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MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY ANALYSIS β-GLUCAN CONTENT VARIABILITY IN GRAIN OF OAT (*Avena sativa* L.)

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In grain of ten genetically divergent oat cultivars (Merkur, Minor Abed, Flaming-Kurz, Nuptiele, Prode, Pellerva, Emperor, Astor, Osmo, Simo) the variability *β*-glucan content were investigated. The different value of content of *β*-glucan was found. Among analyzed oat cultivars, the highest *β*-glucan contents had Pellerva (6.597%), while the least had Simo (2.971%). The contents of *β*-glucans were determined by ICC standard Method No 168. The value of *β*-glucans varied and indicated the differences and similarities between analysed cultivars. The degree of cultivar similarity was determined by dendrogram on which was discriminated two clusters of similar cultivars toward to contents of *β*-glucan . Within cluster 1, a small group of oats, are five cultivars with small distance (Merkur, Minor Abed, Flamings-Kurz, Nuptiele and Prode). The highest similarity in the range of 88 or the least distance in the range of 12. Within cluster 2 was four oat cultivars (Emperor, Astor, Osmo, Pellerva) in which the least differences was between Emperor and Astor with average distance in range 27. Cluster 1 and cluster 2 differed with an average distance of 63. The cultivar Simo expressed the greatest distance to all analysed oat cultivars grouped in two clusters.

Key words: *β*-glucan, content, divergence, oat, cultivars

INTRODUCTION

Oat has continuously promoted as a health food but oftenly without clear knowledge of its specific health related effects. However, today it is known that oat fiber influence to satiety

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and retarded absorption of nutrients as well as a deterrent of various disorders of the gastrointestinal tract. Also, soluble oat fiber is able to attenuate blood postprandial glycemic and insulinemic responses (WOOD *et al*., 2000; HOODA *et al*., 2010; REGAND *et al*., 2011; DONG *et al*., 2011; TIWARI & CUMMINS, 2011), can help in lowering blood total cholesterol and low-density lipoprotein (LDL) cholesterol (BAE *et al*., 2010; DROZDOWSKI *et al*., 2010; TIWARI & CUMMINS, 2011), and to improve high-density lipoprotein (HDL) cholesterol and blood lipid profiles as well as to maintain body weight. Oat *β*-glucan has effect in the prevention, treatment, and control of diabetes and cardiovascular diseases. *In vitro* experiments on animals and human clinical trials indicate the significance of *β*-glucans in the prevention and treatment of allergies (JASENAK *et al*., 2012).

In addition, oat *β*-glucan can stimulate immune functions by activating monocytes/macrophages and increasing the amounts of immunoglobulin, NK cells, killer T-cells, and so on, which will improve resistance to cancer (YANG, 2008) and infectious and parasitic diseases, as well as increase biological therapies and their prevention (MÄLKKI, 2001). All these health benefits of oat *β*-glucan can be explained by its physico chemical properties (viscosity, molecular weight) which can be affected by extraction methods and its behavior in gastrointestinal tract. Today oats is among the richest and most economical sources of soluble dietary fiber. Beta glucan is a polysaccharide that is found in seed of the cereal. Among cereals, oat and barley have the greatest concentrations of *β*-glucan, while rye and wheat have lesser content of *β*-glucan. In oat grains, *β*-glucan is a cell wall polysaccharide found mainly in the endosperm and the subaleurone layer of seeds (WOOD, 1993). Oat bran contains about 7% of beta glucan, and is inexpensive, but only good as a food (VETVICKA *et al*., 2010). It is too weak to use as a supplement or in Whole wheat and rye contain about 2%. Chemically in oat and barley glucans are 1,3/1,4 *β*-glucan positions on the glucose chain, while in yeast and mushroom glucans are 1,3/1,6 positions. Oat *β*-glucan is mainly mixed-linkage (1-3, 1-4)- *β*-D-glucan and is often referred to as *β*-glucan. It is a hemicellulose that makes up about 70% of cereal endosperm cell walls (CARPIITA, 1996).

β-glucans of oat are non-starch polysaccharides, glucose polymers with *β*-(1-3) and *β*-(1-4) glucosidic bonds (DUSS *et al*., 2004). The presence of two types of glucosidic bonds in *β*-glucans molecules influences their physical and chemical properties, such as viscosity and solubility. Viscosity depends on soluble *β*-glucans concentration and on their molecular mass. In relatively small concentrations (1%) *β*-glucans have high viscosity which is present in wider pH range. That is why oat *β*-glucans have very good rheological properties and can be used as food elements in the form of hydrocolloids (ANTTILA *et al*.,2004; HAVRLENTOVA *et al*., 2011).

Increased oat *β*-glucan has been a major target for breeding operations because of its positive and consistent health implications when oat is consumed as a whole grain. Most notably, it has been demonstrated to improve health with respect to blood pressure (KEENAN *et al*., 2002), diabetes (JENKINS *et al*., 2002), cholesterol (MAKI *et al*., 2003) and the immune response (HONG *et al*., 2004), all of which are important given the increases in human health cases related to diet over the past few decades. The development of oat cultivars with greater groat *β*-glucan contents should increase the nutritional and economic value of the oat crop.

Oat *β*-glucan content is a polygenic trait under the control of genes with mainly additive effects. Heritability estimates for *β*-glucan content have ranged from 0.27 to 0.58 (HOLTHAUS *et al*., 1996; KIBITE AND EDNEY, 1998). *β*-Glucan content is affected by environmental factors, including soil nitrogen level and precipitation (BRUNNER & FREED, 1994). Although genotype/environment interaction sometimes is a significant source of variation for *β*-glucan content, the ranking of genotypes is generally consistent over environments (SAASTAMOINEN *et al*., 1992; TONOKA *et al*., 2009).

The objectives of this research are to estimation of *β*-glucan concentration in grain of oat cultivars, determination of genetic divergence and distance among the oat cultivars according to contents of *β*-glucan.

MATERIALS AND METHODS

In this paper, grain samples of 10 geneticaly divergent oat cultivars (Merkur, Minor Abed, Flaming-Kurz, Nuptiele, Prode, Pellerva, Emperor, Astor, Osmo, Simo) were used for analysis of contents of *β*-glucans.

The samples of oat seed were grinded by laboratory grinder. Particles of <500 µm size were used in the experiment.

The contents of *β*-glucans were determined by Megazyme method (ICC Standard Method No 168). The assay is specific for mixed-linkage [(1-3)(1-4)- *β*-D-glucan]. Method principle is: samples are suspended and hydrated in a buffer solution of pH 6.5 and then incubated with purified lichenase enzyme and filtered through Whatman No.41. An aliquot of the filtrate is then hydrolyzed to completion with purified *β*-glycosidase. The produced D-glucose is assayed using a glucose oxidase/peroxidase reagent. Then the absorbances were measured at 510 nm for each sample A1, A2 and blank. The contents of *β*-glucan were calculated by using the factor for the conversion of absorbance values to µg of glucose and factor for the conversion from free D-glucose, as determined, to anhydro-D-glucose, as occurs in β-glucan (MCCLERY *et al*., 1991).

RESULTS AND DISCUSSION

The contents of *β*-glucans in analyzed oat cultivars variate between 2.971% in cultivar Simo and 6.597% in cultivar Pellerva (Tab. 1). Among the remain analyzed oat cultivars the high concentration of *β*-glucans was found in Astor (5.776%), Emperor (5.237%), Nuptiele (4.948%). Other oat cultivars Merkur (4.403%), Flaming Kurz (4.024%), Minor Abed (4.334%), Prode (4.494%) and Osmo (4.505%) had the higher percentage compared with the cultivar Simo, but somewhat smaller percentage in relation to the average value of *β*-glucan contents in analyzed cultivars which is 4.729%.

The contents of *β*-glucans in grains depend on various factors which acting the period of endosperm development: effects of 1,3-1,4- *β* glucanase enzyme which enables degradation of endosperm cell wall in germination period, nitrogen level, temperature, precipitations (BRUNNER and FREED, 1994). Barley and oat contain larger quantities of *β*-glucans than any other grain.

Based on *β*-glucans contents, all analyzed oat cultivars were compared with each other and similarity dendrogram with Euclidean distance was made. By the analysis of dendrogram, small groups (clusters) of mutually similar cultivars could be observed (Figure 1).

Differences exist in the pair-wise relationships between clusters as seen in the PC scatter plots. Quantitative results for genetic distances between cultivars are graphically representated in Figure 1.

Table1. The contents of β-glucans in analyzed oat cultivars

Oat	Absorbances (510nm)			β-glucan
samples analyzed:	A1	A2	ΔA	% (w/w)
Merkur	0.836	0.812	0.824	4.403
Flaming-Kurz	0.768	0.738	0.753	4.024
Minor Abed	0.821	0.802	0.811	4.334
Nuptiele	0.865	0.987	0.926	4.948
Simo	0,327	0.786	0.556	2.971
Prode	0.839	0.843	0.841	4.494
Pellerva	1.220	1.249	1.234	6.597
Emperor	0.945	1.016	0.980	5.237
Astor	1.064	1.099	1.081	5.776
Osmo	0.877	0.810	0.843	4.505

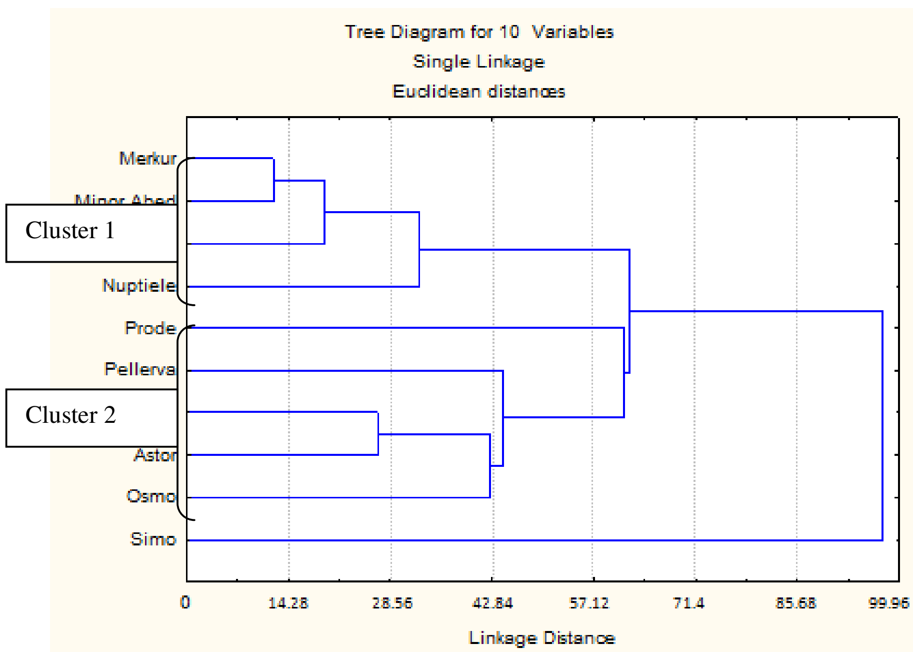

Figure 1. Dendogram of the genetic distances between clusters of oat cultivars for *β*-glucan content

Differences exist in the pair-cultivars relationships between clusters as seen in the PC scatter plots. Quantitative results for genetic distances (measured as Euclidean distance) between clusters are shown in Table 1; a graphical representation is shown in Figure 1. By analysis two cluster of oat cultivars formed on dendogram. Within cluster 1, a small group of oats, are five cultivars with small distance (Merkur, Minor Abed, Flamings-Kurz, Nuptiele and Prode). The highest similarity in the range of 88 or the least distance in the range of 12 expressed between oat cultivars Merkur and Minor Abed (Fig. 1). The most similar to this pair according to value of *β*-glucans was Flamings-Kurz with distance in the range 18. In relation to this three cultivars the similar is Nuptiele cultivars with distance in the range 33. Similarities among this cultivars in Cluster 1, is the higher than among cultivars grouped in cluster 2. Within cluster 2 the least distance was between Emperor and Astor in the range of 27. The smallest distance to this pair expressed cultivars Osmo in the range 43 and Pellerva in the range of 45 and Prode cultivar in the range of 63 approximately. Cluster 2 is closely related to clusters 1 with an average distance of 63. However, the cultivar Simo stands out due to the greatest distance (98) compared with all the other oat cultivars.

It possible say that, the genetic distances are in connection to genetic specificity and/or adaptation of the analyzed cultivars. These results suggest that clustering can be efficient in separating genotypes of existing germ plasm.

In comparison with all other fibres, the positive effects of *β*-glucans on health have been most extensively documented, while no reports of harmful effects of food rich in beta-glucans from oat or barley flour or their extracts. Many researches of biological activity of *β*-glucans (RONDANELLI *et al*., 2009; KA-LUNG *et al*., 2013) have confirmed their potential application in functional food production, but also in pharmaceutical industry and medicine due to physiological effects (VETVICKA AND VANCIKOVA, 2010).

The increased interest in *β*-glucans in the last few decades is the consequence of its functional and bioactive properties. *β*-glucans have positive effect on human metabolism and reduction of cholesterol and sugar in blood (HAGGARD *et al*., 2013; PARK *et al*., 2009; SCHULZE *et al*., 2004), they reduce the risk of cardiovascular diseases (PEREIRA *et al*., 2004).

CONCLUSIONS

In this study established wide diversity of oat cultivars according to identified β-glucan contents in grain. Considering the fact that the contents of β-glucans can be increased by cultivation (and application of proper agro-technical measures), the aim of our researches was determination and comparison of β-glucans contents in 10 oat genotypes and selection of cultivars with higher contents of β-glucans, as a modest contribution to oat cultivation. Obtained data of β-glucans and estimated similarity/distance can use for different practices in exchange of germ plasm among breeding programs, or breeding methods that more frequently utilize crosses among different oat types.

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VARIJABILNOST SADRŽAJA BETA GLUKANA U SEMENU OVSA (*Avena sativum* L.)

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Izvod

U radu je izučavano variranje sadržaja β-glukana u zrnu sorti ovsa (*Avena sativum* L.). U izučavanjima je obuhvaćeno 10 genetički divergentnih sorti ovsa: Merkur, Minor Abed, Flaming-Kurz, Nuptiele, Prode, Pellerva, Emperor, Astor, Osmo, Simo. Uzorci semena ovsa su mleveni na laboratorijskom mlinu. Čestice <500μm su korišćene u eksperimentu. Metodom Megazyme (ICC standardna metoda 168) je određen sadržaj β-glukana u semenu ovsa. U izučavanjima je ustanovljeno variranje sadržaja β-glukana u semenu sorti ovsa. Prosečan sadržaj β-glukana za 10 ispitivanih sorti je iznosio 4.729%. Vrednosti koncentracije β-glukana je varirala od 2.971% kod sorte Simo do 6.597% kod sorte Pellerva. Kod sorti Astor (5.776%), Emperor (5.237%) , Nuptiele (4.948%) sadržaj β-glukana je bio niži u odnosu na sortu Simo, ali veći sadržaj u odnosu na prosečanu vrednost koncentracije izračunate za sve analizirane sorte. Grupa sorti Merkur (4.403%), Flaming Kurz (4.024%), Minor Abed (4.334%), Prode (4.494%) i Osmo (4.505%) su imale manju vrednost koncentracije u odnosu na prosečnu vrednost izučavanih sorti. Na osnovu dobijenih vrednosti sadržaja β-glukana izučavana je sličnost izmedju analiziranih sorti. Ustanovljene su dve grupe medjusobno sličnih sorti ovsa i to: Merkur, Minor Abed, Flaming-Kurz, Nuptiele i Prode, a dugi klaster sorti: Emperor, Astor, Osmo, Pellerva. U klasteru 1, sorte Merkur i Minor Abed su se najmanje razlikovale sa distancom od 12, dok u klasteru 2 najmanja razlika je bila izmedju sorti Emperor i Astor sa distancom od 27. Generalno je veća sličnost između sorti u prvom klasteru, čija distanca je izmedju 12 i 33, dok je distanca izmedju sorti unutar drugog klastera varirala u rasponu od 27 do 63. Klaster 1 i klaster 2 se razlikuju medjusobno sa prosečnom distancom 63. Od dve grupe medjusobno sličnih sorti najveću distancu je imala sorta ovsa Simo sa prosečnom distancom 98.

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