The quality and content of anthocyanins in purple- and blue-grained wheat in the south of Western Siberia

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Abstract. The development of wheat cultivars with an increased content of anthocyanins is one of the topical areas of modern plant breeding. Flavonoid pigments are natural antioxidants and are extremely beneficial to human health. We studied the collection of spring bread wheat with purple and blue grains in the south of Western Siberia (the Russian Federation). Samples were taken from the genetic bank of N. I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) (St. Petersburg). Blue A (New Zealand), Fioletovozernaya (Russia) and Konini (Canada) can be sources of high anthocyanins, protein and gluten to improve commercial bread wheat cultivars.

1. Introduction

One of the trends in the modern structure of the world food market is the emergence of a wide group of new high-tech products for specialized and functional nutrition with high value added. The intensive development of this market is confirmed by its annual growth of more than 15-20%. Such a high indicator fully reflects the desire of the population for a healthy and balanced diet. Functional food products contain an increased (or sharply reduced) amount of basic nutrients, vitamins, antioxidants, and adaptogens in comparison with conventional food components [1-2].

The development of cultivars with an increased content of flavonoids is one of the topical areas of modern plant breeding focused on the creation of functional food products [3-6]. Bioflavonoids are secondary metabolites of a phenolic nature, which are synthesized in various parts of plants, including cereals [7-9]. These compounds belong to the group of natural antioxidants [10]. Products made from such crops are a source of substances that are beneficial to human health [3; 11-14]. For example, anthocyanins (flavonoid pigments) have a hypoglycemic effect, reduce the risk of cardiovascular diseases, atherosclerosis, increase immunity, improve the synthesis of visual pigments, activate metabolic processes, and also provide the prevention of cancer and Alzheimer's disease [11; 15-20]. In this regard, there is currently an increased interest in the use of products made from colored cereal grains. For example, in various countries of America and Asia, food products are produced from colored cultivars of corn and rice. In recent decades, breeding has also been actively carried out to create wheat cultivars with similar properties, since it is the main cereal crop in most countries of the world [5-6; 12; 21-26].
Due to the biosynthesis of flavonoid pigments in different parts of the caryopsis, the grain of bread wheat (*Triticum aestivum* L.) acquires a red, blue or purple hue [8-9]. Red-grained cultivars are widespread, while colored cultivars, which have the highest antioxidant content in blue and purple grains, are still rare. They are not cultivated at all in the Russian Federation. Until now, no bread wheat cultivars suitable for the production of products with anthocyanins have been registered in the State Register of Breeding Achievements Approved for Use [27]. At the same time, the introduction into commercial wheat cultivars of genes that control the biosynthesis of anthocyanins in grain can give a new status to the products produced from these cultivars. In addition, coloration can play a key role in plant adaptation to various biotic and abiotic stresses. Anthocyanins are thought to play an important role in signaling and regulation of development in response to oxidative stress [28-29]. This aspect is of great interest for breeding cultivars resistant to drought, salinity, phytopathogens, and other negative influences.

The creation of new cultivars of wheat with an increased content of anthocyanins is possible using effective donors of the traits. It is known that the quality of crops is determined primarily by genotype and environmental conditions. High quality cultivars may not show their properties when grown in a different climatic zone [30-33]. This fact forces researchers to study potential sources and donors of useful traits in a specific region where the breeding program is planned to be implemented. The purpose of this study was to assess the physical and chemical properties of a collection of spring bread wheat with different grain colors, in the conditions of the south of Western Siberia (the Russian Federation).

2. Materials and methods

Eight samples of spring bread purple- and blue-grained wheat of various ecological and geographical origins from the collection of N. I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) (St. Petersburg, Russia) were studied. Three local varieties served as controls. These are Altajskaya 70 (standard for mid-early cultivars), Altajskaya Zhnica (standard for mid-season cultivars), Stepnaya Niva (standard for mid-late cultivars). The samples were grown in the Altai Territory, Barnaul (the south of Western Siberia, Russia) on the experimental plot of the Federal Altai Scientific Centre of Agro-BioTechnologies (53°25′ N, 83°31′ E, elevation: 174 m) in the summer of 2019.

The weight of 1000 grains, the protein and gluten content in the grain were evaluated. Thousand-grain weight was determined by the standard method [34]. Data on the content of protein, gluten in the grain and grain moisture were obtained using an infrared spectrometer InfroLum FT-10M (“Lumex”, St. Petersburg). The moisture index (14%) was taken into account to convert the protein content on dry matter. The total anthocyanin content (TAC) in the grain was determined according to the method described by Abdel-Aal and Hucl [35]. Absorbance was measured on a Shimadzu UV-1800 spectrophotometer (Shimadzu Europa GmbH) at 535 nm against a reagent blank. TAC in the sample (mg kg⁻¹) was calculated as cyanidin-3-glucoside. Statistical data processing was carried out using the Microsoft Excel 2010 software. The differences in the data were assessed by ANOVA and LSD at p ≤ 0.05.

3. Results

One of the most important indicators of the technological properties of the cultivar is the weight of 1000 grains. It depends on the genotype, cultivation conditions and is determined by the size and density of the seeds. The ratio between the endosperm and other components in large caryopses is higher than in puny and small ones. It has a positive effect on the flour yield. Two purple-grained samples (Mutant 1 and Purple feed) formed the grain weight at the level of the mid-late standard and exceeded the mid-early and mid-season cultivars. Other forms gave way to local genotypes. Blue wheat had the smallest grain (26.7 g) (table 1).

One of the main properties of wheat quality is the mass fraction of protein in the grain. The protein content determines the nutritional value of foods. The protein content in grain in all studied colored samples was statistically significant higher than the control cultivars. The maximum value was
observed in Blue А, which was 30% higher than the standard. Three purple-grained samples (Purple feed, Konini and Fioletovozernaya) accumulated over 20% protein in grain. The baking quality of bread wheat grain largely depends on the quantity and quality of gluten. The gluten content determines the taste of the bread, as well as the characteristics of the crumb such as its porosity, tenacity and elasticity. Cultivars with good gluten are categorized as "strong" wheat. This parameter for all colored wheat, with the exception of Mutant 2, was significantly 10-34% higher compared to mid-late cultivar Stepnaya Niva. The highest value was observed in blue wheat (35.9%).

Table 1. Physical and chemical properties of bread wheat grain in the south of Western Siberia.

| Sample          | Catalogue number | Country of origin | Grain color | 1000 grains weight, g | Protein content, % | Raw gluten content, % |
|-----------------|------------------|-------------------|-------------|-----------------------|-------------------|-----------------------|
| Mutant 1        | k-65585          | Byelorussia       | purple      | 43.4                  | 19.5              | 29.8                  |
| Mutant 2        | k-65799          | Byelorussia       | purple      | 38.0                  | 18.1              | 26.5                  |
| Purple feed     | k-49426          | Canada            | purple      | 42.9                  | 21.1              | 29.4                  |
| Omskaya kormovaya | k-64472       | Russia            | purple      | 39.1                  | 19.2              | 29.8                  |
| Konini          | k-59948          | New Zealand       | purple      | 38.6                  | 20.3              | 32.9                  |
| Fioletovozernaya | k-55583         | Russia            | purple      | 34.4                  | 20.0              | 30.6                  |
| K-59158         | k-59158          | Russia            | purple      | 34.1                  | 19.5              | 29.5                  |
| Blue А          | k-43091          | Canada            | blue        | 26.7                  | 21.7              | 35.9                  |
| Altajskaya 70   | Russia           | red               |             | 41.4                  | 16.0              | 24.0                  |
| Altajskaya Zhnica | Russia          | red               |             | 41.3                  | 16.5              | 26.4                  |
| Stepnaya Niva   | Russia           | red               |             | 44.6                  | 17.2              | 26.8                  |
| LSD<sub>0.05</sub> |                 |                   |             | 1.38                  | 0.48              | 1.52                  |

TCA extracted from whole-meal grain varied significantly depending on the wheat genotype. Red-grained cultivars contained 6-7 times less anthocyanins compared to blue wheat. Among the purple-grained forms, the maximum accumulation of these compounds was noted for the sample Fioletovozernaya (figure 1). However, it was inferior to blue wheat by 36% in this respect.

Figure 1. The total content of anthocyanins in the grain of spring bread wheat, mg kg<sup>-1</sup>

Analysis of variance confirmed the reliability of the variability of all the studied characters. The F-tests were 119.0, 146.27, and 47.13 for 1000 grains weight, protein content and raw gluten content, respectively.
4. Discussion
Breeding of crops with high yield and high quality grain is crucial for the successful production of agricultural products. Unfortunately, in Russia for a long time the priority was a high grain volume, which often led to a decrease in its quality. Having the largest genetic resources in the world, which makes it possible to obtain high quality grain in all regions, our country produces wheat of I and II classes less than 1% of the total volume. Russian wheat is inferior in quality to the best cultivars of Canada, the United States and Australia, which reduces its competitiveness in the global grain market [36]. At the same time, having large reserves of extensive production, Russia is a potential producer of ecologically clean grain products. According to the modern Strategy of Scientific and Technological Development of Russia, one of the priority areas is the transition to a highly productive and environmentally friendly agricultural sector, as well as the creation of safe and high-quality food products including functional ones [3; 13-14; 25]. In this regard, the development of new cultivars of bread wheat with a high content of flavonoids is very timely [3; 5; 10].

The colors of bread wheat grain are due to the accumulation of flavonoid pigments in different layers of the seed coat. Various genetic systems control the trait. The synthesis of proanthocyanidins results in a reddish-brown hue (trait "red grain"). It is controlled by the $R$ genes. The blue hue appears due to the synthesis of anthocyanins in the aleurone layer (trait "blue aleurone"). The $Ba$ genes regulate it. The purple color is due to the synthesis of anthocyanins in the pericarp cells (trait "purple pericarp"), which is regulated by two complementary dominant genes: $Pp-D1$ (chromosome 7D) and $Pp3$ (chromosome 2A) [8-9]. In addition, anthocyanin pigments can also be synthesized in various vegetative organs, for example, coleoptile (controlled by $Rc$ genes), stems ($Pc$ genes), leaf blades ($Plb$ genes) [37]. Most European and American wheat cultivars have red grains, because this type of pigmentation causes lower hydrolytic enzyme activity and increases the grain's resistance to germination [38]. Wheat cultivars with anthocyanin grain color are extremely rare, and in Russia, they are generally absent. Progress in breeding wheat with increased antioxidant activity is impossible without screening the world collection.

Both genotypes and environmental conditions determine grain quality. It is possible to select the best cultivars and lines for certain environmental conditions only by assessing their phenotypes in the same ecological zone [10; 30; 32]. To search for sources and donors of genes for the biosynthesis of flavonoid pigments in different parts of the bread wheat caryopsis, we studied a collection of samples of various ecological and geographical origins. Bread wheat cultivars are compared primarily in terms of protein content and physical properties of gluten, which, in addition to research importance, is of great practical one. These traits affect both the nutritional value of bread and the technological properties of wheat.

The growing weather conditions in the Altai Territory in 2019 were quite favorable for the formation of good quality grain. In June, the amount of precipitation was close to the mean annual value. In July, there was a deficit of precipitation (42 mm), which partially covered the period of heading–the beginning of milk ripeness. The average daily air temperature during grain filling was 19.9°C, which corresponded to the average long-term norm. August was characterized by high temperatures (+ 1.9°C to norm) and a slight deficit of precipitation (81% of the norm). It contributed to the rapid maturation of plants. Dry and warm weather in the middle of summer suppressed the development of leaf diseases. In the conditions of our region, blue wheat has formed a high content of anthocyanins and protein in the grain. We also found this sample to have the highest gluten content. An increase in the gluten content of more than 30% leads to an increase in the strength of the flour. This usually leads to an increase in the volumetric yield of bread. Thus, the Blue A sample from Canada can serve as a source of high protein, anthocyanin and gluten for the wheat in our region. The disadvantage of this form is the low weight of 1000 grains because of small kernels. Therefore, when this sample is involved in crossings, constant control over these traits is necessary. The sample Fioletovozernaya also showed high quality. The protein and gluten contents were 20 and 30.6%, respectively. This sample can also be a source of anthocyanins for bread wheat. The trait level when
wheat grown in the field reached 72 mg kg\(^{-1}\). Also noteworthy is the New Zealand Konini cultivar, which has formed a good grain, high in protein and gluten. The anthocyanin level was 66 mg kg\(^{-1}\).

5. Conclusion
In this experiment, the grain quality of colored wheat was studied in the field conditions of the south of Western Siberia. Evaluation of the content of anthocyanins, protein, and gluten in grain made it possible to identify forms that are promising for use in breeding bread wheat cultivars with high antioxidant activity. Blue A, Violetovozernaya and Konini accumulate the maximum level of anthocyanins, protein and gluten in the grain. The latter cultivar also forms large kernels with a high weight of 1000 grains.

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