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THE INFLUENCE OF GENOTYPE AND ENVIRONMENT ON ARABINOXYLAN AND BETA-GLUCAN CONTENTS IN GRAIN OF SPRING BARLEY (*HORDEUM VULGARE* L.)

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Abstract

MACHÁŇ PAVEL, EHRENBERGEROVÁ JAROSLAVA, CERKAL RADIM, BENEŠOVÁ KAROLÍNA, VACULOVÁ KATEŘINA. 2014. The Influence of Genotype and Environment on Arabinoxylan and Beta-glucan Contents in Grain of Spring Barley (*Hordeum vulgare* L.). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 62(3): 553–560.

Arabinoxylan and beta-glucan contents are limiting factors for a wider use of barley production. Arabinoxylan and beta-glucan contents were assessed in grain samples in sets of seven malting hulled varieties, three hull-less lines and one hull-less spring variety grown in the localities of Branišovice, Žabčice, and Kroměříž in 2009 to 2011. Further, the effect of growing technologies on the level of these non-starch polysaccharides was studied. Variability of arabinoxylan contents was affected most significantly by a genotype and growing technology whereas variability of beta-glucan contents was mostly affected by a genotype and growing environment (interaction of year with locality). The highest values of arabinoxylans and beta-glucans were determined in the grain samples of hull-less lines (KM 1057: 6.16% of arabinoxylans and KM 2084: 6.41% of beta-glucans) and on the contrary, the lowest values of arabinoxylans were found in the grain of hull-less variety AF Lucius (3.85%) and lowest amounts of beta-glucans were found in malting variety Radegast (3.92%). The samples of the growing technology without fungicide treatment had on average more arabinoxylans and beta-glucans than the fungicide non-treated ones.

Keywords: hull-less barley, hulled barley, non-starch polysaccharides, growing technology, grain quality

INTRODUCTION

Barley grain is traditionally used as a basic raw material for malt production; it is also used as feed for livestock. Currently its use as food or raw material for production of functional food has been restored (Baik & Ullrich, 2008; Havrlentová *et al.*, 2011). For this use, content of non starch polysaccharides is a limiting quality parameter. The most important non-starch polysaccharides include hemicelluloses, namely arabinoxylans and beta-glucans, basic construction substances of cell walls. Cell walls of the starch endosperm of grain contain 70% of beta-glucans and 25% of arabinoxylans (Lazaridou *et al.*, 2008; Jamar *et al.*, 2011).

Arabinoxylans (also known as pentosans) have a main chain formed by (1→4)-ß-D-xylanopyrosyl units substituted with α-L-arabinofuranose (Niño-Medina *et al.*, 2009). Specific character of barley arabinoxylans is their esterification with ferulic acid (approximately 0.07% to 0.11% of total mass) (Ehrenbergerová *et al.*, 2012) conferring them antioxidant properties.

Beta-glucans of barley grain are formed by β-glycosyl residues polymerized by β-(1→3) and β-(1→4) bonds. Solubility of beta-glucans declines with their growing molecular weight (Cyran *et al.*, 2002) and increases with higher degree of disruption of regular β-(1→4) structure by β-(1→3) bonds (Lazaridou & Biliaderis, 2007). Arabinoxylan solubility is subject to their molecular weight, arabinose-to-xylose ratio (at least 0.60 of soluble arabinoxylans) and amount of ferulic acid (Li *et al.*, 2005; Izydorczyk & Dexter, 2008).

Arabinoxylans and beta-glucans are capable to create in water viscous solutions and become thus a valuable component of food fiber, on the other side; they can also cause problems at wort filtration during brewing and reduce the nutritional values of feed in livestock (Iji, 1999; Lu & Li, 2006; Baik & Ullrich, 2008; Newman & Newman, 2008; Zavřelová, 2014).

On the other hand, arabinoxylans and beta-glucans have been shown to have a positive impact on human health (Chandalia *et al.*, 2000; Keogh *et al.*, 2003; Behall, *et al.*, 2004; Slavin, 2005; Behall, 2006; Salas-Salvadó *et al.*, 2007; Shimizu *et al.*, 2008; Chen & Raymond, 2008; Babio *et al.*, 2010).

The aim of the study was to determine the ratio of the effect of varieties/lines, environment (localities and years) and growing technologies on beta-glucan and arabinoxylan content and variability in the set of hulled and hull-less varieties and lines of spring barley.

MATERIALS AND METHODS

Arabinoxylans and beta-glucans were assessed in grain (Tab. I) of seven malting hulled varieties, three hull-less lines and one hull-less spring barley variety grown at the localities of Branišovice, Žabčice, and Kroměříž in 2009 to 2011 (Tab. II). At each of the localities, field trials were established using a triplicate randomized block design with orthogonally arranged variations. Production of varieties/lines from three blocks was mixed and consequently cleaned and screened by sifting. Fractions selected by a 2.5 mm sieve opening for the hulled, and 2.0 mm for the hull-less genotypes were used for the analyses. Grain was homogenized with laboratory mill (Super Jolly SJ 500). The course of weather in the individual localities is shown in Fig. 1.

The experimental materials were grown using two systems: basic technology (co-called "Basic"; fertilizer dose of 30 kg N/ha of pure nutrients, grain dressing and application of herbicides and insecticides: Granstar 75 WG, Mustang Forte, and Nurelle D, without fungicides), and basic technology plus fungicides (so-called "Basic+"; Fandango 200 EC, Prosaro 250 EC, Archer Top 400 EC).

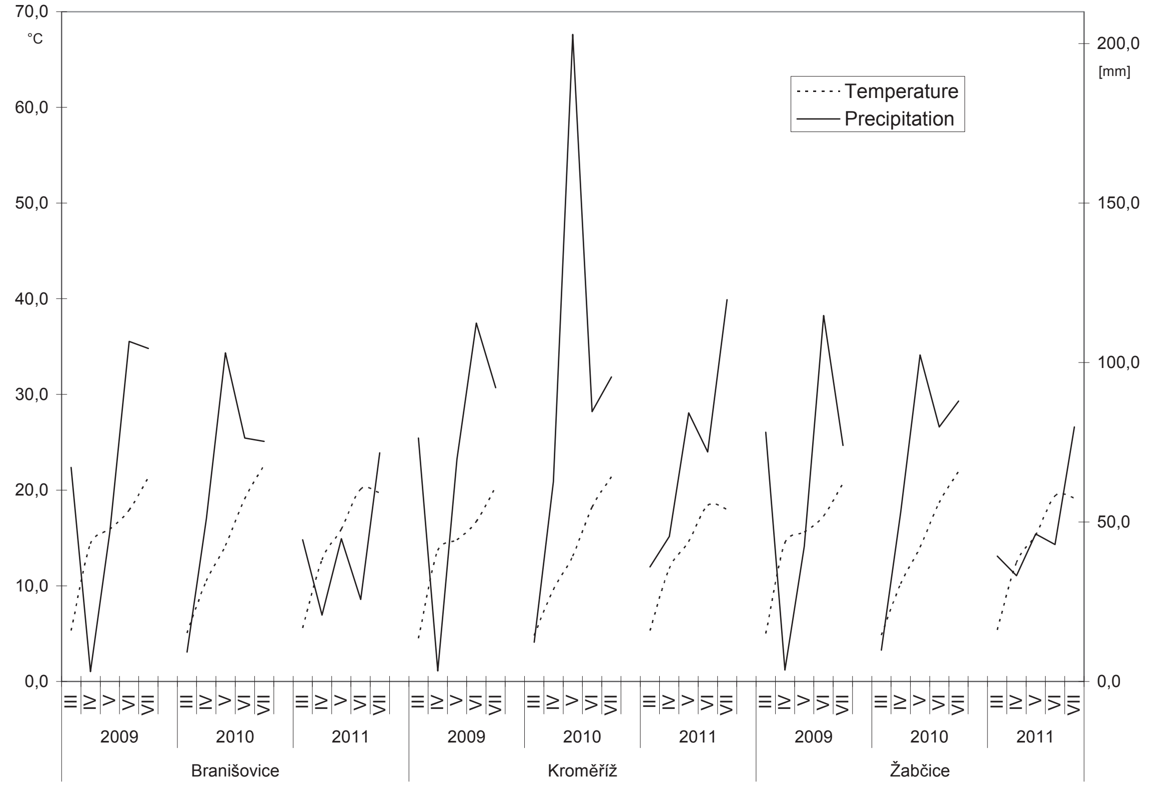
Arabinoxylans were determined by a method described by Douglas (Douglas, 1981). The method is based on a reaction of the sample with acid extract solution. The reaction mixture is heated in a water bath for 25 minutes. Developed reddish-brown to reddish-orange color is measured spectrophotometrically at the wavelengths of 552 and 510 nm. The samples were hydrolyzed with sulfuric acid, subsequently stained with the dye Calcofluor White M2R. Beta-glucans were assessed using a modified method for flow injection analysis (FIA) with spectrofluorimetric detection (Aastrup & Jorgensen, 1988).

I: *Varieties/lines*

Variety	Type of grain	Basic characteristics
Aksamit	hulled	malting variety, recommended for Czech Beer
Blaník	hulled	malting variety, recommended for Czech Beer
Bojos	hulled	malting variety, recommended for Czech Beer
Jersey	hulled	malting variety
Kangoo	hulled	malting variety
Radegast	hulled	malting variety, recommended for Czech Beer
Sebastian	hulled	malting variety
AF Lucius	hull-less	variety recommended for food and feed use
KM 1057	hull-less	line with higher beta-glucan content, higher arabinoxylan content
KM 2084	hull-less	line with higher beta-glucan content
KM 2283	hull-less	line with higher beta-glucan content

II: *Basic characteristics of test localities*

Locality	Production region	Altitude [m]	Long term average temperature [°C]	Long term average sum of precipitation [mm]
Branišovice	Maize	190	8.8	460
Kroměříž	Sugar beet	235	8.7	599
Žabčice	Maize	187	9.2	480



1: *The course of weather in the experimental locations in 2009–2011 (according to Walter-Lieth)*

III: *The analysis of variance of arabinoxylan and beta-glucan contents*

Source of variability	d. f.	M. S.	Effect [%]	M. S.	Effect [%]
		Arabinoxylans		Beta-glucans	
Genotype	10	13.601***	15.57	25.337***	23.64
Technology	1	9.202***	12.81	1.320***	5.40
Locality	2	3.404***	7.79	4.223***	9.65
Year	2	3.719***	8.14	6.448***	11.92
Genotype*Technology	10	1.382***	4.97	0.126***	1.66
Genotype*Locality	20	1.407***	5.01	0.364***	2.83
Technology*Locality	2	1.214***	4.65	0.199***	2.10
Genotype*Year	20	1.277***	4.77	0.472***	3.23
Technology *Year	2	1.857***	5.75	0.582***	3.58
Locality*Year	4	1.115***	4.46	19.350***	20.66
Multiple interactions	124	7.859***	24.56	2.083***	14.63
Error	198	0.470	1.50	0.23	0.71

Note: p = 0.001 – ***, M. S. – mean square, d. f. – degrees of freedom.

Water content grain samples was determined by gravimetric method (EBC 3.2). All chemical analyses were conducted twice and values converted to 100% dry matter of grain. Results were evaluated with program STATISTICA 9 with four-factor analysis of variance, the significances of differences between mean values of different varieties and lines, technologies, years and locations were tested at the 5% significance level with the LSD test (Fisher's exact test). The share of the studied factors and their interactions was expressed as the ratio of the standard deviation of the factor and the standard deviation of the total variance.

RESULTS AND DISCUSSION

Variability in arabinoxylan and beta-glucan contents (Tab. III) was statistically highly significantly (p = 0.001) affected by the variety/line, locality, year, growing technology and their mutual interactions (only the interaction of growing technology with the locality showed

IV: *Average content of arabinoxylans and beta-glucans in the set of varieties/lines (%)*

	Main effects							
	Growing technology		Locality			Year		
	Basic+	Basic	Branišovice	Kroměříž	Žabčice	2009	2010	2011
Arabinoxylans [%]	4.48 a	4.79 b	4.70 b	4.45 a	4.76 b	4.83 b	4.52 a	4.56 a
Beta-glucans [%]	4.63 a	4.74 b	4.83 c	4.48 a	4.74 b	4.79 b	4.43 a	4.84 c

Note: The values marked with different letters in the same rows in the individual groups of main effects differed significantly at p = 0.05.

the significance level of p = 0.01). The highest effect of the factor on variance of arabinoxylans was found for the genotypes (15.57%), growing technology (12.81%), and multiple interactions (24.56%). The largest share in the total variability in beta-glucan content in barley grain was found for genotypes (23.64%), environment (interaction of a locality and year, 20.66%) and multiple interactions (14.64%). Each share of multiple interactions in the variability of both arabinoxylans, and beta-glucans consists a sum of share of four three-way and one four-way interaction.

Treatment and Environmental Effects

The samples from the Basic technology (4.79% and 4.74%) provided statistically significantly more arabinoxylans and beta-glucans versus the Basic+ technology (4.48% and 4.63%) (Tab. IV). When comparing growing technologies, in the Basic+ malting varieties provided generally less arabinoxylans, and hull-less genotypes provided generally more arabinoxylans. For example malting variety Blaník provided in the Basic+ growing technology significantly more arabinoxylans, than in the Basic technology (5.26 versus 4.30%), on contrary hull-less line KM 2084 provided significantly more arabinoxylans in Basic+ technology, than in Basic technology (4.29 versus 3.82%). In terms of growing malting barley as a raw material for malt production or feed for livestock, conventional farming (with use of fungicides) with significantly lower levels of arabinoxylans and beta-glucans appears as more advantageous. Similarly, the results from study conducted by Iji (1999) support the opinion about the improper effect of non-starch polysaccharides on digestion of feed in monogastric animals. Positive effects of non-starch polysaccharides in foods, on the other hand, have been confirmed by Havrlentová & Petruláková (2011), and Shimizu *et al.* (2008). Due to the unregulated exposure of plants to pathogens, we can assume not only a greater degree of plant infestation but also a higher subsequent formation of harmful secondary metabolites of the pathogens (mycotoxins). Lower contents of non-starch polysaccharides in variants untreated with fungicides compared to the fungicide treated variants have also been described in other studies (Havlová *et al.*, 2006; Belcredi *et al.*, 2009; Dickin *et al.*, 2011; Macháň *et al.*, 2011). As evident from Tab. IV the localities affected beta-glucan and arabinoxylan contents statistically significantly. On the average of genotypes, growing technologies, and years of growing, the lowest average arabinoxylan content (Tab. IV) was detected in grain from the locality Kroměříž (4.45%). The locality Kroměříž also provided statistically significantly lower content of beta-glucans (4.48%). The highest beta-glucan content was found in the grain samples from Branišovice (4.83%), the highest arabinoxylan content was determined in samples from Branišovice and Žabčice (4.70% and 4.76%, respectively). The sugar-beet locality (Kroměříž) with its significantly lower values of non-starch polysaccharides thus appears more suitable for malt production than the maize production area (Branišovice, Žabčice). On average of the genotypes, localities, and growing technologies, the samples from 2009 (Tab. IV) gave statistically significantly more arabinoxylans (4.83%) than the samples from 2010 and 2011 (4.52% and 4.56%, respectively). Arabinoxylan contents in the samples from 2010 and 2011 did not differ statistically significantly. Statistically significantly lowest average beta-glucan content was detected in the samples from 2010 (4.43%), versus the samples from 2009 (4.79%) and 2011 (4.83%).

The lowest amount of arabinoxylans on average of the genotypes and growing technologies (Tab. V) was determined in the samples from 2011 from the locality Kroměříž (4.29%) and in the samples from 2010 from Kroměříž and Branišovice (4.38 and 4.40%, respectively). The highest arabinoxylan content was recorded in the samples

V: *Average contents of arabinoxylans and beta-glucans (%) in the studied localities in two intensities of chemical treatment of stands in 2009 to 2011*

Year	Locality	Arabinoxylans	Beta-glucans
2009	Branišovice	4.97 d	5.05 d
	Kroměříž	4.69 bc	4.11 b
	Žabčice	4.83 cd	5.20 f
2010	Branišovice	4.40 a	5.13 e
	Kroměříž	4.38 a	4.29 c
	Žabčice	4.79 bc	3.87 a
2011	Branišovice	4.73 bc	4.30 c
	Kroměříž	4.29 a	5.05 d
	Žabčice	4.66 b	5.15 ef

Note: The values marked with different letters in the same columns differ significantly at p = 0.05.

from the locality Branišovice from 2009 (4.97%). Statistically significantly lowest beta-glucan content versus the other variants was identified in the samples from the locality Žabčice from 2010 (3.87%). The highest level of beta-glucans in the set was determined in the samples from the locality Žabčice from 2009 and 2011 (5.20 and 5.15%, respectively); no statistically significant difference was found between these two samples.

The higher effect of the interaction between the localities and years was also observed in beta-glucan content by Zhang *et al.* (2001) and Grausgruber *et al.* (2010). According other authors, beta-glucan content in grain is affected by the course of weather; hot and dry weather during formation of caryopses is subsequently reflected in higher beta-glucan content in grain (Güler, 2003; Ehrenbergerová *et al.*, 2008; Tiwari & Cummins, 2008; Dickin *et al.*, 2011).

On average of all varieties, a significantly higher level of beta-glucans than in 2010 was found in Žabčice (Fig. 1) in 2009 and 2011 when drought was recorded during the vegetation periods.

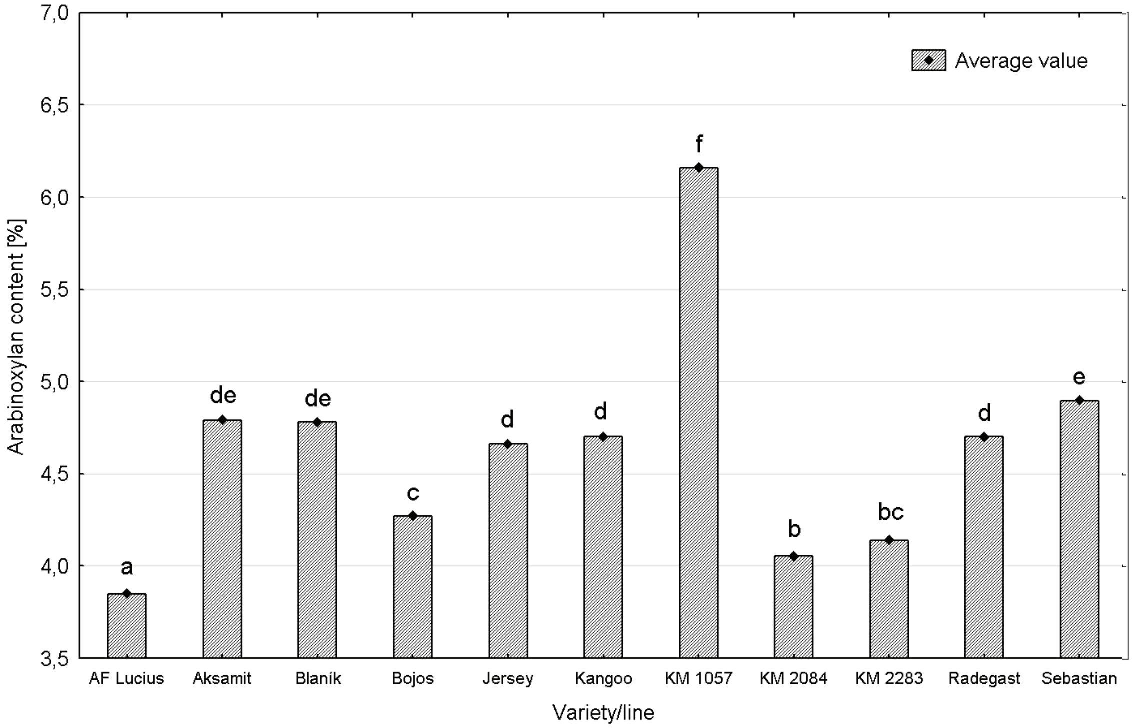
Genotypic Variation

Figs. 2 and 3 show the variability of arabinoxylan and beta-glucan contents in average of the individual localities, growing years and technologies. Of the malting varieties, the variety Bojos exceeded in its statistically significantly lowest arabinoxylan content (4.27%, Fig. 2), than other malting varieties. Low levels of arabinoxylans and lowest levels of beta-glucans among malting varieties were detected in the variety Radegast (4.70%, Fig. 2; resp. 3.92%, Fig. 3). Relatively low average values of arabinoxylans were further detected in the malting varieties Kangoo (4.70%) and Jersey (4.66%). Of the malting varieties, Bojos had the lowest beta-glucan content (4.03%, Fig. 3).

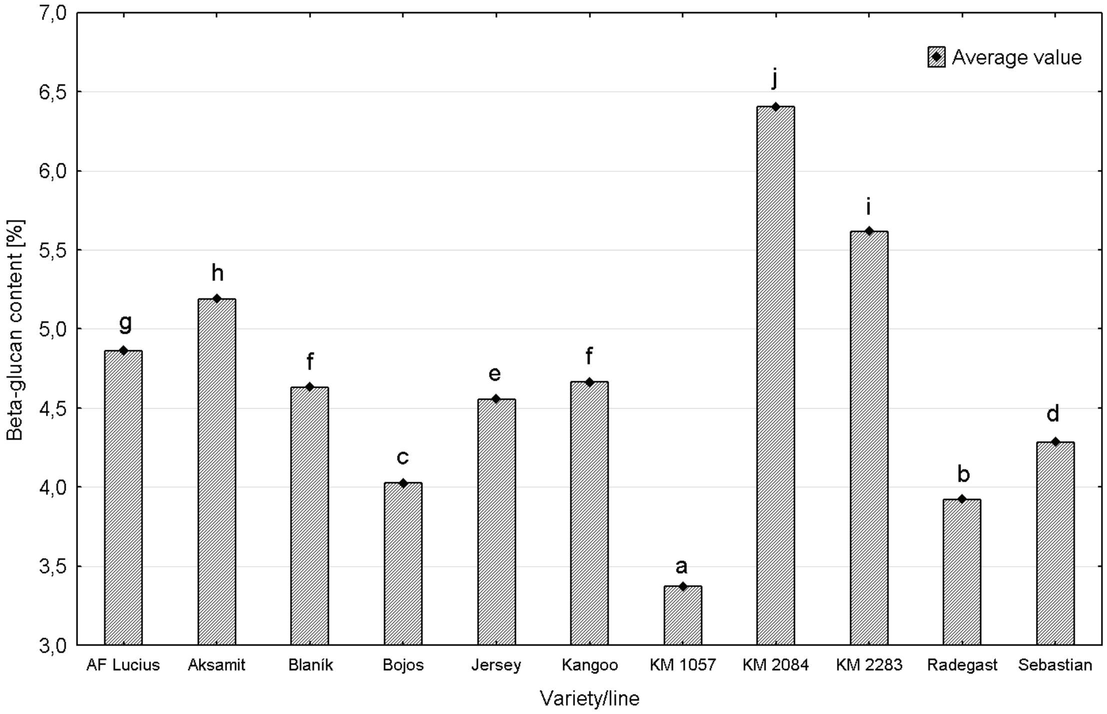
The set of the studied varieties also included hull-less genotypes intended for food production – the variety AF Lucius and lines KM 1057, KM 2084, and KM 2283. The line KM 2084 provided statistically significantly more beta-glucans (6.41%) than all other genotypes. Similarly, Prýma *et al.* (2000) and Grausgruber *et al.* (2010) found higher beta-glucan content in the hull-less waxy genotypes and some genetic resources. The line KM 2283 also exceeded in higher content of beta-glucans (5.62%, Fig. 3). On the other hand, the line KM 1057 gave the most arabinoxylans (6.16%) and less beta-glucans (3.37%). Due to a low content of beta-glucans in grain, the line KM 1057 appears as perspective for livestock feeding. The hull-less variety AF Lucius contained statistically significantly the least arabinoxylans (3.85%) and higher content of beta-glucans (4.86%).

CONCLUSIONS

Variability in arabinoxylan and beta-glucan contents was statistically highly significantly affected by the variety/line, locality, year, technology and by most of their mutual interactions. The highest



2: *Average values of arabinoxylan content in set of barley varieties and lines*
Note: Average values marked with different letters represent statistically significant differences at the significance level of 95%.



3: *Average values of beta-glucan content in set of barley varieties and lines*
Note: Average values marked with different letters represent statistically significant differences at the significance level of 95%.

effect of the factor on variance of arabinoxylans was found for the genotypes, growing technologies, and multiple interactions. The largest share in the total variability in beta-glucan content in barley grain was found for genotypes, interaction of a locality and year, and multiple interactions. Since genotype had the highest share on variability of both non-starch polysaccharides than all the other factors and their interactions, selection of proper genotype is the most crucial for desired ammount of arabinoxylans and beta-glucans in barley grain. Of the malting varieties, the variety Bojos exceeded in its arabinoxylan content (4.27%), this variety contained also low amounts of beta-glucans (4.03%). Variety Radegast exceeded in low levels of arabinoxylans and beta-glucans (4.70%, resp. 3.92%). Relatively low average values of arabinoxylans were further detected in the malting varieties Kangoo (4.70%) and Jersey (4.66%). The line KM 2084 intended for food production provided statistically significantly more beta-glucans (6.41%) than all other genotypes. The line KM 2283 also contained higher amount of beta-glucans (5.62%). On the other hand, the line KM 1057 gave the most arabinoxylans (6.16%) and least beta-glucans (3.37%). Due to a low content of beta-glucans in grain, the line KM 1057 appears as perspective for livestock feeding. The hull-less variety AF Lucius contained statistically significantly the least arabinoxylans (3.85%) and higher content of beta-glucans (4.86%). This hull-less variety can be also recommended for feed production. When grown in the Basic+ technology malting varieties provided generally less arabinoxylans, and hull-less genotypes provided generally more arabinoxylans.

Results showed in this work give overview about concentration and variability of arabinoxylans and beta-glucans, therefore can be used to prefer suitable varieties and growing technologies for diverse uses of barley grain production (malt, food, and feed).

SUMMARY

Barley is traditionally used for malt production, but also as feed and food. For this use, content of non-starch polysaccharides is a limiting quality parameter. Non-starch polysaccharides are capable to create in water viscous solutions and become thus a valuable component of food fiber, but on the other hand they can cause problems at wort filtration during brewing and reduce the nutritional values of feed in livestock. This work deals with determination of contents of non-starch polysaccharides in the set of eleven different barley genotypes with hulled malting varieties, hull-less lines and hull-less variety. Set of eleven barley genotypes was grown in Branišovice, Kroměříž, Žabčice, in two different growing technologies, in the years from 2009 to 2011. Arabinoxylans were detected by method described by, beta-glucans were determined after sample hydrolysis in sulfuric acid with flow injection analysis with spectrofluorimetric detection. Levels of significance and share of variability for named factors and their interactions was determined. A significant difference between growing technologies, localities, years and genotypes was found. Genotypes suitable for malt production, food and feed usage were identified. The highest values of arabinoxylans and beta-glucans were determined in the grain samples of hull-less lines (KM 1057: 6.16% of arabinoxylans and KM 2084: 6.41% of beta-glucans) and on the contrary, the lowest values of arabinoxylans were found in the grain of hull-less variety AF Lucius (3.85%) and lowest amounts of beta-glucans were found in malting variety Radegast (3.92%). Lines KM 2084 and 2283 can be recommended for production of functional foods. Both these lines are also potentially suitable as a genetic material for further breeding of varieties with good adaptability capable of producing high concentrations of beta-glucans in grain. Barley grown in system with fungicide treatment provided in average grain with significantly lower levels of both non-starch polysaccharides (4.48% arabinoxylans and 4.63% beta-glucans), than the system without fungicide treatment (4.79% arabinoxylans and 4.74% beta-glucans)

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