Rossnagel, B.G. and Denyer, K. (2002). *Plant Physiology,* 130: 191-198.

Polakova, K., Vaculova, K. and Kucera, L. (2004). In: *9th International Barley Genetics Symposium*, Brno, pp. 566-573.

Rohde, W., Becker, D. and Salamini, F. (1988). *Nucleic Acids Research,* 16: 7185–7186.

Seguchi, M., Yasui, T., Hosomi, K. and Imai, T. (2000). *Cereal Chemistry,* 77: 339-342.

Swanston, J.S. (1997). *Cereal Chemistry,* 74: 452-455.

Vaculova, K. and Machova-Polakova, K. (2004). In: *Advances in Molecular Biology: Methods for Genotype Identification, Plant Breeding and Product Control*, Prague, 3: 61-62.

Washington, J.M., Box, A., Karakousis, A. and Barr, A.R. (2000). In: *Barley Genetics VIII*, *8th International Barley Genetics Symposium*, Adelaide, pp. 303-306.

Yun, S.H. and Matheson, N.K. (1990). *Starch/Starke,* 42: 302-305.