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A Survey of Total β-glucan Content in Croatian Barley Varieties

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Although β-glucans in cereals are desirable as healthy diet components, high levels of β-glucans in malting barley are unacceptable because they can cause unsatisfactory degradation of cell walls during malting. The aim of this study was to investigate the β-glucan content in twelve Croatian and two German barley varieties at three representative locations in Eastern Croatia over three consecutive seasons (2012–2014). Total β-glucan content in barley samples was determined using enzymatic method. Most of the investigated barley varieties had total β-glucan content lower or significantly lower than 4%. Furthermore, a distinct and clear genotype influence was noticed. No significant difference was found between years, but between locations Osijek and Tovarnik.

Keywords: *Hordeum vulgare,* brewing, feed barley, malting barley

Introduction

In recent years, researchers have shown an increased interest in β-glucans because of their beneficial effects on human health. Degradation products of β-glucans larger than glucose will not be absorbed in the human body, so they represent a valuable source of soluble fibre (Kanauchi et al. 2011). From a human nutrition perspective, β-glucans can be characterised as prebiotics (Bamforth and Gambill 2007). However, from brewers' perspective, β-glucans are not desirable in cereals intended for malting and brewing.

β-Glucans are non-starch polysaccharides characterized by (1→3), (1→4) β-D-glucose linkage. They are the main components of the endosperm cell walls and can be mostly found in barley and oats, while in other cereals they are present to a significantly lesser extent (Vis and Lorenz 1997). Total content of β-glucans in barley normally ranges from 2 to 8% (Marconi et al. 2014) and depends predominantly on genetic factors, but other factors (e.g. climatic conditions, agro-technical measures, soil type) also contribute to the total β-glucan content in barley (Aastrup 1979; Narziss et al. 1989; Zhang et al. 2001).

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β-Glucans are considered useful when they appear in small amounts in malted cereals used for brewing, because they contribute to beer foam stability and improve beer organoleptic properties (i.e. beer mouth feel) (Havlová et al. 2006). However, when present in higher amounts they cause serious problems during both malting and brewing. During malting, high β-glucan content can lead to unsatisfactory degradation of cell walls, which disrupts the germination and reduces the malt extract (Wang et al. 2004). β-Glucan residues in malt can lead to poor mash conversion, resulting in highly viscous wort. This ultimately can cause problems during the filtration process (Vis and Lorenz 1998; Wang et al. 2004) and induce haze in the final product, i.e. beer (Jin 2002).

For that reason, barley with low to moderate β-glucans content is preferable for malt production (Vis and Lorenz 1998). Furthermore, the existing research results suggest the use of six-rowed barley that seems to have somewhat lower β-glucan content than two-rowed varieties (Lehtonen and Aikasalo 1987; Zhang et al. 2001). Based on their intended use in respect to their characteristics, barley varieties in Croatia can be classified as 'malting', 'feed' and 'malting-feed'. Most of the varieties are winter varieties with somewhat higher yields (+30%) and lower costs compared to spring barley. However, in order to be acceptable for malting/brewing, the main quality parameters have to be suitable, such as β-glucan content, protein content, friability, glassy grains percentage.

Because of the quality parameters that are important for brewers, some winter barley varieties are labelled as malting-feed varieties (Kunze 1996). Dual-purpose and higher yields, compared to malting barley varieties, make these varieties attractive to barley growers. Upon the entry into the EU the Croatian market has opened to malting barley varieties originating from EU countries which meet the strict quality criteria set for malt and beer. According to the above-mentioned criteria, domestic malting varieties were not suitable for brewing. Therefore, the Croatian Varietal Commission has allowed dual-purpose labelling of varieties that were primarily registered as livestock feed.

The objective of this study was to determine the total β-glucans content in 14 barley varieties collected at three locations over three consecutive seasons (2012–2014). The acquired data could serve maltsters as important input information upon the admission of barley to the Croatian malting industry.

Materials and Methods

Samples

Croatian winter barley varieties of different traits (e.g. maturity, plant height, straw strength, lodging tolerance, biotic and abiotic stress tolerance, etc.) and grain properties were selected based on previous studies (Lalić et al. 2006, 2007; Kovačević et al. 2008) and on their importance for industry over the last decade.

Samples of 14 different varieties were collected over three consecutive seasons (2012–2014) from the variety trials of the Agricultural Institute, Osijek. The barley varieties were grown under field conditions on three localities, i.e. Osijek (OS), Slavonski Brod (SB) and Tovarnik (TO) over three growing (vegetation) seasons (2011/12, 2012/13 and

Table 1. Soil properties, precipitation and mean temperature during the growing season of winter barley (October–June) at the three test sites

	Osijek	Slavonski Brod	Tovarnik
Latitude	45°27' N	45°10'N	45°10'N
Longitude	18°48'E	18°01'E	19°09'E
Soil type	eutric cambisol	alluvial soil	hipogley
Soil pH	6.25	4.2	7.1
Rainfall (mm)			
2011/12	392.3	419.5	332.4
2012/13	679.0	647.2	625.3
2013/14	573.2	567.9	460.9
Mean temperature (°C)			
2011/12	8.3	8.3	8.9
2012/13	9.1	9.1	9.6
2013/14	10.2	9.9	10.8

2013/14). The experiments were conducted in randomized block designs (RCBD) with six replications; plot size was 7.56 m^2. Soil properties and climatic conditions during the growing seasons at the respective locations are displayed in Table 1.

Sampling (5 kg per sample) was performed on cleaned and processed barley grains (according to the EBC 3.3.1. method) and the samples were kept refrigerated in sterile dry containers. Almost all varieties are two-rowed varieties from the Agricultural Institute Osijek, except Lord which is six-rowed; Tiffany and Vanessa on the other hand were originally bred in Germany.

Determination of total β-glucan content

First the barley samples were milled using a standard laboratory mill with a 1 mm sieve (MF10.2 basic, IKA Labortechnik, Germany) and after that using a kitchen coffee grinder (Braun KMM 10). The grinded samples were kept in sealed plastic bags until the enzymatic determination of total β-glucan content (AOAC 1995) using a commercial assay kit (Mixed linkage β-glucan assay kit, Megazyme Int., Bray, Ireland).

Statistical analysis

Analysis of variance (ANOVA) was carried out using the GLM procedure of SAS Vers. 8 software (SAS Inst., Cary, NC). Mean comparisons were done using Fisher's least significant difference (LSD) test.

Values of the main effects and the first interaction axis for interpretation of genotype×environment interaction were presented in graphic representation–biplot anal-

Table 2. Mean values of β-glucan content in chosen barley varieties across intended purpose, year and location

Year/ Locality	β-Glucan g/100 g dry weight														
	Rex	Barun	Bingo	Bravo	Vanessa	Tifanny	Maxim	Premium	Gazda	Lukas	Maestro	Merkur	Trenk	Lord	Mean
Intended purpose	M/F	M/F	F	F	M	M	M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F	
2012	4.41	3.90	4.67	4.73	3.14	3.82	4.10	4.21	3.53	4.33	3.48	4.10	3.94	4.32	4.412^{a}
2013	4.21	3.78	4.27	4.39	2.64	3.4	3.67	3.65	3.44	3.93	3.61	3.83	3.78	4.11	4.20^{a}
2014	4.30	3.84	4.28	4.40	2.53	3.54	3.75	3.57	3.06	3.73	3.49	3.74	3.55	4.20	4.21^{a}
LSD 5%															0.316
Tovarnik	4.47	4.11	4.62	4.59	2.60	3.73	3.94	3.99	3.25	3.96	3.39	3.81	3.82	4.06	4.06^{b}
Slavonski Brod	4.23	3.68	4.28	4.48	2.84	3.53	3.79	3.72	3.39	4.06	3.63	3.96	3.73	4.30	4.15^{ab}
Osijek	4.29	3.54	4.19	4.61	2.76	3.60	3.79	3.73	3.45	4.36	3.83	4.12	3.79	4.41	4.41^{a}
LSD 5%															0.316
Mean	4.32^{b}	3.81^{ef}	4.39^{ab}	4.53^{a}	2.75^{i}	3.60^{fg}	3.84^{e}	3.81^{ef}	3.35^{h}	4.06^{cd}	3.57^{g}	3.93^{de}	3.77^{efg}	4.23^{bc}	4.24
LSD 5%															0.476

Significant difference ($P \leq 0.05$) is indicated by different letters.
M/F = malting/feed; F = feed; M = malting.

ysis of GGE model, and biplot for 9 environments and 14 cultivars of winter barley was constructed.

The software PBTools, version 1.4. 2014. (Biometrics and Breeding Informatics, PBGB Division, International Rice Research Institute, Los Baños, Laguna, Philippines) was used for GGE biplot analysis.

Results

Mean values of total β-glucan content are presented in Table 2. ANOVA revealed no significant difference between years, but significant differences between genotypes and locations.

Figure 1 gives GGE-biplot for 14 barley genotypes grown in 9 diversified environments. The G×E visualization by the GGE biplot revealed that Vanessa is less influenced by the locations and exhibits a high negative PC1 score which indicates the lowest β-glucan content. Tiffany, the second German winter barley suitable for malting/brewing showed also stable performance across locations, however, somewhat higher β-glucan content. A similar performance showed the Croatian varieties Gazda, Trenk and Maestro and with respect to the β-glucan content also Premium, Maxim and Barun. The latter two, however, were somewhat more sensitive to the environmental influence. Therefore, these Croatian varieties had β-glucan contents satisfactory for the malting industry (i.e. < 4%).

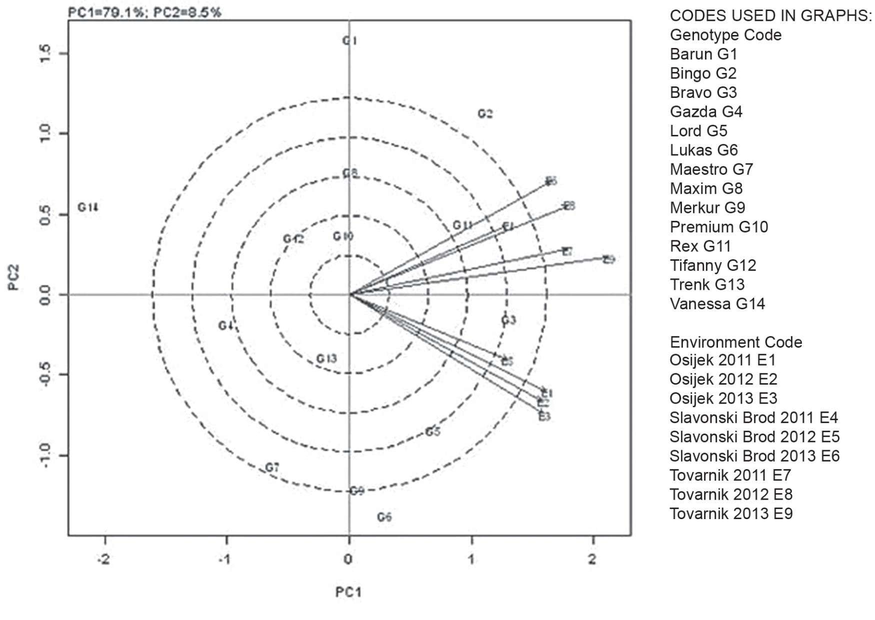

Figure 1. GGE-biplot for 14 barley genotypes grown in 9 diversified environments

Dual purpose (malting/feed) varieties Lord, Lukas, Merkur and Rex had not only somewhat higher β-glucan contents but also a more unstable performance. The varieties which were registered as feed varieties (i.e. Bingo and Bravo) had not only the highest β-glucan contents, but also an unstable performance across locations. Results also indicate that Osijek is most suitable for barley growing, as far as low β-glucan content is concerned.

Discussion

The adaptability of a variety to different growing conditions and a genotype's reaction to biotic and abiotic stresses are significant factors for the success of a variety. In Croatia, high temperatures during May and June can affect the grain filling period and cause premature ripening of the grain (Lalić et al. 2006). In barley, premature ripening can cause an increase of the total β-glucan content (Passarella et al. 2002). The content of β-glucans is affected by both the genotype and the environment, genetic factors, however, are more important (Molina-Cano et al. 1997; Zhang et al. 2001). Furthermore, it is considered that after longer cultivation under the same growing conditions β-glucan high-molecular components (the most problematic in brewing) become genotypically determined, i.e. become a variety trait (Narziss 1989). Although the above-mentioned statement is commonly accepted by maltsters and is often cited in professional literature, geneticists and barley breeders think this should be confirmed by scientific research methods.

Considering the total β-glucan content, it should be taken into account that during malting β-glucan content decreases proportionally to the increase of grain friability (Henry 1988). β-Glucan degradation takes place at germination (malting) temperatures which is the only period in which enzymes degrade β-glucans. The solubility of β-glucans is greatly affected by the structure and interrelations of certain β-glucan fractions. High-molecular compounds are especially problematic for brewing (Kanauchi et al. 2011), therefore, low β-glucan content in grains is required by maltsters and brewers. The β-glucan content in grains correlates with β-glucan concentration in wort. Brewing technology itself contributes also to the final β-glucan content in wort. Edney et al. (2012, 2014) reported wort β-glucan content ranging from 60 to 140 mg/L for Canadian barley varieties. Even though there are no recommendations for brewers regarding total β-glucan content in malt, for wort it is recommended it should not exceed 200 mg/L (Davis 2006).

The American Malting Barley Association is even more stringent and recommends β-glucan concentrations in wort of <100 mg/L for two-row barley and <120 mg/L for six-row barley (Davis 2014). In everyday practice higher values are also tolerated, e.g. IGB (Institute & Guild of Brewing) sets the limit at < 200 mg/L (O'Rourke 2002), while EBC (European Brewery Convention) sets it at < 250 mg/L. However, often these recommended values are not achieved because the total β-glucan content of the raw material is often around 4% (MEBAK 1997; EBC 1998).

The results of this study showed that Croatian barley varieties classified as feed varieties had the highest total β-glucan content, while German malting varieties had the lowest total β-glucan content. The total β-glucan content of the majority of Croatian winter barley varieties which were classified as dual-purpose malting-feed varieties was < 4%, however, some showed an increased instability with respect to their reaction to the environment. Gazda, Trenk and Maestro were identified as suitable for malting with respect to their low β-glucan content and their environmental stability.

Looking at environmental stability, it is visible from the results of GGE-biplot (Fig. 1) that Osijek (eutric cambisol) is most suitable for barley growing, showing almost no fluctuations across the years. Tovarnik (hypogley) also gave good results for barley stability, but Slavonski Brod (alluvial soil) was most unstable across the years. Eventhough β-glucan content is considered to be genotypically determined trait, this research showed that soil type in Osijek, an eutric cambisol (pH 6.25) appeared to be most suitable soil for barley growing if low β-glucan content is desirable. Since rainfall and temperatures (Table 1) were similar at all three locations across the years, it can be concluded that pH values and the soil type made a slight impact on the difference in β-glucan content of chosen barley genotypes. This is a very important piece of information for barley growers indicating that Osijek is most suitable for barley growing in order to realize stable β-glucan concentrations. Also, the genotypic stability of chosen varieties was not influenced by Osijek's and Tovarnik's soil as much as by Slavonski Brod's soil. However, more and extensive research should be conducted on this note.

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