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SHORT COMMUNICATION

Divergence of barley and oat varieties according to their content of *β*-glucan

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Abstract: Barley and oat have been promoted for years as good sources of dietary fiber, especially *β*-glucan. Studies have shown a positive effect of barley and oat *β*-glucan on human health and as a result, there has been an increasing demand for *β*-glucans in the past decade. Thus, the variability of the *β*-glucan content was investigated in the grain of 10 barley and 10 oat varieties. The *β*-glucan contents were determined by the ICC Standard Method No 168. The content of *β*-glucan in the analyzed barley varieties were within the range 3.52–7.81 % and in analyzed oat varieties, the content of *β*-glucan were within the range of 3.15–7.28 %. Among analyzed barley varieties, Novosadski 314 contained the highest content of *β*-glucan (7.81 %), while Tomba had the highest content of *β*-glucan (7.28%) in the analyzed oat varieties. Based on the results, it could be concluded that there is genetic diversity of oat and barley varieties with respect to their *β*-glucan content. This fact enables varieties with a high a nutritional capacity to be selected.

Keywords: *Avena sativa*; *Hordeum sativum*; selection; variability.

INTRODUCTION

Barley and oat have been promoted for years as good sources of dietary fiber, especially *β*-glucan.[1,2] *β*-1,3/1,4-glucans are polysaccharides present in the bran of some grains, such as oat and barley but to a much lesser degree in rye and wheat.[3] In seeds of barley and oat, the highest concentrations of *β*-glucans are found in the endosperm and the subaleurone layer of the seed.[4,5] Numerous studies are increasingly showing a positive effect of *β*-glucan from barley and oat on human health. It was found that *β*-glucans present in barley and oat stimulate the immune system, and thus have a positive effect in the fight against infections[6–8] and in the prevention of cancer.[9–11] The intake of oat and barley *β*-glu-

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cans through different food products significantly decreases the total cholesterol and low-density lipoprotein cholesterol, and it may lead to improvement of lipid serum status and weight reduction.[12–14] Moreover, the intake of oat and barley β-glucan may affect a reduction in blood glucose levels in diabetics,[15] and the digestion of starch, thereby reducing glycemic and insulin response.[16–18] Furthermore, β-glucans may represent an important component of food in the modification of metabolic irregularities and the prevention of metabolic syndrome.[19,20] Based on *in vitro* experiments, and animal and human clinical studies, there is considerable evidence concerning the importance of β-glucan in the treatment and prevention of allergic diseases, which opens new perspectives in the use of this group of natural substances.[21]

The physiological effects of β-glucans are mainly attributed to their physicochemical structure, such as molecular weight, viscosity, solubility and interaction with the gastrointestinal tract.[22,23] In addition, the physicochemical characteristics, and the physiological effects of β-glucan may be affected by method of extraction, concentration of β-glucan, storage and with the food preparation method.[24–26] Among other things, β-glucans, which exist as indigestible polysaccharides in barley and oat, have been shown not only to have positive effects on health, but also the potential to be a possible new source of prebiotic.[27,28]

Knowing these beneficial effects of β-glucan, it is important to determine the availability and the best potential sources of β-glucan and possibly to increase them, in order to promote their wider application and accessibility in everyday life.

Considering that the content of β-glucan in oat and barley could be increased by breeding and using appropriate agro-technology (effect of fertilization – application of nitrogen (N) affects positively by increasing the content of β-glucan)[29–31] whereby the effect of genotype is dominant and has been confirmed,[29,32] the goals of this research were to define the content of β-glucan in genetically divergent cultivars of 10 oat and 10 barley varieties, comparison of the β-glucan content in different oat and barley varieties, and identification of the varieties with higher contents of β-glucan as a modest contribution to the breeding of oat and barley.

EXPERIMENTAL

Plant material

The plant material used for the field trials consisted of 10 varieties of spring barley (*Hordeum sativum* J. ssp. *distichum* L.) and 10 varieties of spring oat (*Avena sativa* L.). These genotypes originated from the Gene Bank collection of Field and Vegetable Crops in Novi Sad. Two rows of spring barley varieties were used for investigation, *i.e.*, Novosadski 296, Novosadski 297, Novosadski 300, Novosadski 301, Novosadski 306, Novosadski 310, Novosadski 312, Novosadski 314, Novosadski 316 and Novosadski 318, and 10 varieties of spring oat, *i.e.*, Flaemingsterne, Vok, Mozart, Juha, Hannes, Alden, Aslak, Wasa, Tomba and Kaempe gul. The barley and oat cultivars used for analysis were employed in increase of the production of cultivar seeds, because of their high genetic potential of yield, quality and adaptability. Nowadays, these cultivars are used in cross hybridization and represent the core gene source in breeding programs for the creation of new enhanced cultivars.

Field experiment

The experiment design in 3 replications was a basic plot with 5 rows of 1 m length with an inter-row spacing of 0.2 m and in row distance of 0.05 m between the seeds. Sowing was performed by hand. The trial was realized in the location Kragujevac (44°00′51″ N, 20°54′42″ E), during two growing seasons – 2011 and 2012. In the first year of the experiment, phenotypes were estimated and biotypes for each variety removed. In the second year of the experiment, the seed material was used for the determination of *β*-glucans. Mineral fertilizers (NPK 15:15:15, MAP – monoammonium phosphate) were applied before seeding according to the recommendations, based on the chemical properties of the soil and the available content of P, K and N reserves. The following fertilizers were applied for nutrition: urea (46 % N), KAN (27 % N) and AN (34 % N). The crop was protected adequately against pests and weeds by the appropriate use of pesticides and herbicides, with their efficacy being monitored. For analysis of the grain, full maturity stage of 30 plants (10 plants per replication) was used.

Soil characteristics

The growing of barley and oat genotypes was realized on an experimental field on the soil type pseudogley having poor physical properties, an acid pH (pH_{H2O} 4.1) and the following content: humus 2.34 %, readily available phosphorus 7.8 mg 100 g^{-1} soil and potassium 14.2 mg 100 g^{-1} soil.

Climatic conditions during growing seasons

The values of temperature and precipitation in two years of experiment were different in relation to the average values of the previous ten years (Table I). During the period January/June, the average values of the temperature was 9.28 °C in the first and 9.38 °C in the second year of the experiment. In both years of the experiment, the average temperature was lower than in the long-term period (10.08 °C). The precipitation was more favorable in the year 2011, with sums of 327.9 mm, than in 2012 (378.9 mm). In the second year during the grain filling stage (April–May months), the amount of precipitation (174.6 mm) was higher than in the first year (120 mm) and in the second year, the amount of precipitation was similar to the sum of the long term precipitation (373.7 mm).

TABLE I. Monthly and mean temperatures and monthly and cumulative precipitation; *Xm* – average values of temperature and precipitation

Period	January	February	March	April	May	June	*Xm*	Total
			t / °C					
2011	0.3	0.6	6.6	12.2	15.6	20.4	9.28	55.7
2012	–0.1	–4.2	8.8	12.7	16.0	23.1	9.38	56.3
2000–2010	0.9	2.4	7.6	12.0	17.2	20.4	10.08	60.5
			Precipitation, mm					
2011	28.1	59.2	48.9	37.1	82.9	71.7	54.65	327.9
2012	107.1	54.9	24.5	69.1	105.5	17.8	63.15	378.9
2000–2010	42.8	44.7	52.5	66.6	74.9	92.2	62.28	373.7

Seed sample

After harvesting all the genetically divergent barley and oat varieties, 5 g of seeds were used for grinding and for analysis of their content of *β*-glucans.

Laboratory analysis

The β-glucan contents were determined by the ICC Standard Method No 168, which was adapted to measure the content of β-glucan in barley, oat and their products. Particles smaller than 500 μm were used in the experiment.

Accuracy. In barley containing 4.0 % total β-glucan, the method was accurate to 4.0±0.1 %.

Processing of the results

Results for content of β-glucan of analyzed barley and oat varieties were obtained by spectrophotometrically reading the absorbance at λ=510 μm for each of the three test tubes (Table II). The final content of β-glucan was obtained by applying the following formula for grains of barley and oat:

$$\beta\text{-glucan (mass \%)} = \Delta A \times F \times 94 \text{ (or 64)} \times 0.10w \times 162/180 = \Delta A \times F/W \times 8.46 \text{ (or 5.76)} \quad (1)$$

where ΔA is the absorbance after β-glucosidase treatment (reaction); F – a factor for the conversion of absorbance values to μg of glucose:

$$F = \frac{100\ (\mu\text{g of D-glucose})}{\text{Absorbance of } 100\ \mu\text{g of D-glucose}}$$

and 94 is a volume correction factor (0.1 ml out of 9.4 ml was analyzed for cereal samples); 64 is a volume correction factor (0.1 ml out of 6.4 ml was analyzed for cooked, toasted of extruded cereal products); 0.10w is a factor to express the β-glucan content as a percentage of dry flour weight; W is the calculated dry weight of the sample analyzed in mg; 162/180 is a factor to convert from free D-glucose, as determined, to anhydro-D-glucose, as occurs in β-glucan[33] and w is the grain weight.

RESULTS AND DISCUSSION

The content of β-*glucans in barley*

In the analyzed barley varieties, the contents of β-glucans ranged from 3.52 to 7.81 % (Table II). The highest content of β-glucan among analyzed barley varieties was found in the variety Novosadski 314, with a concentration of 7.81 %, while the lowest concentration was found in the variety Novosadski 312 (3.52 %). Among the other analyzed barley varieties, high contents of β-glucan were observed in Novosadski 318 (7.8 %), Novosadski 310 (7.39 %), Novosadski 306 (7.38 %), Novosadski 297 (7.23 %) and Novosadski 301 (7.18 %). A lower amount of β-glucans was found in the variety Novosadski 300 (6.77 %), while the barley varieties Novosadski 296 (5.85 %) and Novosadski 316 (5.08 %) together with the variety Novosadski 312 (3.52 %) had lower contents of β-glucan than the average of 6.6 %.

Based on the β-glucans contents, all the analyzed barley varieties were compared with each other and a similarity dendrogram with Euclidean distances was made (Fig. 1). An analysis of the dendrogram showed two clusters (groups) with mutually similar varieties (Fig. 1). In the first cluster were the two barley varieties Novosadski 296 and Novosadski 316, with a distance of about 41 % (the degree of similarity was 59 %). The second cluster consisted of a larger number of varieties and pairs showing a high percentage of similarity (Novosadski 297,

TABLE II. The content of β-glucan in oat and barley varieties (mass %)

Analyzed oat variety	β-Glucan content	Analyzed barley variety	β-Glucan content
Flaemingsterne	4.79	Novosadski 296	5.85
Vok	5.60	Novosadski 297	7.23
Mozart	4.28	Novosadski 300	6.77
Juha	4.71	Novosadski 301	7.18
Hannes	4.25	Novosadski 306	7.38
Alden	3.38	Novosadski 310	7.39
Aslak	3.15	Novosadski 312	3.52
Wasa	4.71	Novosadski 314	7.81
Tomba	7.28	Novosadski 316	5.08
Kaempe gul	4.37	Novosadski 318	7.80

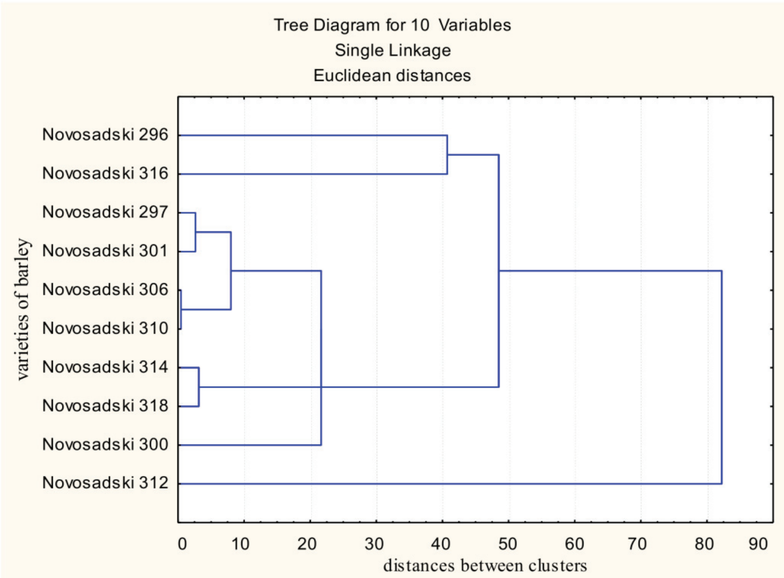

Fig. 1. Dendogram of the genetic distances between the clusters of barley varieties for their content of β-glucan (the dendogram was obtained with Statistica for Windows, version 10).

Novosadski 301, Novosadski 306, Novosadski 310, Novosadski 300, Novosadski 314 and Novosadski 318). In the second cluster, the greatest similarity, 99 %, was observed for the varieties Novosadski 306 and Novosadski 310. Another pair consisted of the varieties Novosadski 297 and Novosadski 301 with a similarity of 97 %. With the previous pair of varieties of the second cluster, they form a small group with a distance of 8 % (degree of similarity of those pairs is 92 %). The most similar to this group according to values of β-glucans was Novosadski 300 with distance in the range of 22 %. The second cluster with a distance of 22 % also contains a pair of varieties, Novosadski 314 and Novosadski 318, which are 97.5 % similar. The degree of similarity of the first and second cluster is 51 %

(49 % distance). The variety Novosadski 312 stands out because of the greatest distance (82.5 %) compared to all other barley varieties.

The content of β*-glucans in oat*

The content of *β*-glucan in the analyzed oat varieties ranged from 3.15 to 7.28 % (Table II). The highest content of *β*-glucan was found in the variety Tomba, with a concentration of 7.28 %. A lower content of 5.6 % was found in the variety Vok. The oat varieties in which the content of *β*-glucan ranged from 4 % to 5% were Flaemingsterne 4.79 %, Juha 4.71 %, Wasa 4.71 %, Kaempe gul 4.37 %, Hannes 4.25 % and Mozart 4.23 %. Among the remaining analyzed oat varieties that have a *β*-glucan concentration of less than 4 % are Alden (3.38 %) and Aslak (3.15 %), in which the lowest concentration of *β*-glucan was observed. The average value of the *β*-glucans contents in the analyzed oat varieties was 4.65 %. Based on the *β*-glucans contents, all the analyzed oat varieties were compared with each other and a similarity dendrogram with Euclidean distances was made (Fig. 2). An analysis of the dendrogram shows two clusters (groups) with mutually similar varieties (Fig. 2). The first cluster consisted of two small groups of varieties, which together show a high percentage of similarity. The first group includes the varieties Flaemingsterne, Juha and Wasa, with the highest percentage of similarity (99 %) within the first cluster. Within the second group, there is a pair of varieties Mozart and Hannes with a degree of similarity of 98 %. The variety Kaempe gul joins this pair with a distance of 8 %. The degree of similarity between the first and second group is 80 % (the distance between mentioned groups is 20 %). The second cluster includes the two oat varieties Alden and Aslak with a mutual distance in the range of 14 %. The degree of similarity of the first and second cluster is 50 %. The variety Vok stands out from the two clusters by a distance in the range of 52 %, while the most notable variety is Tomba due to the greatest distance (97 %) compared to all other varieties of oat.

Many studies have shown that the grains of barley and oat are characterized by higher concentrations of *β*-glucans compared to other grains, whereby barley has a higher content of *β*-glucan with respect to oat. The average concentration of *β*-glucan in barley is approximately in the range of 3 to 7 %, whereas the concentration of *β*-glucan in oat is approximately in the range of 2 to 6 %.[34–36] Havrlentova and Kraic noted a large variation in the content of *β*-glucan in barley and oat varieties in all genotypes and indicated that barley and oat may be convenient sources of *β*-glucan.[34]

Based of the biochemical analysis of the *β*-glucan content in the investigated oat and barley varieties, it could be concluded that the content of *β*-glucan was higher in the barley varieties than in the oat varieties, which could be observed through the average value of the *β*-glucan contents (6.6 % of the dry weight of the grain in the analyzed barley varieties, but only 4.65 % of the dry weight of the grain of the analyzed oat varieties). In the tested barley varieties, the β-glucan content ranged from 3.52 to 7.81 % of the dry grain weight, while the β-glucan content of analyzed oat varieties ranged from 3.15 to 7.28 % of the dry grain weight. The highest contents of β-glucan in the analyzed barley varieties were found in Novosadski 314 (7.81 %) and Novosadski 318 (7.8 %), while the highest content of β-glucan in analyzed oat varieties was observed in Tomba, with a concentration of 7.28 %.

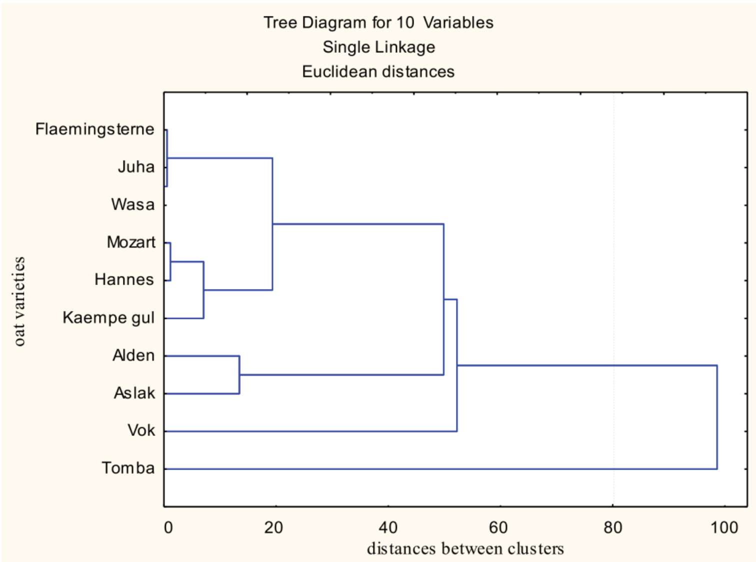

Fig. 2. Dendogram of the genetic distances between clusters of oat varieties for their content of β-glucan (the dendogram was obtained with Statistica for Windows, version 10).

Numerous studies suggest the beneficial effects of β-glucans on human health as immunostimulants,[3,7,8] they can act both anti-mutagenic and anti-carcinogenic,[10,11,37] they can be used for lowering cholesterol levels and improvement of serum lipid status,[38–40] they can have a positive effect on glycemic and insulin response,[17,18] and among others, β-glucans can represent a new source of prebiotics.[27,28] As a result of their positive effects on health, there has been an increasing demand for β-glucans in the last decade. For this reason, it is necessary to find the most affordable sources of β-glucans to enable their application and ensure their availability.

The variability of the β-glucan content is controlled by both genetic and environmental factors and their interaction, which acts during the period of endosperm development.[36,41,42]

Among the environmental factors, warm and dry weather during grain-filling results in higher levels of β-glucan, while high precipitation or irrigation are detrimental.[42,43] Nitrogen (N) fertilizer was shown to influence the level of the biochemical constituents in the grain, and to lead to increased contents of *β*-glucan.[29–31]

Considering that genotype had the greatest influence on variability than all the other factors and their interactions, the dominant effect of which has been confirmed in numerous studies,[29,32,41] the selection of the proper genotype is the most crucial for desired amount of *β*-glucan in grain.[32] Specific programs of cultivation, selection and breeding are the key steps to obtain the appropriate barley and oat varieties for the production of foods rich in *β*-glucans and of high nutritional value.[37–39]

CONCLUSIONS

Based on the obtained results, it could be concluded that genetic diversity influences the content of *β*-glucan in oat and barley varieties, giving the possibility of choosing varieties for the selection of lines of high nutritional capacity required to improve nutrition and to use as a source for obtaining useful preparations in the pharmaceutical industry for further implementation.

The varieties that could be recommend for hybridization and breeding are barley varieties Novosadski 314 and Novosadski 318 and oat varieties Tomba and Vok, as varieties with the highest content of *β*-glucans.

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ИЗВОД

ДИВЕРГЕНТНОСТ СОРТИ ЈЕЧМА И ОВСА ПРЕМА САДРЖАЈУ *β*-ГЛУКАНА

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Јечам и овас се промовишу годинама уназад као добар извор дијететских влакана, а поготову *β*-глукана. Бројне студије показују позитиван ефекат *β*-глукана јечма и овса на здравље људи и као последица јавља се све већа потражња за *β*-глуканима у последњој деценији. У зрну 10 сорти јечма и 10 сорти овса испитиван је садржај β-глукана. За одређивање садржаја *β*-глукана коришћена је ICC стандардна метода бр. 168. У испитиваним сортама јечма садржај *β*-глукана кретао се у интервалу 3,52–7,81 %, а у испитиваним сортама овса садржај *β*-глукана кретао се у опсегу 3,15–7,28 %. Највећи садржај *β*-глукана код анализираних сорти јечма поседује сорта Новосадски 314 (7,81 %), док је највећи садржај *β*-глукана код анализираних сорти овса уочен код сорте Tomba (7,28 %). На основу резултата, можемо закључити да постоји генетичка дивергентност у садржају *β*-глукана између испитиваних сорти овса и јечма, на основу које постоји могућност одабира сорти за селекционе линије високог нутритивног капацитета, као и за побољшање захтева исхране.

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