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# β-GLUCAN CONTENT VARIABILITY IN SEED OF BARLEY CULTIVARS

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**ABSTRACT.** Barley is an important source of β-glucans because they have a positive influence on human health and potential application in the food industry. The aim of this manuscript was to determine the variability of ten barley cultivars based on β-glucan content. The percentage of β-glucan varied in the range of 4.44% to 8.17%, with a mean value of 6.57%. Based on β-glucan content, the degree of cultivar similarity was determined by the dendrogram. Among analyzed barley cultivars, the highest concentration of β-glucan was found in the cultivar *Lav* and *Ukras,* while the lowest concentration was observed in the cultivar *Vihor* (4.44%). Compared with all the other cultivars mentioned, a pair of cultivars expressed the greatest distance. Statistical analysis showed that there is a significant difference between analyzed barley cultivars. The analysis of β-glucans in varieties of barley in two growing seasons aims to develop an agricultural strategy and develop barley varieties with higher β-glucans content.

**Keywords:** *Hordeum vulgare*, β-glucan, genetic variability, genotypic similarity/distance, nutritive quality

## INTRODUCTION

Today's food doesn't represent only nutrient sources, it also represents a source of compounds that may improve an individual's health. Prebiotic dietary fiber (β-glucans) should be consumed because β-glucans can stimulate beneficial bacteria in the human gut. Also, they participate in the production of short-chain fatty acids (CARLSON *et al.,* 2017). Consumers are generally well informed that dietary fibers are part of a balanced diet, which has led to an increase in demand for fiber-rich foods. β-glucans are a significant part of fibers that are usually concentrated in the internal aleurone and subaleurone endosperm cells walls of cereal seeds such as barley, oat, wheat, rye, buckwheat, amaranth, and millet (HAVRLENTOVÁ *et al.*, 2011). Over the last few decades β-glucans have been widely researched because of their demonstrated beneficial effects on human health and potential for utilization in various food products (RAHMANI *et al.*, 2019).

β-glucans from oat and barley, or β-glucans from dietary supplements or some food products, significantly reduce lipoprotein and total cholesterol concentrations (AMES *et al.*, 2018), which are the major risk factors associated with cardiovascular diseases. The capacity of β-glucans to decrease postprandial glucose and insulin levels was evaluated by many scientists (BOZBULUT and SANLIER, 2019; ANDREEA *et al.*, 2021; CHEN *et al.*, 2022). Besides that, the consumption of β-glucan in a diet can increase the feeling of satiety and reduce appetite compared to food that does not contain β-glucan (AOE *et al.*, 2014). Studies on animals have shown that β-glucans influence reducing obesity and are also capable of stimulating immune functions, which allows them to have a significant impact on resistance to bacterial and parasitic infections (CHOI *et al.*, 2018). Further, β-glucan is a potential probiotic. SHEN *et al.*, (2012) represent that cereal β-glucan leads to an increased number of the species *Lactobacillus* sp. and *Bifidobacterium* sp. in rats, while the number of the species from fam. Enterobacteriaceae decreased. Considering these health benefits, worldwide efforts have been undertaken to extract and purify β-glucans that can be incorporated into foods or consummated as supplements.

Barley is a cereal with a very high content of β-glucan that ranges from 3% to 11% in whole barley seed (HASSAN *et al.*, 2017). Other commercially significant cereals such as oat (2.2–7.8%), rye (1.2–2.0%) and wheat (0.4–1.4%) also contain β-glucan but lower levels of β-glucan (LAZARIDOU *et al.*, 2007). The variability of β-glucan content in barley is determined by the genotype of the species but also depends on environmental conditions and agricultural practices (VAEZI *et al.*, 2019). It was shown that higher rainfall during the development of cereals, as well as a lower temperature had negative effects on the concentration of β-glucans. Further, the use of optimal nitrogen fertilization (up to 180 kg N/ha) and some fungicide have a negative effect on β-glucan content in barley seed, while irrigation has the opposite effect (JANSEN *et al.*, 2013). The negative correlations between β-glucan, on the one hand, and arabinoxylans, amylose and amylopectin were also observed (BŘEZINOVÁ *et al.*, 2009).

β-glucans represent linear homopolysaccharides composed of D-glucopyranosyl residues linked mostly via two or three consecutive β-(1→4) linkages that are separated by a single β-(1→3) linkage (BETA *et al.*, 2012). The solubility and viscosity of β-glucans are due to the presence of mixed linkages in cereal β-glucan (molecules important for their physical properties). The possibility of increasing viscosity is a significant property of β-glucans which is linked to many health benefits making them of particular importance to human nutrition. The tendency of β-glucans to form a very viscous solution may cause a problem during the brewing process and be synonymous with higher production costs in the brewing industry (*et al.*, 2011). β-glucans are also considered an anti-nutritional factor in the diet of broiler chickens, because the highly viscous environment that they create in their gastrointestinal tract interferes with the digestion and absorption of nutrients (CHOTINSKY, 2015). Therefore, the development of a wide range of barley types, according to β-glucan content, allows the selection of specific barley cultivars intended for specific users.

In our research, ten samples of barley cultivars were used. Research involves analysis of β-glucan content, determination of cultivars with a significantly higher content of β-glucans which can be used in breeding programs for improving this property in barley. We also tried to determine the cultivars with a significantly lower content of β-glucans which are suitable for use in the brewing industry.

## MATERIALS AND METHODS

Seed samples of ten genetically divergent barley cultivars (*Horizont*, *Orijent*, *Ukras*, *Ural*, *Osvit*, *Vihor*, *Lav*, *Lazar*, *Novosadski 292*, *Novosadski 294*) were used for the analysis of β-glucan content. Varieties originate from Gene Bank collection of Institute of Field and Vegetable Crops Novi Sad. Varieties chosen for research are commercially used and are important for agricultural production.

### *Field trial*

The seed samples have been collected from experimental field location in Kragujevac (44º00′51″ N, 20º54′42″ E) during two growing seasons (2011 and 2012). A field experiment was designed in 3 replications of the basic plot with 5 rows of 1 m length with an inter-row spacing of 0.2 m and in a raw distance of 0.05 m between seeds. The soil type was meadow valley chernozem. Before seeding for plant nutrition fertilizers were applied (NPK 15:15:15, MAP – monoammonium phosphate), based on the soil chemical properties. Appropriate pesticides were applied in the aim of plant protection against pests, diseases, and weeds.

### *Soil characteristics*

Specific barley genotypes were collected from the experimental field on the soil type pseudogley. The soil has poorer physical properties: acid pH ($pH_{H2O}$ = 4.3), humus 2.26%, readily available phosphorus 7.4 mg 100 g/soil and potassium 13.7 mg 100 g/soil.

### *Climatic conditions*

During the growing season in the years of the experiment, the values of temperature and precipitation were different. For knowledge of whether environmental factors are typical for the location, a comparison with computed average values for 10 previous years (2001 – 2010) was conducted and differences in average values of temperature amount and distribution of precipitation were established (Tables 1 and 2). In the growing seasons 2010/11-2011/12 the average temperatures (8.6°C) were slightly lower than the average values of ten years period (9.08°C) (Table 1). The amount of precipitation was extremely low in the period of summer, while from December to ripening time (June) distribution of participation was favorable at each stage of plant development. At the phase of seed filling was a favorable amount of precipitation (April – 47.7 mm, May – 76.7 mm) as well the end of maturity stage amount of precipitation was favorable (in June – 45.1 mm) for the synthesis of proteins and polysaccharides (Table 2).

Table 1. Monthly average values of temperature (°C).

| **Month / Year** | **October** | **November** | **December** | **January** | **February** | **March** | **April** | **May** | **June** | **Average** |
|---|---|---|---|---|---|---|---|---|---|---|
| **2010/11** | 9.4 | 11.3 | 2.4 | 0.8 | 0.6 | 6.5 | 12.0 | 16.0 | 20.9 | 8.9 |
| **2011/12** | 10.4 | 3.0 | 4.4 | 0.6 | -3.9 | 8.3 | 12.9 | 16.0 | 22.9 | 8.3 |
| **Average** | 9.9 | 7.2 | 3.4 | 0.7 | -1.7 | 7.4 | 12.5 | 16.0 | 21.9 | 8.6 |
| **2001/2010** | 12.2 | 7.0 | 2.0 | 0.9 | 2.4 | 7.6 | 12.0 | 17.2 | 20.4 | 9.08 |
| **Δt** | >2.3 | <0.2 | <1.4 | >0.2 | >4.1 | >0.2 | <0.4 | >1.2 | <1.5 | >0.5 |

Δt – difference of temperatures between two analyzed periods (2010/11-2011/12 and 2001-2010)

### *Seed samples*

At full maturity stage, 30 plants (10 plants per replication) were used for harvest. Seed samples were prepared by standard method representative for milling. Seed samples were milled (Disc mill CM 290 Cemotec™) to a particle size of less than 500 microns. Samples are suspended and hydrated in a buffer solution of pH 6.5, then incubated with purified lichenase enzyme and filtered. An aliquot of the filtrate is then hydrolyzed to completion with purified β-glucosidase. The D-glucose produced is assayed using a glucose/oxidase peroxidase reagent. The contents of β-glucans were determined by Megazyme method ICC Standard Method No. 166 (McCleary and Codd, 1991).

Table 2. Monthly average values of the amount of precipitation (mm).

| Month / Year | October | November | December | January | February | March | April | May | June | Average | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **2010/11** | 86.9 | 27.9 | 50.1 | 29.1 | 46.7 | 30.9 | 20.8 | 66.0 | 32.3 | 43.4 | 390.7 |
| **2011/12** | 33.3 | 2.0 | 45.3 | 95.4 | 60.4 | 6.3 | 74.6 | 87.3 | 57.8 | 51.4 | 462.4 |
| **Average** | 60.1 | 15.0 | 47.7 | 62.3 | 53.6 | 18.6 | 47.7 | 76.7 | 45.1 | 47.4 | 426.6 |
| **2001/2010** | 64.3 | 57.4 | 48.5 | 42.8 | 44.7 | 52.5 | 66.6 | 74.9 | 92.2 | 60.4 | 543.8 |
| **Δmm** | >4.2 | >42.5 | >0.8 | <19.5 | <8.8 | >33.9 | >18.9 | <1.8 | >47.2 | >13.0 | >117.3 |

Δmm – difference in precipitation between two analyzed periods (2010/11-2011/12 and 2001-2010)

### *Measurement of absorbance at spectrophotometer*

The absorbance was measured at λ=510 nm for each sample A1 (the first measurement of sample absorbance values), A2 (the second measurement of sample absorbance values) and blank. The contents of β-glucan were calculated by using formula according to McCleary and Codd (1991).

### *Statistical analysis*

Statistical analysis (analysis of variance (ANOVA), followed by Fisher's LSD test and standard deviation) for β-glucan was performed using the SPSS program (IBM SPSS Statistics, Version 20, Inc. 1989-2011, USA).

## RESULTS

The contents of the isolated β-glucan in ten barley cultivars differ substantially, as can be seen in (Fig. 1). Among analyzed barley cultivars, the highest concentration of β-glucan was measured in the cultivar *Lav* (8.18%), while the lowest concentration was observed in the cultivar *Vihor* (4.44%). High levels of β-glucans were also found in *Ukras* (7.56%), *Osvit* (7.33%) and *Novosadski 292* (7.03%). Other cultivars (*Horizont*, *Orijent*, *Ural*, *Lazar*, *Novosadski 294*) deviate in β-glucan concentrations from 4.67% to 6.72%. For all barley cultivars, the average value of β-glucan contents was 6.57%.

In Figure 1 and Table 3, the results of the two-factorial analysis of variance (ANOVA) followed by Fisher's multiple range LSD test were presented. Barley cultivars *Vihor* and *Lazar* had significantly ($p < 0.05$) lower β-glucan content, compared to others. Significant differences were not found between *Horizont*, *Orijent*, *Ural* and *Novosadski 294*. A significant difference was found among cultivars *Horizont*, *Orijent*, *Ural* and *Novosadski 294* compared to others. Cultivars *Novosadski 292* and *Lav* were significantly different compared to other analyzed cultivars. *Ukras* and *Osvit* showed no significant difference between them.

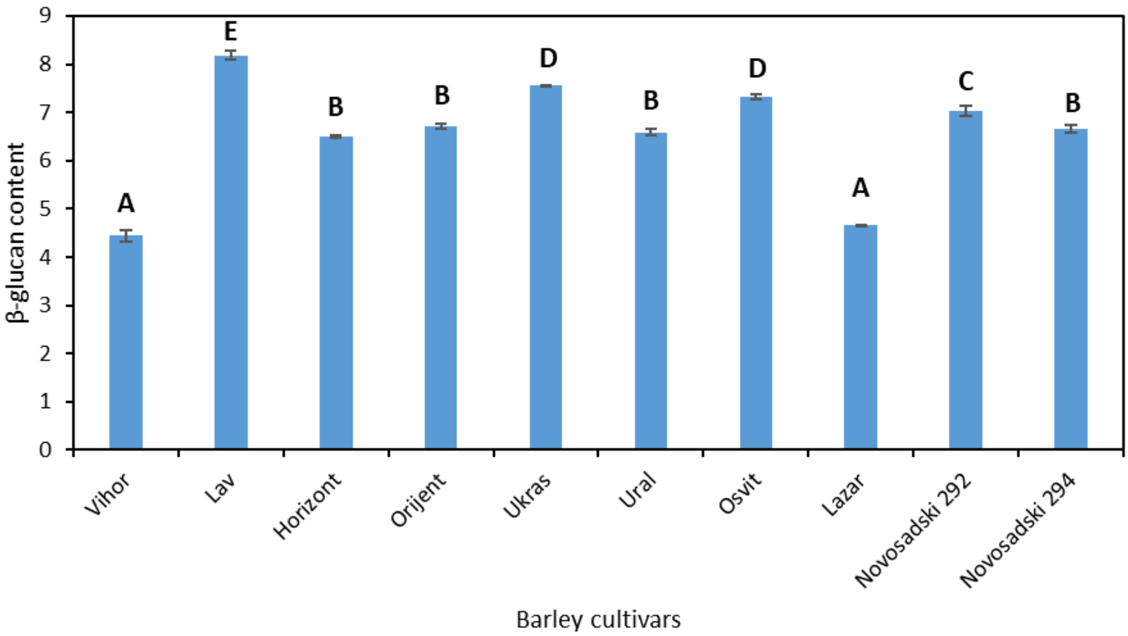


Figure 1. The mean value of β-glucan of ten barley cultivars over two growing seasons. The results are expressed as means for two independent determinations ± standard deviation. Values indicated by different letters differed significantly ($p \leq 0.05$).

Table 3. Analyze of variance (ANOVA) followed by Fisher's multiple range LSD test for analysis of β-glucan in ten analyzed barley cultivars

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Between Groups** | 37.62 | 9 | 4.18 | 242.86 | 0.05 |
| **Within Groups** | 0.34 | 20 | 0.02 | - | - |
| **Total** | 37.96 | 29 | - | - | - |

Df – The degrees of freedom; F– ratio of two variances – Fisher test; Sig. (significance level) ≤ 0.05 – significant difference was found between analyzed cultivars when compared to mean values of β-glucan content in seed.

Based on the identified β-glucan contents, all results of barley cultivars were compared with each other and a dendrogram of similarity with Euclidean distance was represented (Fig. 2). Within cluster 1 are cultivars *Vihor* and *Lazar* with a small distance in the range of 6, or with the highest similarity in the range of 94. This pair of cultivars show a higher distance (95) compared with all the other barley cultivars. Within cluster 2 are cultivars *Horizont*, *Ural*, *Orijent* and *Novosadski 294*. The highest similarity in the range of 98.5, or the least distance in the range of 1.5, is expressed between barley cultivars *Orijent* and *Novosadski 294*. The most similar to this pair, according to the value of β-glucans, were the pair of cultivars *Horizont* and *Ural* with a small distance in the range of 5.5. The cultivars *Horizont* and *Ural* showed a small distance in the range of 2.5 or the greatest similarity in the range of 97.5. Cluster 3 includes a small group of barley cultivars made up of the 3 cultivars *Ukras*, *Osvit* and *Novosadski 292*. Within this cluster, the smallest distance in the range of 5 was shown between the cultivars *Ukras* and *Osvit*. The most similar to this pair is the cultivar *Novosadski 292* with a distance in the range of 13. The least distance in the range of 57.5, or the highest similarity in the range of 42.5, compared with cultivars from clusters 2 and 3, expressed the cultivar *Lav*. Clustering of the cultivars can be efficient in separating genotypes of existing germplasm according to these results. Genetic distance represents genetic specificity and adaptation of the species.

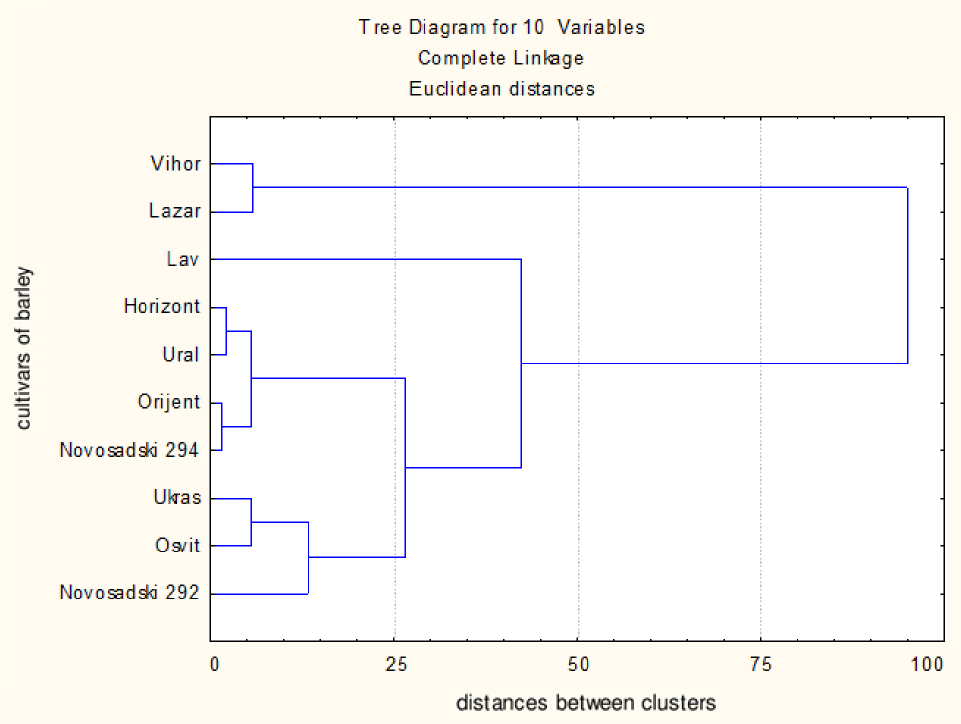


Figure 2. Genetic distances between clusters of barley cultivars. Based on the identified β-glucan contents in analyzed cultivars, all results were compared with each other and a dendrogram of similarity with Euclidean distance was constructed.

## DISCUSSION

According to literature data, barley β-glucan contents in different varieties have ranged mostly from about 2.5% to 11.3%. DJUKIĆ and KNEŽEVIĆ (2014) found that in oat varieties content of β-glucan ranged from 6.597% to 2.971%. In research by MARKOVIĆ *et al.*, (2017), β-glucan in barley cultivars deviates from 3.52% to 7.81%. The average value found in our study is 6.57%. Barley β-glucan content ranged from 1.81% to 7.18% as reported by CHOI *et al.*, (2020). MARTÍNEZ *et al.* (2018) showed that was no significant difference in concentration of β-glucan in barley when grown under different environmental conditions. On the other hand, results obtained by KRSTANOVIĆ *et al.*, (2016) showed that environmental and genetic factors may have an influence on the concentration of β-glucan in barley. They show that there are differences in β-glucan content for fourteen varieties of barley grown during three annual seasons of 2012-2014 in three different locations. BŘEZINOVÁ *et al.*, (2009) reported that β-glucan content in a set of 8 malting varieties of spring barley and 4 hulless lines (KM lines) in the range of 2.78-6.08%, which is a lower value compared with our results. In their study is a strong negative correlation between the content of β-glucans and arabinoxylans. Our investigation established differences in barley β-glucan levels. Our results are in agreement with the investigation of VAEZI *et al.* which showed the differences in β- glucan levels due to different growing conditions and genotype specificity and the interaction of these two factors (VAEZI *et al.*, 2019). Cultivars *Vihor* and *Lazar* start to develop earlier in comparison to the remaining investigated barley cultivars. The developing period can impact the amount of β-glucans in the grain of *Vihor* and *Lazar*, compared to long-time ripening cultivars (*Ukras*, *Osvit*, *Novosadski 292* and *Lav*). This indicates that is possible to increase the concentration of β-glucan in the seed of barley by breeding, with the aim of enhancing the food value. The β-glucan in seed endosperm consists of chain confirmation and due to β-(1→3)-linkages is a hygroscopic compound (DEMIRBAS, 2005). However, relationships between the level of β-glucan content and hydration are not in the same direction. So, an investigation FAST-SEEFELDT *et al*. (2007) showed that higher water content and water mobility influence β-glucan content, while GAMLATH *et al*. (2008) found relationship between high β-glucan content and slower hydration, which is contrary to the reported data of study MAYOLLE *et al.* (2012). The significant interaction between cultivar and growth temperature on the total β-glucan content was also observed by ANKER-NILSSEN *et al.* (2008). In their research, waxy cultivar *Cindy* showed the largest β-glucan content of 7.4% at 18°C and the lowest content of 5.8% at 9°C, while cultivar *Annabell* had the lowest β-glucan content of 4.0% at 9 and 12°C. Although the relative contribution of cultivars, environmental conditions and their interactions are not possible to fully quantify precisely, we can conclude that the adequate selection of cultivars to obtain a barley grain with the appropriate level of β-glucan is very important.

## CONCLUSION

β-glucan content, which was investigated, can be increased by the cultivation and application of proper agro-technical measures and growing conditions. Although we have observed high variation of β-glucan content within the analyzed barley cultivars, our results indicate that barley is a suitable source of the health beneficial β-glucan. The barley cultivars *Lav* (8.18%), *Ukras* (7.56%), *Osvit* (7.33%) and *Novosadski 292* (7.03%) with the highest β-glucan levels can be used in breeding programs and in the food industry as a potential material for the preparation of functional foods. In addition, the variety *Vihor* (4.44%) and Lazar (4.67%) with the lowest β-glucan contents may be appropriate for use in the malting and brewing industry as well as in the production of animal feed.

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