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EVALUATION OF OAT GENOTYPES FOR THE CONTENT OF β-GLUCANS IN GRAIN ON THE BASIS OF ITS PHYSICAL CHARACTERISTICS

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Abstract

Due to the beneficial effect of oat β-glucans on human health and their negative role in the assimilation of feed by non-ruminant animals, the selection of oats for increased (cereal direction) and reduced (feed use) content of these polysaccharides in grain is an urgent task. To perform screening of breeding material for the specified biochemical indicator of oat quality, it is advisable to use simple, express and non-destructive methods of grain analysis. The aim of this work is the development of a rapid method to evaluate oat genotypes for the content of β-glucans in grain based on the measurement of physical characteristics of grain. For the first time, it was found that in oat samples with a high content of β-glucans, the film-free grain was characterized by a higher density ($r = 0.818$). Probably, the mechanism explaining the presence of this correlation is associated with the formation of thicker and more densely packed cell walls in the endosperm, which has a greater number of these chemicals. In the research, 16 accessions of hulled oats and 2 accessions of naked oats from the VIR collection were involved. Concentration of β-glucans in grain was measured by the conventional enzymatic techniques. Physical characteristics (nature, 1000-grain weight, density, volume fraction of water uptake) were studied on the whole and/or hull-less grain. Measuring grain density for each oat accession was performed by the sand replacement method described by D.C. Doehlert and M.S. McMullen (2008); the natural grain weight was measured by the techniques offered by C.K. Walker, and J.F. Panozzo (2011); water uptake by grain was determined by the vacuum infiltration methodology. It is shown that 1000-grain weight of the studied oat accessions was not associated with the level of β-glucans in grain. There is an insignificant positive dependence between β-glucan content on the one hand, and the natural weight, density of whole grain and the volume fraction of water uptake by grain on the other hand. The oat accessions with higher content of β-glucans had higher density of hull-less grain ($r = 0.818$, $p \leq 0.05$). For an approximate calculation of the value of the content β-glucans in grain of genotypes of oats, you can use the formula: SBG = 4.16 × PZ, where PZ is the density of the film-free grain, g/cm^3; SBG is relative content of β-glucans in the grain, %; 4.16 is a coefficient of transition from the grain densities to the values of β-glucans. Minimum content of β-glucans in grain (3.2-3.8 %) and the lowest grain density (1.05-1.10 g/cm^3) were observed in the accessions Pushkinskii, Hondai 8473 and Privet. Maximum values of β-glucan content and grain density (5.7-6.7 % and 1.26-1.31 g/cm^3, respectively) were recorded in the accessions Pomor, Haruaoba and Marion. As a result, a rapid method was offered for evaluation of oat genotypes, which makes it possible to divide accessions into two contrasting groups: with maximum and minimum content of β-glucans in grain, considerably differing in both chemical and physical parameters. This method does not require expensive chemical agents or complex equipment, and may be implemented in any laboratory of a typical breeding center. The effect of possible introduction of the proposed technique involves saving financial and labor resources as well as avoiding complete grain damage, thus providing an opportunity for further utilization of the conserved breeding material in other analyses of grain for its quality.

Keywords: oats, grain, β-glucans, density, test weight, 1000-grain weight, water uptake, evaluation

Oat (*Avena sativa* L.) grain is not only high nutritional valuable, but also contains unique dietary fiber, the (1,3)(1,4)-β-D-glucans [1-3]. These polysaccharides have a beneficial effect on human health, as they can lower food glycemic index [4, 5], reduce blood cholesterol [6], including low-density cholesterol [7], improve liver function, and prevent excess body weight [8-10]. However, the fact that β-glucans act as a negative factor in nutrient assimilation by non-ruminant animals burdens the positive role of these compounds. Thus, experiments with feeding broiler chickens showed significant differences in grain nutritional value of oat varieties, which was negatively dependent on the content of β-glucans in the grain [11]. Since the proportion of barley and oat grain in Russian compound feeds for non-ruminant animals is more than half, a decrease in the concentration of β-glucans in the crop is necessary. In this regard, the selection of food oats for increased level of β-glucans and feed oats for reduced β-glucans in grain remains topical [12].

The content of β-glucans in grain is measured by a standard chemical analysis [13, 14]. The advantages of this method are its accuracy, and the disadvantages are its high complexity and the need to use expensive imported reagents and laboratory equipment. Another currently used physical method is based on the measurement of near-infrared reflection [15] using an automatic grain analyzer, for example, the Infratec™ 1241 Grain Analyzer (FOSS Analytical A/S, Denmark) [16]. Unfortunately, most chemical and physical methods require the complete grain destruction.

Screening of breeding material requires rapid and preferably non-destructive methods that can divide the hybrid population of oats into two extreme groups by the content of β-glucans in the grain. Available publications are mainly devoted to comparing the content of β-glucans in grain with the physical characteristics of whole grain [17, 18]. Since β-glucans are a part of the endosperm cell walls, the estimates of physical characteristics of not only the whole oat grains, but also of grains free from hulls are advisable.

In this work we have discovered that in oat samples having a high content of β-glucans, hulless grains are characterized by a higher density ($r = 0.818$, $p \leq 0.05$). Based on this, we suggest the technique of oat genotype evaluation which allows separation of samples with a maximum and minimum content of β-glucans in grain. Preliminary results were partially presented in our report at 10th International Oat Conference: innovation for food and health (St. Petersburg, Russia, July 11-15, 2016) [19].

The aim of the work was to analyze the relationship of the whole and hulless oat grain physical characteristics with the content of β-glucans.

Materials and methods. The 16 hulled oats and 2 hulless oats (the collection of the Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg) were investigated. To determine the physical characteristics of each sample, both hulled and hulless grains were used. In the latter case, floral glumes were removed manually without destroying fruit glumes and seed glumes.

A 1000-grain weight was calculated by weighing 250 grains. Bushel weight of grains was evaluated by a micro method of Walker et al. [20]. The volume of a known grain weight (about 10 g) was measured in a 50 ml cylinder. The density of hulled and hulless grains of each sample was determined by sand displacement method [21] with white fine sand from the South China Sea coast (Vietnam); the average sand density per 12 measurements was 1.55 ± 0.01 g/cm^3.

Air cavities in the grain deprived of floral films was estimated by vacuum infiltration with water (a 35 ml medical syringe at an air pressure of 50 kPa). The time of contact of grain with water in the syringe was about 1 min. After

removing from the water, grain surface was dried with filter paper. Then the grain was weighed and the volume of absorbed water was calculated as a ratio to the initial volume of dry grain.

All physical parameters of grain were determined in 3 replicates. The content of β-glucans was evaluated by the standard method [13] in 2-3-fold repetition.

Statistical processing was performed with Microsoft Excel 2003. The mean values (*M*), standard errors of the mean (±SEM), and the correlation coefficient (*r*) were calculated. The significance of differences was evaluated using Student's *t*-test at $p \leq 0.05$.

Results. Table 1 describes the oat samples we used.

1. Characterization of oat (*Avena sativa* L.) samples used to compare physical parameters of hulled and hulless grain (collection of Vavilov All-Russian Institute of Plant Genetic Resources — VIR)

No. in VIR catalog	Name	Variety	Origin
		Hulled oats	
k-9978	Marion	*A. byzantina*	USA
k-11840	Borrus	*A. sativa* var. *aurea*	Germany
k-13904	Ogle	*A. sativa* var. *aurea*	USA
k-13918	Kirovets	*A. sativa* var. *aurea*	Russia, Kirov Province
k-13943	Proat	*A. sativa* var. *aristata*	USA
k-13947	Tulancingo	*A. byzantina*	Mexio
k-14373	Fakir	*A. sativa* var. *aurea*	Россия, Kirov Province
k-14597	Sprint 2	*A. sativa* var. *aurea*	Russia, Ekaterinburg Province
k-14648	Argamak	*A. sativa* var. *mutica*	Russia, Kirov Province
k-14787	Privet	*A. sativa* var. *aurea*	Russia, Moscow Province
k-14858	Borot	*A. sativa* var. *mutica*	Russia, Leningrad Province
k-14872	Haruaoba	*A. byzantina*	Japan
k-14877	Hondai 8473	*A. sativa* var. *grisea*	Japan
k-14907	Vernyi	*A. byzantina*	Russia, Adygea
k-15126	Matilda	*A. sativa* var. *aurea*	Sweden
k-15176	Lev	*A. sativa* var. *mutica*	Russia, Moscow Province
		Hulless oats	
k-14717	Pushkinskii	*A. sativa* var. *inermis*	Russia, Leningrad Province
k-15117	Pomor	*A. sativa* var. *inermis*	Russia, Kemerovo Province

2. Correlation coefficients (*r*) of parameters in hulled grain and grain deprived from hulls in 18 oat (*Avena sativa* L.) samples (collection of Vavilov All-Russian Institute of Plant Genetic Resources — VIR)

Parameter	Hulled grains			Hulless grains		
	bushel weight	1000-grain weight	density	absorbed water	density	β-glucans
Hulled grain:						
bushel weight	1					
1000-grain weight	0.347	1				
density	0.780*	0.176	1			
Hulless grain:						
absorbed water	0.101	–0.197	0.082	1		
density	0.101	–0.239	0.278	0.382	1	
β-glucans	0.299	–0.096	0.495	0.237	0.818*	1

* *r* values are statistically significant at $p \leq 0.05$.

Moderate insignificant positive correlations occur between the content of β-glucans in grain and the density of whole grains, between the density of hulls and the volume of water absorbed by hulless grain, and between bushel weight of whole grains and 1000-grain weight (Table 2). Weak positive correlations are between the content of β-glucans in grain and its bushel weight and also water absorbed by hulless grain. The bushel weight and the whole grain density show strong positive correlation, while the dependence of the bushel weight on the density of de-hulled grain is practically absent. The density of the de-hulled grain and the content of β-glucans correlate strongly and positively.

The increased density of de-hulled grain of the oat samples with a higher content of β-glucans, apparently, could be due to the formation of thicker (and probably more densely packed) cell walls in the endosperm or a smaller volume of air cavities between the fruit and seed glumes and the hard part of the grain. Our results (see Table 2) indicate the absence of a negative relationship between the relative volume of absorbed water under negative pressure (and, consequently, the volume of air cavities) in grain deprived from floral glumes and the density of such grain. We assume the first mechanism to be more likely. This is indirectly supported by the close relationship between the density of barley grain and the hardness of its endosperm (20).

Based on statistically proven data that oat genotypes with a higher content of β-glucans had a higher density of de-hulled grain, the coefficients were calculated of transition from density values to the content of β-glucans in grains measured by the standard chemical method [13]. The average value of this dimensionless indicator is 4.16±0.12.

Therefore, for an approximate calculation of the content of β-glucans (CBG) in grain of oats genotypes, one can use the formula: CBG = 4.16 × GD, where GD is the density of de-hulled grain, g/cm^3, CBG is the relative content of β-glucans in grain, %, and 4.16 is a coefficient of transition from grain density to the content of β-glucans.

The obtained experimental data allowed us to distinguish among the examined 18 oat genotypes two contrasting groups of three samples each with a maximum and minimum content of β-glucans in grain, above 5.6% and less than 3.9%, respectively. These groups differed significantly not only in the content of β-glucans, but also in grain density. With an average density of de-hulled grain of 1.17±0.04 g/cm3 and 4.88±0.47% β-glucans, Pushkinskii, Privet and Hondai 8473 samples had the lowest indicators (1.07±0.02 g/cm^3 and 3.57±0.18%, respectively), while the maximum values (1.28±0.02 g/cm^3 and 6.13±0.30%) were characteristic of Pomor, Marion and Haruaoba samples. Differences in the density of de-hulled grains and the content of β-glucans between the genotypes of different groups were statistically significant at $p \leq 0.05$.

Currently, the possible relationships between the amount of β-glucans in grain and various physicochemical, morphological, and agronomic characters of oat genotypes are being actively investigated. A negative correlation was reported between the content of β-glucans and the total amount of dietary fiber and crude fiber, as well as a positive relationship with protein accumulation [22]. In oats, a close relationship between grain β-glucans and fat content has been demonstrated [16]. A significant positive correlation was found between grain β-glucan concentrations and bushel weight, on the one hand, and 1000-grain weight, on the other, while there were significant negative correlations of the β-glucan content with protein content and grain hoodness [17].

Our results on 1000-grain weight of whole grain for oats samples contrasting in the content of β-glucans support experimental data of C. Griffey et al. [18], demonstrating the absence of a relationship between these indicators for barley, as well as the results of M. Saastamoinen et al. [17] about a positive correlation between grain β-glucans and bushel weight. However, unlike the reports of the latest authors, our data show this correlation to be weak.

Over the years, in some countries, work has been done both on the search for oat samples with a high or low content of β-glucans in grain among existing varieties, and on the creation of forms with different accumulations of this polysaccharide depending on the intended use [23-25]. A few years ago, un-

der the auspices of the European Commission, the European project "Avena Genetic Resources for Quality in Human Consumption" was implemented, in which 658 varieties of oats were studied. It was confirmed that the genetic component has a significant effect on the content of β-glucans in oat grain [26]. In the Russian Federation, such studies are rare. Particularily, in a joint project between VIR (Russia) and the Nordic Gene Bank (NordGen, Nordic Genetic Resource Center, Sweden), the content of β-glucans in oats varieties was studied. The value of the considered indicator in the grain ranged from 3.3 to 6.2% [27]. The existing genotypic diversity of this biochemical trait (from 1.9 to 8.5%) [26-30] is quite sufficient for the progress of selection of oats for an increased or decreased content of β-glucans in grain for food and fodder use, respectively [31].

Thus, in oat samples with a high content of β-glucans, de-hulled grains had a higher density ($r = 0.818$, $p \leq 0.05$). Probably, the mechanism explaining the presence of this correlation is associated with the formation of thicker and more densely packed cell walls in the endosperm, which has a greater amount of these chemicals. Based on the statistically proven relationship between the content of β-glucans in grain and the density of de-hulled grain, an approach is proposed for evaluating oat genotypes, which allows us to divide the tested samples into two contrasting groups, with a maximum and minimum content of β-glucans. The method does not require expensive chemicals, sophisticated equipment and can be implemented in any conventional laboratory. In addition to saving material resources and labor costs when assessing oat genotypes, the proposed method avoids complete grain destruction. This makes it possible to further use the stored breeding material for grain quality analysis.

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