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VARIABILITY IN ENDOSPERM β-GLUCAN CONTENT OF HUSKED AND NAKED OAT GENOTYPES

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Oat (*Avena sativa* L.) is one of the major cereal crops in temperate climate zones. Naked oat characterizes with good agriculturally features and could be use to produce high quality biological foodstuff and complete feed for animals. The health benefits of oat are mainly associated with its mixed β-glucan. The main tasks of the study were to characterize the variation in β-glucan content between different types of oat (husked and naked) and to the variability of β-glucan content for oat varieties and breeding lines bred in Latvia. Grain samples of 102 oat genotypes (82 genotypes of husked and 20 genotypes of naked oat) were used for investigation β-glucan content. The genotypes were grown at the State Stende Cereal breeding institute in the years 2007 and 2009. β-glucan content was determined enzymatically. Statistical analysis of variance showed that a significant difference (p-value<0.001) between genotypes in β-glucan content was observed for both types of oat. Statistically significant difference (p-value<0.0001) was obtained for the β-glucan content variation between the years of investigation only for husked oat. On average, naked oat had slightly higher β-glucan content than husked oat. For naked genotypes it ranged from 38.5 to 43.1 g kg-1, but for husked oat – 24.9 to 35.2 g kg-1. Standard deviation of the mean values of β-glucan content for hulled genotypes was lower than for the naked ones. From husked breeding material of Latvian origin the highest and less variable β-glucan content was found in oat variety 'Arta' (mean value 34.2±2.5 g kg-1) and breeding lines '32224', '31231', '31959' - 34.6±2.9, 34.2±3.4 and 34.2±2.1 g kg-1 respectively. The best results within naked oats showed breeding lines '31353' and 'S-156' - 43.1±1.2 and 41.9±0.9 g kg-1 respectively. This material could be used as a promising material in the breeding work of oat varieties.

Key words: oats, naked oats, husked oats, genotypes, β-glucan, variability.

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INTRODUCTION

Avena sativa (Linnaeus, 1753) or common oat is one of the major crops in temperate climate zones. It is an annual crop used both for human and animal nutrition. Before being used as a food, it was used for medicine purposes. With the development in field of nutrition, oat was recognized as a healthy food, it was signifying that a substance in it helped prevent heart disease and therefore it became more popular for human nutrition (Butt et.al. 2008).

Oat can be broadly classified as husked and

naked; the naked oats are nutritionally superior compared to conventional husked oats. Some scientists argue that the oats classifications are only an artificial one created by the oat breeders. However there is a difference by weight because the husk contains negligible amounts of β-glucan compared to the endosperm of the grain thus naked oats contains a higher percentage β-glucan compared to husked oats. Naked oats have a thin non-lignified husk on the outside of the grain which falls off during harvesting, resulting in a grain of higher energy, protein and lipid and lower fibre content compared with conventional oats (Tiwari et.al. 2009). Naked oat characterizes with good agriculturally features and could be use to produce high quality biological foodstuff and complete feed for animals (Biel et.al. 2009, Welch 1995).

Among the main compounds associated with health-promoting effects in cereals is dietary fiber. Dietary fiber is found only in plant foods. It consists of both soluble and insoluble fiber. Soluble fiber dissolves while insoluble fiber does not dissolve in water. Both types are important for health in different ways (Grausgruber et.al. 2004, Manthey et.al. 1999). Water-soluble fiber in cereals is composed of non-starchy polysaccharides such as β-glucan. Some of the oat constituents are valuable as ingredients or starting materials for several types of products (Brindzova et.al. 2008). Comparing to other cereals, oat as well as barley endosperms have relatively higher β-glucan contents compare with other cereals. Oat β-glucan has received the most attention and has a number of uses and potential uses. β-glucan is included in the soluble dietary fiber fractions of oat that participates in the glucoregulation and causes a decrease in serum cholesterol levels in humans (Wood 2007). Comparing to other cereals, oat as well as barley endosperms have relatively higher β-glucan contents compare with other cereals (Queenan et.al. 2007).

A relatively small amount of information is available in the literature describing the effects of environment and genotype on oat grain quality. Dietary fiber depends on genotypes, meteorology and growing technologies (Doehlert et.al. 2001). That is why oat breeders should find genotypes with highest quality parameters. It would be useful to include these genotypes as source material in breeding programs.

This study aimed to analyse oat varieties, lines and hybrids to appraise its potential to serve as a raw material for new varieties with use in high value dietetic food production. The research includes breeding material selected from oat breeding program of State Stende Cereals breeding institute.

MATERIALS AND METHODS

There were arranged field trials in State Stende Cereals breeding institute in the years 2007 and 2009, for estimating biological, biochemical and agronomical importance of 102 oat genotypes (int. al. 82 husked and 20 naked). The objects of research for determine the phenotypic variation are varieties and perspective lines of Latvian oat breeders (54 varieties and breeding lines) and foreign varieties (48 varieties).

To obtain equal research background all genotypes were grown in plant breeding crop rotation fields three years, in the use of similar growing conditions (seed-time, fertilizer, plant protection activities), which agree with generally accepted technology of oat cultivation in Latvia. Treatments and varieties were laid out in four replications. Recorded plot area was 2 m^2 seed rate - 500 seeds per m^2. Plots were laid randomized. The soil at state was sod-podzolic sandy loam. Trial conditions are interpreted in table 1.

Field trial conditions were highly similar, precrop was the same. There was no significant difference among soil parameters. Manure was similar and seed-time at the end of second and start of third decades of April.

The temperature and moisture conditions provided good oat field germination in 2007. In June the average temperature was by 2,2 ^{0}C higher than the long-term average, but the sum of precipitation was only 1.7 mm. July was cool and wet (average daily temperature 0,1 ^{0}C below long-term

Table 1.Basic agrochemical soil parameters and field trial conditions

Parameters	Year of field trial		
	2007	2008	2009
Soil pH	6.5	6.5	6.3
Humus content %	1.8	1.8	2.6
P in soil, mg kg-1	313	313	334
K in soil, mg kg-1	249	249	250
Manure (NPK)	51, 30, 42	56, 56, 56	56, 56, 56
Precrop	Winter wheat	Winter wheat	Winter wheat
Seed-time	April 16	April 24	April 20

Table 2. The meteorological characteristics profile of field trial from 2007 till 2009 year

Month	temperature, ^{0}C				moisture, mm			
	Long-term average	2007	2008	2009	Long-term average	2007	2008	2009
April	4.2		6.5	6.6	37		45.1	11.5
May	10.2	13.5	10.2	11.0	45	65.2	16.1	28.8
June	14.2	16.0	14.0	13.5	57	40.8	72.0	94.3
July	16.3	16.0	15.9	17.1	87	113.6	95.6	147.5
August	15.5	17.4	15.0	15.7	87	121.2	159.5	83.4

average, precipitation was 120% of long-term average). Mean temperature in August was by 2,2 ^{0}C higher than the long-term average. April of 2008 was comparatively warm with mean daily temperature by 2,2 ^{0}C higher than the long-term average. The moisture deficiency was observed in May (36% from long-term average) and also in the first decade of June. In June and July, the mean daily temperature was lower than the a norm (by 0,2 ^{0}C and 0,4 ^{0}C respectively). The second decade of July was rich with precipitation (58,4 mm). Very high amount of precipitation was observed also in August (183% from long-term average). In general in April of 2009 average daily temperature was by 2,3 ^{0}C higher than long-term average, bet moisture 11,5 mm, only 31,1% from long-term average. Average daily temperature and moisture of May and July was slightly higher then long-term average. In August daily temperature and moisture was close to long-term average. Monthly temperature and moisture average and long-term average values are shown in table 2.

β-glucan content was determined enzymatically following the oat grains procedures of the commercial kits from Megazyme (Megazyme International Ireland Ltd.) according to the method developed by McCleary and Glennie-Holmes (1985) and performed at the State Stende Cereals breeding institute. In the procedure, highly purified enzymes were employed. A sample (0.5 g) of flour was weighted and β-D-glucan was depolimerized with lichenase to oligosaccharides and then hydrolyzed to glucose with a specific purified β-glucsidase. The β-glucan content (mg kg-1) was calculated using the glucose quantity found in formula (1):

$$\beta\text{-glucan} = \Delta E \times F/mg \times 270, \quad (1)$$

where

ΔE – the absobance difference at 510 nm in a UV-spectrophotometer after β-glucosidase treatment – blank absorbance;

mg – weight of sample;

F – a factor for conversion of absorbance value to μg glucose

ANOVA procedures were used for data analysis.

RESULTS AND DISCUSSION

There are only several biochemical studies of oat

as potential raw material for human diet. Oats are mostly studied as food stock and parameters like yield from hectare, volume weight of cereals tonne, husk content of yield (Zute S. et.al. 2008). But unfortunately these parameters does not describe oats nutritive and dietary value, which is important criterion describing the quality of food. Oats differ from others cereals by balanced EAA structure in protein, oil rich with unsaturated fatty acids, easily available starch and comparatively high amount of β-glucan, E and B5 vitamin in grain. Oats is one of the cereals, which is possible to use in multifunctional product production, because its biochemical composition can ensure not only nutrition of organism, but also serve in prevention of several diseases and recuperation promotion (Martinez et.al. 2001). Plant breeders should pay attention to cereals biochemical meters, wile developing new oat varieties for food production. Till now in oat breeding program of Latvia protein and lipids was the selection criterions to evaluate breeding material after biochemical composition. To improve oat breeding program, plant breeders want to include dietary fiber as selection criterion. In laboratory of institute there is acquired method to determine β-glucan amount in oat grains.

β-glucan is one of the dietary valuable substances of oat grains and it composes the main part of soluble fiber. Studies of Sweden and Finland, there are established that β-glucan content in dry matter of husked oat genotypes vary from 2.2 till 4.5%, but oat groats – from 3.9 – 6.3% (Saastamoinen et al. 1992). Studies of Mexico showed the range of β-glucan amount even 4.8 till 7.1% (Martinez et.al. 2002).

β- glucan amount in husked oat varieties and lines cultivated in Latvia during three years varied from 28.90 till 41.4 mg 100 g^{-1}, but naked oat – 35.7 – 47.2 mg 100 g^{-1}. Analysing mean values of β- glucan in husked and naked oats can conclude that β- glucan amount by meteorological conditions of Latvia is comparatively stabile and little vary during years (coefficient variation - CV <10%) (table 3.).

Greater diversity of genotypes after β- glucan amount in grains established to analysed naked oat varieties and lines ($CV_{n=20}$ = 13.3%), but husked oat genotypes after this parameter varied less ($CV_{n=82}$ = 3.3%). Consequently selected husked oats are considered less varied than naked oats after β- glucan amount.

Description of husked oat varieties and lines

In this study there are included 82 husked oat varieties and breeder lines inter alia 42 Latvian and 40 foreign oat varieties and lines – 24 from Western Europe, ten from USA and Canada, six from Russia and Belarus. Foreign oat varieties include genotypes, which at present are grown in Latvia or others European countries (for example, 'Ivory', 'Hecht', 'Margarett', 'Vendela', Kazstan' etc.), and genotypes which were wide grown previously (for example 'Selma', 'Kirovec', 'Skakun', 'Astor' etc.). Such selection let to compare the changes of β- glucan in oat grains today's and former made varieties.

Although husked oat genotypes after β- glucan amount in grains by year in foreign varieties and breeding lines was less various ($CV_{n=40}$=3.1%), for separate varieties the values were significantly higher or lower then β- glucan mean value of all varieties. The highest level of β- glucan was for oat variety 'Freja' (Sweden) oat breeding line BOR 88322 (Finland) and variety 'Bug' (Belarus) (table 4). In addition β- glucan amount of oat line BOR 88322 and variety 'Bug' varied less (CV<5%). The least amount of β- glucan was in oat varieties 'Ivory' and 'Hecht' from Germany. Results obtained showed that nowadays recommended for breeding and production of dietary food variety 'Ivory' has tendency to show lower amount of β- glucan in grains than varieties 'Freja' and 'Bug', recommended before 20 years.

Comparing β- glucan changes in oat grains by year, conclude that the greater changes between oat genotypes were observed in 2007 (CV=7.6%), but significantly lesser – 2008 and 2009 (CV respectively – 3.0 and 2.4%). Comparing β- glucan changes between oat genotypes, established that the amount of β- glucan mainly varied during years for varieties 'Panther' and 'Skakun' (CV

Table 3. β- glucan amount of oat grains cultivated in Latvia, mg 100 g^{-1} dry matter, 2007 - 2009, SSCBI

Oat group	β- glucan, mg 100 g^{-1}, mean/min-max				CV, % between	
	2007	2008	2009	average	years	genotipes
Husked oat, n=82	33.8 28.9–41.2	30.6 28.9–32.5	33.5 30.9–34.8	32.6 30.2–35.3	6.7	3.3
Naked oat, n=20	41.94 36.5–47.2	40.5 37.1–42.6	39.9 35.7–42.6	40.8 37.9–43.3	4.3	13.3

Table 4. Comparison of oat varieties and breeding lines from different countries (except Latvian) after β-glucan amount in grains dry matter, 2007 – 2009, SSCBI

Oat varieties and lines	Country of origin	β-glucan level in oat grains, mg 100g^{-1}				CV, % between years
		2007	2008	2009	mean± sd	
Highest β-glucan values						
Freja	SWE	36.2	31.6	34.6	34.1±2.3	6.8
BOR 88322	FIN	35.8	32.4	34.3	34.1±1.7	4.9
Bug	BLR	35.2	32.0	34.4	33.9±1.7	4.9
Mean between varieties and lines, n=40		33.5	30.5	34	32.7±1.9	5.8
Lowest β-glucan values						
Vendela	SWE	30.02	31.6	33.1	31.6±1.5	4.9
Ivory	DEU	29.32	30.6	33.4	31.1±2.1	6.7
Hecht	DEU	29.99	29.2	31.5	30.2±1.2	3.9

*CV – coefficient of variation

Table 5.Comparison of Latvian oat varieties after β-glucan amount in grains dry matter, 2007 – 2009, SSCBI

Oat varieties and lines	β-glucan level in oat grains, mg 100g^{-1}				CV, % between years
	2007	2008	2009	mean± sd	
Highest β-glucan values					
Arta	36.3	31.5	34.8	34.2±2.5	7.2
Laima	34.2	30.8	33.2	32.7±1.7	6.1
Mean between varieties, n=8	33.3	30.7	33.6	32.6±1.7	5.3
Lowest β-glucan values					
Stendes dzeltenās	32.5	29.5	33.2	31.9±1.9	6.2
Stendes Darta	30.5	31.1	33.0	31.5±1.3	4.1
Stendes Liva	30.7	30.8	33.4	31.6±1.5	4.8

respectively 13.3 and 11.2%), but the most stable values for varieties 'Margarett' and 'Hecht' (CV respectively 3.3 and 3.8%).

There were included also eight oat varieties of Latvian breeders, established during last 50 years in this study. Between Latvian varieties should mention oat variety 'Arta', which showed the highest β- glucan level in grains (table 5.), the parameters of this variety during three years were equivalent to parameters of variety 'Freja' and were significantly higher then other Latvian varieties.

There were included 34 breeding lines from oat breeding program in this study. Also variability of these genotypes after β-glucan amount in grains on average during three years was low ($CV_{n=34}$=3.6%), though slightly higher by the side of variability of foreign varieties and lines (+0.5%). The results of analysis detect that a few breeding lines from oat breeding program after β-glucan level was equal to oat variety 'Arta', for example, breeding lines 32224, 31231, 31959, 30104. The best mean values of β-glucan during three years showed breeding lines 32190 and 31824 (respectively average during three years 35.2 and 34.8 mg $100g^{-1}$), though the parameters of these breeding lines substantially varied by year (coefficient of variation respectively 14.7 and 17. 6 %). In breeding process one of the primary aims is to select breeding lines with the highest level of β-glucan, but it is also important, that the variance of these parameters were little. Comparing the changes of β-glucan level in oat grains by year, the greater differences were obtained among breeding lines in 2007 (CV=8.8%), but noticeably little difference in 2008 and 2009 (CV respectively – 2.8 and 2.0%)

Estimating turnover of every breeding line by year, established that only for ten of 34 oat breeding lines variability of β-glucan level by year was lower then 5%. Unfortunately the β-glucan level of the best breeding lines varied from 6.1 till 9.9% (table 6.).

Description of naked oat varieties and lines

Literature studies showed that β- glucan level in naked oat grains is higher than in husked oat grains. (Biel et.al. 2009). Comparing biochemical parameters of husked and naked oats after their amount in 100 grams, however should pay attention that 25g of grain mass take husk. Wood-fibre is the main component of the husk, which has been removed from grain in food industry. For correct comparing of nutritive value of husked and naked oats as raw material for food, hull from husked oats should be removed. As biochemical parameters were detected for whole grain (husked oat with husk) in this study, naked oats was compared inter-group.

In general there were estimated eight varieties and breeding lines of oats from different countries (Belarus, Czech Republic, Germany, USA, Canada), which were assessed after several agronomical parameters within the framework of breeding program and acknowledged as suitable for Latvian agrometeorologal obstacles. Estimating level of β- glucan in grains of naked oats, there were established, that the highest results by year were achieved for varieties 'Izak', 'Beloruskij golozjornij' and for breeding line OA-504-5 (respectively 42.4, 41.9 and 43.3 mg 100 g^{-1}) (table 7). Variety 'Izak' showed very low variation of β- glucan by year (CV=1.5%). In general foreign naked oat varieties and breeding lines after β- glucan level was comparatively similar, coefficient of variation among genotypes by year varied from 4.7% in 2007 till 3.8% - 2009. The highest variation by year vas obtained for breeding line OA-504-5 and variety 'Pennuda', respectively 8.5 and 7.5%.

There were estimated also 12 Latvian breeding lines of naked oats. The best results by year were obtained by breeding lines 31353 (Arta/Kirovec// Nova), 28156 (Selma/Nos nacht), P4690 (Arta/ Skakun//Pennuda), (respectively 43.1, 41.9 and 41.9 mg 100 g^{-1}) (table 8.). The variation after β-glucan amount among mean values of three

Table 6. Comparison of Latvian oat breeding lines after β-glucan amount in grains dry matter, 2007 – 2009, SSCBI

Oat lines	β-glucan, mg 100g^{-1}				CV, % between years
	2007	2008	2009	Mean ± sd	
Highest β-glucan values					
32190	41.1	31.2	33.4	35.2±5.2	14.7
32184	41.2	29.1	34.0	34.8±6.1	17.6
32224	37.7	31.9	34.3	34.6±2.9	8.4
31959	36.3	32.2	34.1	34.2±2.1	6.1
31231	37.6	30.8	34.2	34.2±3.4	9.9
30104	36.7	31.2	34.1	34.0±2.8	8.1
31929	36.3	31.0	34.6	33.9±2.7	8.0
Mean between lines, n=34	35.0	30.7	33.6	32.8±2.3	7.1
Lowest β-glucan values					
31869	30.3	30.5	32.7	31.1±1.3	4.2
31709	30.9	29.8	32.6	31.1±1.4	4.5
32013	29.6	29.6	32.9	30.7±1.9	6.2
31769	28.9	29.6	32.9	30.5±2.2	7.2

Table 7. Comparison of naked oat varieties and breeding lines from different countries (except Latvian) after β-glucan amount in grains dry matter, 2007 – 2009, SSCBI

Oat varieties and lines	Contry of origin	β-glucan daudzums auzu graudos, mg 100g^{-1}				CV, % between years
		2007	2008	2009	mean± sd	
Highest β-glucan values						
OA -504-5	CAN	47.2	42.9	39.9	43.3±3.7	8.5
Beloruskij golozjornij	BLR	45.7	40.9	39.3	41.9±3.3	7.9
Izak	CZE	41.9	41.6	41.7	41.4±0.6	1.5
Mean between varieties and lines, n=8		43.5	40.4	39.0	40.9±2.3	5.7
Lowest β-glucan values						
Adam	DEU	43.6	38.8	40.6	40.6±2.6	6.3
Nos Nacht	DEU	42.5	39.6	39.5	40.5±1.7	4.2
Pennuda	USA	41.2	37.1	35.7	37.9±2.9	7.5

Table 8. Comparison of Latvian naked oat breeding lines after β-glucan amount in grains dry matter, 2007 – 2009, SSCBI

Oat lines	β-glucan, mg 100g^{-1}				CV, % between years
	2007	2008	2009	Mean ± sd	
Highest β-glucan values					
31353	44.4	42.9	41. 9	43.1±1.3	2.9
S-156	40.8	42.2	42.6	41.9±0.9	2.2
P4690	46.1	40.0	39.6	41.8±3.8	8.9
Mean between lines, n=12	41.4	40.4	40.4	40.8±1.6	3.9
Lowest β-glucan values					
28167	36.7	40.5	40.9	39.4±2.3	5.9
30629	39.2	38.5	37.9	38.5±0.7	1.8

year data was insignificant - $CV_{n=12}$= 3.9%. The greatest differences among genotypes were obtained in 2007 - CV=6.9%, the lowest in 2008 - 2.9%. The stable amount of β-glucan in grains showed breeding lines 31353 and S-156, for which this parameter varied between 2.9 and 2.2%. These lines are acknowledged as most perspective in oat breeding program and are possible to use as raw material for new oat varieties further.

CONCLUSIONS

1. Consequently selected husked oat mean value are considered less varied than naked oat after β- glucan amount (respectively $CV_{n=20}$ = 13.3% and $CV_{n=82}$ = 3.3%).

2. From foreign oat varieties and breeding lines varieties 'Freja' and 'Bug' and line BOR 88322 had the highest level of β- glucan (respectively 34.1±2.3, 33.9±1.7 and 34.1±1.7 mg $100g^{-1}$). From Latvian oat varieties variety 'Arta" showed the highest level of β- glucan (respectively 34.2±2.5 mg $100g^{-1}$), but the lowest 'Stendes Darta' (respectively 31.5±1.3 mg $100g^{-1}$).

3. Comparing β- glucan changes between oat genotypes, established that the amount of β-glucan mainly varied during years for varieties 'Panther' and 'Skakun' (CV respectively 13.3 and 11.2%), but the most stable values for varieties 'Margarett' and 'Hecht' (CV respectively 3.3 and 3.8%).

4. The results of analysis detect that a few breeding lines was equal to oat variety 'Arta', the best mean values of β-glucan by year showed breeding lines 32190 and 31824 (respectively average during three years 35.2 and 34.8 mg $100g^{-1}$), though the parameters of these breeding lines substantially varied by year (coefficient of variation respectively 14.7 and 17. 6 %).

5. Estimating level of β- glucan in grains of naked oats, there were established, that the highest results by year for foreign varieties and line were achieved for varieties 'Izak', 'Beloruskij golozjornij' and for breeding line OA-504-5 (respectively 42.4, 41.9 and 43.3 mg 100 g^{-1}) and Latvian breeding lines 31353, 28156, P4690 (respectively 43.1, 41.9 and 41.9 mg 100 g^{-1}6.

6. In the breeding process of husked oats, there were picked breeding lines with better yield and other agronomic features, unfortunately the biochemical parameters of these lines are in lower level. But exactly these parameters estimate the nutritive value of oat diet and development of new products.

7. Well-known that husked oats are genetically related and interbreed, it would be advisable in selection process perform breeding between husked oats with high yield and naked oat lines with high nutritive value, to improve quality parameters of husked oats.

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