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THE EFFECT OF THE CHANGED AMYLOSE AND AMYLOPECTIN RATIO ON THE SELECTED QUALITATIVE PARAMETERS IN SPRING BARLEY (*HORDEUM VULGARE* L.) GRAIN

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Abstract

BALOUNOVÁ MARTA, VACULOVÁ KATEŘINA, HLOŽKOVÁ LENKA, MIKULÍKOVÁ RENATA, EHRENBERGEROVÁ JAROSLAVA: *The effect of the changed amylose and amylopectin ratio on the selected qualitative parameters in spring barley (Hordeum vulgare L.) grain.* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2013, LXI, No. 3, pp. 577–585

In 2008–2011, grain yield and chemical composition were assessed in a set of spring barley genotypes with standard and changed amylose/amylopectin ratio (non-waxy and waxy, respectively) at two locations (Kroměříž, Žabčice). Genotypic as well as environmental effects were important for the variations in contents of chemical compounds. The waxy genotypes had statistically significantly higher levels of β-glucans and protein (6.9% d.m., 12.1% d.m., respectively) than genotypes with the standard starch character (5.4% d.m., 11.5% d.m., respectively). The hull-less waxy genotype KM2619.413.4 was statistically significantly different from all other genotypes in the set due to its highest content of β-glucans (10.80% d.m.). We found statistically significant differences between genotypes divided by the type of grain (hulled vs. hull-less) and also by the starch character (standard vs. waxy) in average starch contents as follows: hull-less genotypes with the standard starch composition (64.8% d.m.) > hull-less waxy genotypes (63.7% d.m.) > hulled genotypes with standard starch composition (61.4% d.m.) > hulled waxy genotypes (60.0% d.m.). Genotypes with elevated grain constituents (higher starch and BG contents) could be important from the point of a prospective use of barley for food production as was already found for example in the hulless waxy genotypes KM2645.412.3.4.6 (63.7% and 8.0%, respectively) and KM2551.469.1-2 (64.1% and 7.0%, respectively).

waxy, β-glucan, arabinoxylans, new breeding genotypes

Barley (*Hordeum vulgare* L.) is an ancient cereal traditionally used for malting and in food and feed industries all over the world. In recent years, its almost forgotten nutritional qualities have been rediscovered. Mixed-linkage β-glucans are unique to the cell walls in grasses (Poaceae). Barley and oat (*Avena sativa* L.) grains have relatively high (l→3),(1→4)-β-D-glucan concentrations when compared to other cereals. Typically, three to six percent of (l→3),(1→4)-β-D-glucan is accumulated in barley kernel, but higher concentrations are present in some genetic resources (Cory *et al.*, 2012). Approximately 75% of (l→3),(1→4)-β-D-glucans occur in the endosperm, and the remaining 25% in the aleurone layer. β-glucans together with arabinoxylans belong to the group of non-starch polysaccharides (Newman & Newman, 2008). The levels of β-glucans and arabinoxylans are influenced by both genetic and environmental factors; the genetic factors appear to be of greater importance (Zheng *et al.*, 2011). Actual levels of β-glucan may differ dramatically between barley varieties. The amount of (l→3),(1→4)-β-D-glucan accumulated in grains is a major factor determining the end-use of barley. Low (l→3),(1→4)-β-D-glucan barley is preferred by the feed, malting and brewing industries. For human nutrition, (l→3),(1→4)-β-D-glucan has become a desirable food ingredient due to its cholesterol-lowering properties, it reduces the risk of cardiovascular disease (Hang *et al.*, 2007), blood pressure (Behall *et al.*, 2006), glucose and insulin responses (Nilsson *et al.*, 2006), lowers the risk for type 2 diabetes (Chen & Raymond, 2008) and controls body weight and prevents obesity (Shimizu *et al.*, 2008; El Khoury *et al.*, 2012). Food processing of barley grain is much easier if forms with hull-less grain are used.

Barley starch is primarily composed of two forms of carbohydrate polymers, amylose and amylopectin. The amylose and amylopectin ratio in the barley endosperm is an important grain characteristic affecting malting, food, and feed quality (Swanston *et al.*, 1995). The three groups of barleys have been identified depending on the ratio of these components: "*waxy*" (low amylose), standard (the ratio of amylose and amylopectin ~ 1:3) and "high-amylose" barleys. The recessive *wax* gene located on chromosome 7HS controls the waxy endosperm character (Nakao, 1950). The *waxy* barley genotypes with changed amylose and amylopectin ratio tend to increased levels of (l→3),(1→4)-β-D-glucan in the grain compared to barley genotypes with the standard starch character. The methods of molecular genetics allow selection of the desired recessive materials from early generations after crossing. Domon *et al.* (2002) detected insertion/deletion polymorphisms in the *waxy* locus and suggested molecular marker (p-197 and p+606) for the detection of the presence of three different *waxy* alleles (*waxy*, *Waxy* and novel *Waxy*) affecting barley starch composition in the barley grain endosperm. This molecular marker can be used in molecular assisted selection (MAS) for a simple selection of genotypes with a reduced portion of amylose.

In the present study, hulled and hull-less spring barley breeding genotypes with the genetically determined presence of *waxy* recessive allele were investigated for their agronomic characteristics and chemical composition of the grain. The aim of this study was to search for new barley genetic resources with high (l→3),(1→4)-β-D-glucan levels and improved agronomic parameters.

MATERIALS AND METHODS

Experimental material

Altogether 50 own spring barley breeding genotypes (developed on the basis of hybridization with Canadian hull-less waxy barley varieties CDC Candle, HB 803 and Merlin in the Agricultural Research Institute Kroměříž, Ltd.) were studied. Xanadu was used as the control variety. A set of breeding genotypes included 4 hulled genotypes with the standard amylose/amylopectin ratio, 6 hulled genotypes with starch waxy character, 10 hull-less genotypes with the standard amylose/amylopectin ratio and 30 genotypes with starch waxy character (Tab. I).

Field experiments

Experimental genotypes were grown in the field plots of 10 m^2 (yield trials, two replications) after oilseed rape as a previous crop at two locations in 3-4 cultivation years (Kroměříž 2008–2011, Žabčice 2009–2011). The standard crop management practice for spring barley was used. The grain yield (in t/ha) and thousand kernel weight (TKW in g) were calculated after harvesting and is set at 14% moisture. Grain yield was determined from unsorted grains. Separated grains above 1,8 mm sieve for hull-less genotypes and above 2,2 sieve for hulled genotypes were used for determination of TKW and chemical analyses.

Chemical analysis

Total (l→3),(1→4)-β-D-glucan content (BG) was determined enzymatically using a Mixed-Linkage β-Glucan Assay procedure: McCleary Method (Megazyme Int. Ireland Ltd., Bray, Wicklow, Ireland). In brief, milled barley samples were suspended and hydrated in a sodium phosphate buffer solution of pH 6.5 and incubated with purified lichenase enzyme. The glucose produced was then analyzed using a glucose/peroxidase reagent.

The spectrophotometric method by Douglas (1981) was used for the determination of arabinoxylan content (AX). This method is based on the hydrolysis of arabinoxylans to pentose sugars, which then react with floroglucinol.

The content of crude protein (further only protein) was determined by the Dumas method (ICC Standard Method No. 167). The nitrogen detected after combustion of each sample was converted into nitrogenous substances by multiplying by a conversion factor of 6.25.

After the hydrolysis of starch with hydrochloric acid, starch content was determined by Ewers method (EN ISO 10520, 1999).

The water content in the samples was determined by CSN EN ISO 712 (2010). All results of chemical analyses are reported as % on dry basis (d.m.).

Statistical analysis

Experimental data were assessed using STATISTICA software, version 10.0 (StatSoft, Inc., Tulsa, Oklahoma, USA). The variation coefficient was determined as a rate of variability (in %). Analysis of variance (main components) was used to assess the effect of individual factors on variability of the observed parameters. The averages were compared by division into homogenous groups (HG) using generalization of Turkey's test to the case of unequal samples sizes at a significance level α = 0.05 (i.e. unequal n HSD test). Mutual relationships between the observed characters were evaluated by using the correlation.

I: *List of the studied genotypes*

Nr.	Genotype	type of grain	char. 1)	Nr.	Genotype	type of grain	char.
1	KM1910-2	hull-less	stand.	27	KM2645.412.1.1.1-1	hull-less	waxy
2	KM2283-1	hull-less	stand.	28	KM2645.412.1.1.1-2	hull-less	waxy
3	KM2460.312.494.3-1	hulled	waxy	29	KM2645.412.1.1.4	hulled	waxy
4	KM2460.312.494.3-2	hulled	waxy	30	KM2645.412.3.4.6	hull-less	waxy
5	KM2530.721-1	hull-less	stand.	31	KM2645.412.5.1.12	hull-less	waxy
6	KM2551.333.6	hull-less	stand.	32	KM2645.412.6.1.13	hull-less	waxy
7	KM2551.469.1	hull-less	waxy	33	KM2646.415.4	hull-less	waxy
8	KM2551.469.1-2	hull-less	waxy	34	KM2646.417.2	hull-less	waxy
9	KM2619.413.4	hull-less	waxy	35	KM2647.418.6	hull-less	waxy
10	KM2619.413.5	hull-less	waxy	36	KM2666.644	hulled	stand
11	KM2620.352.9	hull-less	waxy	37	KM2678.287.7	hull-less	stand
12	KM2624.102	hulled	waxy	38	KM2691.289.2	hulled	stand
13	KM2624.314.4	hull-less	waxy	39	KM2691.290.1	hulled	stand
14	KM2624.316.4	hull-less	waxy	40	KM2691.386.5.10	hulled	stand
15	KM2624.419.3.5	hull-less	waxy	41	KM2693.390.1.5	hull-less	stand
16	KM2640.411.3.2.9	hull-less	waxy	42	KM2696.648.13	hull-less	stand
17	KM2640.411.5.5.2	hull-less	waxy	43	KM2700.307	hull-less	stand
18	KM2640.411.7.2.11	hulled	waxy	44	KM2700.949	hull-less	stand
19	KM2640.411.7.3.12	hull-less	waxy	45	KM2719.718.1	hull-less	waxy
20	KM2641	hull-less	stand.	46	KM2719.718.4	hull-less	waxy
21	KM2642.416.4.3	hull-less	waxy	47	KM2719.718.5	hull-less	waxy
22	KM2642.416.4.5	hulled	waxy	48	KM2719.718.7	hull-less	waxy
23	KM2642.416.5	hull-less	waxy	49	KM2911.623.2	hull-less	waxy
24	KM2642.74	hull-less	waxy	50	KM2911.623.5	hull-less	waxy
25	KM2645.355.1	hull-less	waxy	51	Xanadu, std. 2)	hulled	stand
26	KM2645.412.1.1.1	hull-less	waxy				

1) character of the starch – standard or waxy; 2) control variety

II: *The effect of the individual factors on variability of the studied parameters*

source of variability	df 1)	MS 2)					
		yield	TKW	protein	starch	β-glucans	arabinoxylans
genotype	50	2.34***	65.94***	3.47***	21.00***	7.15***	2.09***
location	1	8.55***	40.70**	37.73***	2.9	5.26***	0.01
year	3	136.16***	286,60***	102.26***	51.92***	3.21***	15.19***
error	234	0.57	4.91	0.78	1.9	0.26	0.33

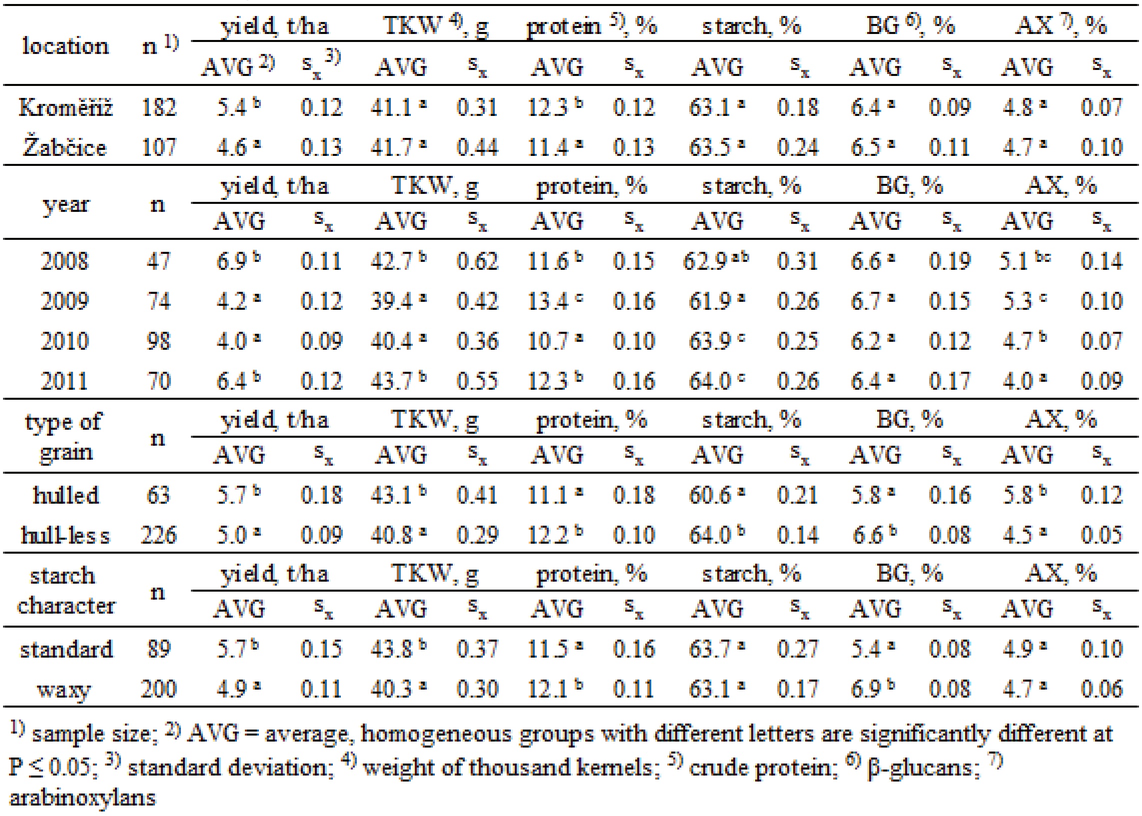
1) df – degrees of freedom; 2) mean square

RESULTS AND DISCUSSION

According to the results of ANOVA, genotype and cultivation year affected all observed parameters statistically significantly. The location influenced grain yield, TKW, protein and BG contents (Tab. II).

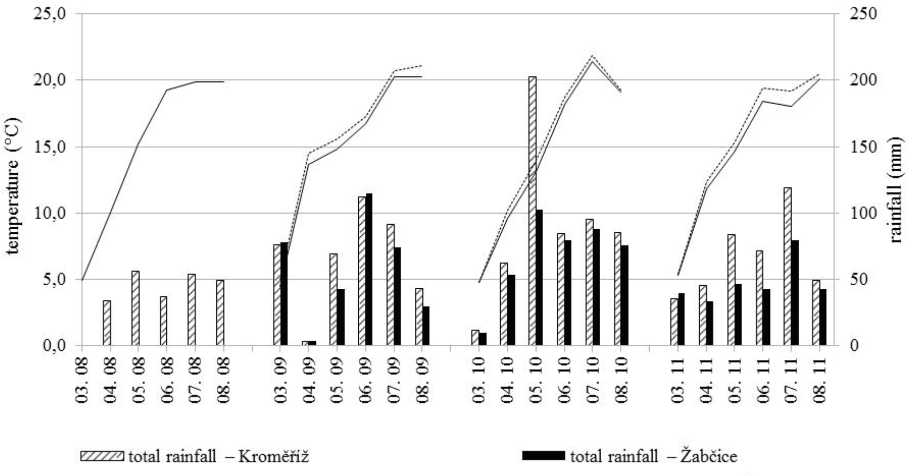
Differences between cultivation years were found for all the studied characters except BG content, while the effect of location (Kroměříž or Žabčice) was statistically significant only for grain yield and content of protein (Tab. III). The group of hull-less genotypes had statistically significantly lower average yield (5.0 t/ha) than the hulled genotypes (5.7 t/ha). The average value of TKW was also statistically significantly lower in the group of hull-less (40.8 g) than in the hulled genotypes (41.7 g). Previous studies reported that these reduced values of grain yield and TKW were partly caused by the absence of the hull in the hull-less genotypes (Barabaschi *et al.*, 2012, Dickin *et al.*, 2012) because the hull comprises from 10% to 13% of barley dry matter (Bhatty, 1999). The hulled and hull-less barley genotypes of the set also statistically differed in their average contents of all studied grain constituents. The waxy genotypes had statistically significantly lower average values of grain yield (4.9 t/ha) and TKW (4.3 g) than those with the standard starch character (5.7 t/ha and 43.8 g). However, they also had higher levels of BG and protein (6.9% d.m., 12.1% d.m., respectively) than genotypes with the standard starch character (5.4% d.m., 11.5% d.m., respectively).

III: *Differences between average values of examined characters of locations and years (Kroměříž, Žabčice, 2008–2011)*



location	n $^{1)}$	yield, t/ha		TKW $^{4)}$, g		protein $^{5)}$, %		starch, %		BG $^{6)}$, %		AX $^{7)}$, %	
		AVG $^{2)}$	s_x $^{3)}$	AVG	s_x	AVG	s_x	AVG	s_x	AVG	s_x	AVG	s_x
Kroměříž	182	5.4 ^{b}	0.12	41.1 ^{a}	0.31	12.3 ^{b}	0.12	63.1 ^{a}	0.18	6.4 ^{a}	0.09	4.8 ^{a}	0.07
Žabčice	107	4.6 ^{a}	0.13	41.7 ^{a}	0.44	11.4 ^{a}	0.13	63.5 ^{a}	0.24	6.5 ^{a}	0.11	4.7 ^{a}	0.10
year	n	yield, t/ha		TKW, g		protein, %		starch, %		BG, %		AX, %	
		AVG	s_x	AVG	s_x	AVG	s_x	AVG	s_x	AVG	s_x	AVG	s_x
2008	47	6.9 ^{b}	0.11	42.7 ^{b}	0.62	11.6 ^{b}	0.15	62.9 ^{ab}	0.31	6.6 ^{a}	0.19	5.1 ^{bc}	0.14
2009	74	4.2 ^{a}	0.12	39.4 ^{a}	0.42	13.4 ^{c}	0.16	61.9 ^{a}	0.26	6.7 ^{a}	0.15	5.3 ^{c}	0.10
2010	98	4.0 ^{a}	0.09	40.4 ^{a}	0.36	10.7 ^{a}	0.10	63.9 ^{c}	0.25	6.2 ^{a}	0.12	4.7 ^{b}	0.07
2011	70	6.4 ^{b}	0.12	43.7 ^{b}	0.55	12.3 ^{b}	0.16	64.0 ^{c}	0.26	6.4 ^{a}	0.17	4.0 ^{a}	0.09
type of grain	n	yield, t/ha		TKW, g		protein, %		starch, %		BG, %		AX, %	
		AVG	s_x	AVG	s_x	AVG	s_x	AVG	s_x	AVG	s_x	AVG	s_x
hulled	63	5.7 ^{b}	0.18	43.1 ^{b}	0.41	11.1 ^{a}	0.18	60.6 ^{a}	0.21	5.8 ^{a}	0.16	5.8 ^{b}	0.12
hull-less	226	5.0 ^{a}	0.09	40.8 ^{a}	0.29	12.2 ^{b}	0.10	64.0 ^{b}	0.14	6.6 ^{b}	0.08	4.5 ^{a}	0.05
starch character	n	yield, t/ha		TKW, g		protein, %		starch, %		BG, %		AX, %	
		AVG	s_x	AVG	s_x	AVG	s_x	AVG	s_x	AVG	s_x	AVG	s_x
standard	89	5.7 ^{b}	0.15	43.8 ^{b}	0.37	11.5 ^{a}	0.16	63.7 ^{a}	0.27	5.4 ^{a}	0.08	4.9 ^{a}	0.10
waxy	200	4.9 ^{a}	0.11	40.3 ^{a}	0.30	12.1 ^{b}	0.11	63.1 ^{a}	0.17	6.9 ^{b}	0.08	4.7 ^{a}	0.06

$^{1)}$ sample size; $^{2)}$ AVG = average, homogeneous groups with different letters are significantly different at $P \leq 0.05$; $^{3)}$ standard deviation; $^{4)}$ weight of thousand kernels; $^{5)}$ crude protein; $^{6)}$ β-glucans; $^{7)}$ arabinoxylans



1: *Overview of weather conditions (Kroměříž 2008–2011, Žabčice 2009–2011)*

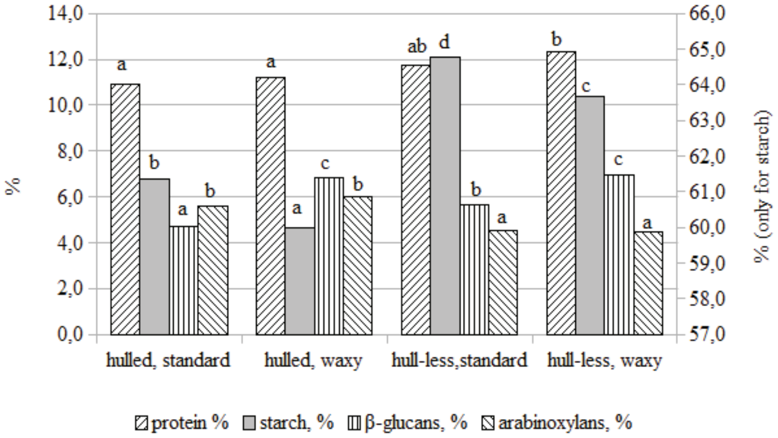
The most variable parameter in the set of spring barley genotypes was grain yield, its coefficient of variation ranged from 10.8% (KM2640.411.7.2) to 53.6% (KM2619.413.4). These high levels of the coefficient of variation were probably caused by large differences between years (Fig. 1). Although the average yields had a large range of values (2.8–6.2 t/ha), no statistically significant differences between genotypes were revealed (Tab. IV).

Genotypic as well as environmental effects were important for the variations in contents of chemical compounds. Average protein contents ranged from 11.0% d.m. to 14.7% d.m. in hull-less genotypes and from 10.8% d.m. to 11.7% d.m. in hulled genotypes. Similar values of protein ranging from 10.2% d.m. to 14.5% d.m. were also measured in the set of hulled and hull-less barley genotypes by Hang *et al.* (2007). The group of hull-less waxy genotypes had statistically significantly higher content of protein (12.3% d.m.) than hulled standard and hulled waxy genotypes (10.9% d.m., 11.2% d.m., respectively) – see Fig. 2.

IV: *Average values of observed parameters and their distribution into the homogenous groups in the set of new breeding genotypes*

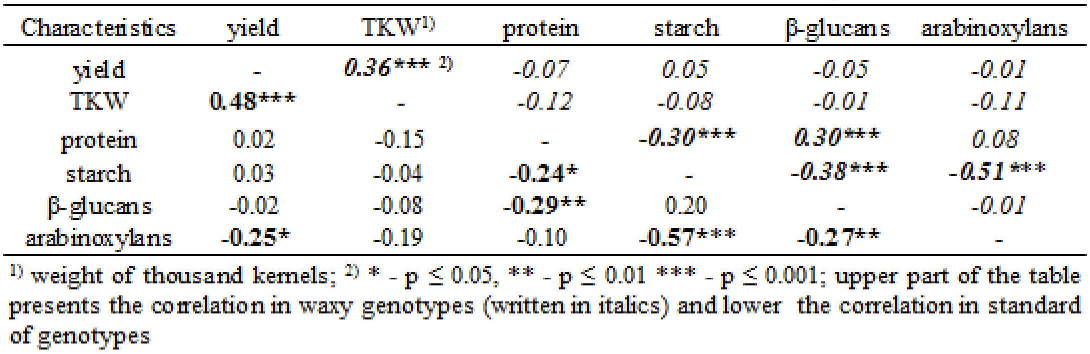
Nr. 1)	yield, t/ha		TKW 4), g		protein 5), %		starch, %		BG 6), %		AX 7), %	
	avg±s_x 2)	HG 3)	avg±s_x	HG	avg±s_x	HG	avg±s_x	HG	avg±s_x	HG	avg±s_x	HG
1	4.5±0.93	a	43.5±1.68	b-j	12.2±0.49	ab	64.9±0.79	l-q	5.0±0.14	a-e	4.7±0.45	a-g
2	5.4±0.43	a	41.5±1.63	a-j	11.7±0.83	ab	64.1±0.61	f-q	5.9±0.22	b-m	5.1±0.36	a-h
3	6.2±0.52	a	40.0±0.52	a-i	11.6±0.57	ab	59.2±0.31	ab	7.8±0.17	qr	5.7±0.40	b-h
4	5.6±0.45	a	40.1±0.20	a-i	11.4±0.49	ab	59.8±0.36	a-e	7.9±0.19	qr	5.6±0.36	b-h
5	5.9±0.61	a	39.9±1.39	a-h	12.1±0.78	ab	66.3±0.92	q	5.3±0.25	a-f	4.0±0.31	a-c
6	6.2±0.60	a	47.4±0.80	j	12.3±0.78	ab	61.8±1.09	a-o	6.4±0.10	e-p	4.5±0.23	a-g
7	5.3±0.71	a	47.0±1.74	ij	13.1±0.54	ab	62.2±0.41	b-p	7.5±0.20	o-r	4.5±0.21	a-f
8	4.7±0.92	a	47.0±1.93	g-j	12.9±1.14	ab	64.1±1.13	c-q	7.0±0.37	h-r	3.9±0.17	a-d
9	2.8±0.68	a	37.9±0.68	a-f	14.7±0.61	b	58.0±0.57	a	10.8±0.41	s	5.1±0.09	a-h
10	4.1±0.92	a	37.6±0.95	a-f	11.6±0.81	ab	65.1±0.81	j-q	7.1±0.29	k-r	4.5±0.12	a-g
11	4.8±0.74	a	39.4±1.44	a-g	12.3±0.47	ab	64.7±0.41	l-q	6.3±0.20	d-p	4.1±0.32	a-c
12	5.4±0.95	a	47.4±1.21	j	11.7±0.34	ab	60.2±0.80	a-f	6.2±0.09	d-n	5.9±0.28	d-h
13	5.1±0.58	a	44.0±1.23	e-j	12.1±0.42	ab	64.5±0.54	h-q	7.1±0.38	j-r	4.3±0.27	a-e
14	4.5±0.98	a	42.9±2.46	a-j	12.3±0.78	ab	61.4±1.38	a-p	7.4±0.63	m-r	4.5±0.14	a-g
15	4.0±0.62	a	44.4±1.92	b-j	12.4±0.44	ab	61.9±0.67	a-q	7.9±0.20	p-r	4.0±0.16	a-f
16	4.8±0.72	a	40.0±1.76	a-i	11.5±0.54	ab	65.5±0.51	pq	6.8±0.25	h-r	4.4±0.23	a-f
17	5.3±0.41	a	42.7±1.28	b-j	11.8±0.58	ab	64.5±0.44	h-q	6.7±0.23	g-q	4.1±0.35	a-c
18	5.5±0.27	a	42.4±0.58	a-j	10.8±0.51	a	60.6±0.20	a-k	6.9±0.29	h-r	6.3±0.27	gh
19	4.7±0.60	a	34.9±1.10	a	11.8±0.40	ab	63.8±0.40	g-q	6.8±0.23	h-r	4.6±0.18	a-g
20	5.9±0.57	a	44.8±1.09	f-j	11.3±0.59	ab	66.3±0.30	q	5.3±0.09	a-f	3.8±0.20	b
21	4.4±0.61	a	38.3±1.19	a-f	12.1±0.59	ab	64.6±0.59	i,k-q	6.4±0.20	e-p	4.3±0.17	a-c,e
22	5.6±0.56	a	40.5±1.04	a-j	11.0±0.80	ab	60.5±0.60	a-i	5.8±0.15	b-l	6.7±0.34	h
23	4.1±0.72	a	40.4±1.15	a-j	13.2±0.81	ab	62,5±0.91	b-q	6.3±0.17	c-p	5.0±0.23	a-h
24	5.2±0.53	a	41.7±1.09	b-j	12.1±0.49	ab	64.7±0.30	k-q	5.4±0.15	b-g	4.5±0.23	a-g
25	4.5±0.77	a	37.2±0.32	a-f	11.7±0.60	ab	64.6±0.64	j-q	6.7±0.16	f-r	4.7±0.20	a-g
26	5.0±0.86	a	39.8±0.68	a-j	11.8±1.06	ab	64.7±0.74	f-q	6.3±0.06	c-q	4.5±0.25	a-g
27	4.4±0.68	a	36.9±1.18	a-e	12.0±0.57	ab	63.7±0.47	e-q	7.0±0.35	h-r	4.4±0.42	a-f
28	5.6±0.47	a	41.0±1.29	a-j	11.6±0.55	ab	64.7±0.46	k-q	6.8±0.21	h-r	4.6±0.30	a-g
29	5.3±0.65	a	42.0±0.89	a-j	10.3±0.49	a	59.6±0.55	a-d	6.2±0.18	c-o	6.0±0.42	e-h
30	5.4±0.65	a	41.3±1.00	a-j	12.2±0.43	ab	63.7±0.60	f-q	8.0±0.16	r	4.1±0.27	a-c
31	4.4±0.66	a	36.0±0.84	ab	12.4±0.42	ab	63.0±0.35	b-q	7.7±0.27	qr	4.4±0.23	a-f
32	5.1±0.83	a	37.0±1.10	a-e	11.8±0.49	ab	63.1±0.53	c-q	7.5±0.19	n-r	4.4±0.15	a-f
33	4.2±0.89	a	43.1±2.04	c-j	13.1±0.81	ab	63.5±0.69	d-q	7.1±0.30	n-r	4.2±0.25	a-c
34	4.2±0.86	a	40.7±0.18	a-j	12.7±0.78	ab	63.7±0.78	e-q	7.1±0.22	k-r	4.6±0.29	a-g
35	5.0±0.55	a	42.0±1.28	b-j	11.5±0.78	ab	65.7±0.64	pq	5.4±0.18	a-g	4.6±0.34	a-g
36	5.0±0.60	a	42.2±0.74	a-j	11.3±0.70	ab	61.1±0.92	a-m	5.0±0.25	a-d	5.8±0.23	c-h
37	5.5±0.60	a	46.9±1.53	h-j	12.2±0.46	ab	65.4±0.49	o-q	5.4±0.12	b-g	4.5±0.31	a-g
38	5.5±0.42	a	41.3±1.47	a-j	11.1±0.92	ab	60.7±0.91	a-n	5.0±0.13	a-e	5.5±0.57	a-h
39	5.9±0.52	a	43.7±0.68	d-j	10.8±0.40	a	61.0±0.58	a-m	5.0±0.16	a-c	6.0±0.30	e-h
40	5.9±0.53	a	44.9±0.99	f-j	11.3±0.81	ab	60.5±0.56	a-g	4.5±0.23	ab	5.6±0.59	b-h
41	5.6±0.44	a	42.9±1.21	b-j	11.7±0.65	ab	63.5±0.89	d-q	6.2±0.29	c-o	4.6±0.40	a-g
42	5.4±0.48	a	42.7±1.77	c-j	11.5±0.49	ab	64.7±0.56	l-q	5.7±0.18	b-i	4.8±0.19	a-g
43	6.1±0.75	a	43.0±0.54	b-j	11.0±0.45	ab	65.3±0.77	o-q	5.7±0.12	b-i	4.4±0.29	a-f
44	6.1±0.66	a	42.5±0.94	b-j	11.5±0.62	ab	65.2±0.71	n-q	5.8±0.23	b-m	4.7±0.43	a-g
45	4.8±0.60	a	36.3±1.05	a-d	13.5±1.07	ab	62.3±0.80	b-q	7.1±0.21	k-r	5.1±0.28	a-h
46	4.8±0.52	a	36.6±0.86	a-c	12.9±0.89	ab	63.9±1.12	f-q	7.1±0.29	k-r	4.8±0.25	a-g
47	4.8±0.67	a	36.1±0.86	a-c	13.0±0.72	ab	63.8±1.19	f-q	7.1±0.19	k-r	4.7±0.30	a-g
48	4.8±0.87	a	36.5±0.71	a-e	12.7±0.69	ab	63.1±0.77	b-q	7.5±0.20	m-r	4.8±0.24	a-g
49	4.6±0.37	a	39.1±1.18	a-j	11.5±0.64	ab	63.5±0.43	b-q	5.7±0.18	b-l	3.6±0.38	ab
50	4.9±0.50	a	39.2±1.26	a-j	11.9±0.44	ab	63.9±0.75	c-q	5.5±0.65	a-h	3.7±0.44	ab
51	6.1±0.71	a	46.8±0.97	h-j	10.2±0.61	a	63.0±0.52	c-q	4.1±0.14	a	5.0±0.29	a-h

1) see Tab. I for origins; 2) average±standard deviation; 3) homogeneous groups with different letters are significantly different at $P \leq 0.05$; 4) weight of thousand kernels; 5) crude protein; 6) β-glucans; 7) arabinoxylans



2: *Average contents and their differences in the groups divided according to the type of grain and starch character (homogenous groups with different letters are significantly different at P ≤ 0.05)*

V: *Matrix of correlation coefficients between the observed characteristics in the set of waxy and standard genotypes*



Characteristics	yield	TKW1)	protein	starch	β-glucans	arabinoxylans
yield	-	***0.36****** 2)	*-0.07*	*0.05*	*-0.05*	*-0.01*
TKW	**0.48*****	-	*-0.12*	*-0.08*	*-0.01*	*-0.11*
protein	0.02	-0.15	-	***-0.30******	***0.30******	*0.08*
starch	0.03	-0.04	**-0.24***	-	***-0.38******	***-0.51******
β-glucans	-0.02	-0.08	**-0.29****	0.20	-	*-0.01*
arabinoxylans	**-0.25***	-0.19	-0.10	**-0.57*****	**-0.27****	-

1) weight of thousand kernels; 2) * - p ≤ 0.05, ** - p ≤ 0.01 *** - p ≤ 0.001; upper part of the table presents the correlation in waxy genotypes (written in italics) and lower the correlation in standard of genotypes

The observed interval of starch contents measured (from 58.01% d.m. to 66.33% d.m.) were in agreement with those previously reported by Helm & Francisco (2004). We found statistically significant differences between genotypes divided by the type of grain (hulled vs. hull-less) and also by the starch character (standard vs. waxy) in average starch contents as follows: hull-less genotypes with the standard starch composition (64.8% d.m.) > hull-less waxy genotypes (63.7% d.m.) > hulled genotypes with standard starch composition (61.4% d.m.) > hulled waxy genotypes (60.0% d.m.), (Tab. IV). Ehrenbergerová *et al.* (2003) found in their set of waxy and non-waxy genotypes significantly higher starch content in the standard varieties than in the waxy types. Based on the correlation analysis (Tab. V) statistically highly significant, nevertheless low negative correlation was found between starch and protein contents, both in waxy genotypes (r = −0.30***) and also in genotypess with a standard starch composition (r = −0.24*). This negative correlation was also found by the authors Hartman *et al.* (2010) in the set of malting spring barley genotypes (r = −0.79***).

The content of BG varied in the range from 4.15% d.m. to 6.39% d.m. in the barley forms with standard starch composition and from 5.37% d.m. to 10.80% d.m. in waxy barley forms. These values are comparable to those achieved by Vaculová *et al.* (2008) and Eticha *et al.* (2010). Our results are in accordance with the claim that barley genotypes with altered ratio of the main starch polysaccharides (amylose and amylopectin) tend to higher BG contents in the grain (Faustnaught *et al.*, 1996, Hang *et al.*, 2007; Baik & Ullrich, 2008 and others). Numerous studies have also found a higher content of BG in hull-less barley compared to hulled barley (Bhatty, 1999; Izydorczyk *et al.*, 2000; Wirkijowska *et al.*, 2012).

This tendency was also recorded in our study; the hull-less genotypes had statistically significantly higher average BG content (6.62% d.m.) than the hulled genotypes (5.85% d.m.). The hull-less waxy genotype KM2619.413.4 was statistically significantly different from all other genotypes in the set due to its highest content of BG (10.80% d.m.). However, this genotype also had the lowest yield (by 55% compared with the average of the set). No effects of the growing year and location on the BG content were found. However, some research papers (Morgan & Riggs, 1981; Swanston *et al.*, 1997; Swanston & Ellis, 2002) reported that the total BG content increased when plants were grown under hotter and drier conditions. In our study, we found a negative correlation between starch and BG contents (r = –0.38***), but only in waxy genotypes. Genotypes with elevated grain constituents (higher starch and BG contents) could be important from the point of a prospective use of barley for food production as already found for example in the hulless genotype KM2645.412.3.4.6 (Tab. IV, No. 30).

The hull-less genotypes had statistically significantly lower content of AX (4.47% d.m.) than the hulled genotypes (5.80% d.m.), but the differences between waxy and standard genotypes in AX content were not found. This is in accordance with findings of Gupta *et al.* (2010) that the presence of the waxy gene in barley does not affect the increase of the AX content as in the case of BG. The genotype and the growing year also affected AX content significantly (Tab. III). The influence of both genetic and environmental factors on the AX content was described by Holtekjkølen *et al.* (2008). Some previous studies focused mainly on malting spring barley genotypes reported statistically significant negative relationship between the levels of BG and AX in the grain (Březinová-Belcredi *et al.*, 2009; Vaculová *et al.*, 2011). Results achieved in our research confirmed this relationship only in genotypes with standard starch composition (r = –0.27**); in waxy genotypes, no important correlation was recorded. In both groups (waxy, standard) a significant negative correlation between AX and starch contents (r = –0.51***; r = –0.57***, respectively) was found.

CONCLUSION

New selected waxy genetic resources are a possible source of β-glucans in health-promoting food products. These genotypes were transferred to the Collection of Genetic Resources of Spring Barley maintained at the Genebank in the Czech Republic – KM2460.312.494.3-1 (No. 3), KM2460.312.494.3-2 (No. 4), KM2551.469.1-2 (No. 8), KM2624.316.4 (No. 14), KM2640.411.7.2.11 (No. 18), and KM2645.412.1.1.1 (No. 26). The basic description of this new genotypes is available on http://genbank.vurv.cz/genetic/resources/asp2/default_c.htm.

SUMMARY

A total of 51 spring barley genotypes with genetically identified allele of the waxy gene (changed amylose/amylopectin ratio) were grown in the field plots of 10 m^2 (yield trials, two replications) after oilseed rape as a previous crop at two locations (Kroměříž and Žabčice) in the period of 2008 (only in Kroměříž) – 2011. The grain yield (in t/ha) and thousand kernel weight (TKW in g) were calculated after harvest. Contents of starch, crude protein, β-glucans and arabinoxylans were determined using chemical analyses. The effects of the genotype, growing location (Kroměříž, Žabčice), year (2008–2011), type of grain (hulled vs. hull-less) and starch character (standard, waxy) on individual characters were studied. The aim of this project was to search for new barley genetic resources with high genotypic (l→3),(1→4)-β-D-glucan levels and improved agronomic parameters.

Genotypic as well as environmental effects were important for the variations in contents of chemical compounds, grain yield and thousand kernel weight. We found statistically significant differences between genotypes classified by the type of grain (hulled vs. hull-less) and starch character (standard vs. waxy) in average starch contents: hull-less genotypes with standard starch composition (64.8% d.m.) > hull-less waxy genotypes (63.7% d.m.) > hulled genotypes with standard starch composition (61.4% d.m.) > hulled waxy genotypes (60.0% d.m.). The waxy genotypes had statistically significantly lower average values of grain yield (4.9 t/ha) and thousand kernel weight (4.3 g) than those with standard character of starch (5.7 t/ha and 43.8 g). They also had higher levels of β-glucans and protein (6.9% d.m., 12.1% d.m., respectively) than the genotypes with standard starch character (5.4% d.m., 11.5% d.m., respectively). No differences between waxy and standard genotypes in arabinoxylans contents were found. New selected waxy genetic resources were transferred to the Collection of Genetic Resources of Spring Barley maintained at the Genebank in the Czech Republic.

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