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COLORED GRAIN WHEAT: BREEDING FEATURES

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The emerging research direction is focused on improving grain nutritional quality through biofortification with anthocyanins, bioactive pigments characterized by strong antioxidant properties. The aim of the work was to reveal the peculiarities of breeding wheat varieties with colored (purple, blue, black) grains based on the content of bioactive components of colored pigments-antioxidants anthocyanins and carotenoids with high antioxidant activity, involve various sources of bioactive pigments in crossbreeding, select elite plants in populations, evaluate promising material with colored grains, and develop new varieties. Crossbreeding of sources of bioactive components with a series of wheat varieties, selection of elite material according to target grain color characteristics, combining target genes that determine grain pigmentation and its technological and baking properties has been carried out. The first varieties of bread wheat and spelt wheat with colored grains have been produced in Ukraine. For the first time, functional whole grain products made from colored wheat and spelt wheat flour with 100 % natural dark brown color have been obtained on the Ukrainian food market. Through crossbreeding sources of bioactive pigments anthocyanins and carotenoids, several hundred populations have been obtained, and promising breeding material has been selected for the development of wheat varieties with black (blue+purple) grains. The breeding of wheat varieties with colored grains is a rather complex technology and involves a number of important features in obtaining and selecting material with different colors and shades of grain. Successful work on the establishment of wheat varieties with colored grains and high grain productivity as well as quality requires the involvement of a large amount of diverse genetic material in terms of grain color, agronomic and technological characteristics.

Key words: wheat, colored (blue, purple, black) grain, anthocyanins, carotenoids, biofortification.

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Colored vegetables and fruits [1, 2], cereals such as brown rice [3], colored sorghum and millet [4], purple corn [5], colored lentils [2], black, purple, and blue barley [6] and wheat [7] are foods rich in bioactive phytochemical polyphenolic compounds that are important for human health, such as anthocyanin pigments with high antioxidant activity, which is perhaps the most important characteristic of the biological value of food products [8, 9]. According to numerous clinical studies, foods rich in anthocyanins are considered to protect the human body against a whole range of pathologies, such as cardiovascular diseases, cancer, diabetes, hypertension, organ inflammation, and obesity. They help slow down aging, protect the body from destructive UV radiation, etc. [10, 11].

Due to the peculiarities of their molecular structure, anthocyanin pigments belong to the class of glycosides and have a complex molecular skeleton in the form of a B-ring with side methyl and hydroxyl groups. The six most common are cyanidins, delphinins, malvidins, pelargonidins, petunidins, and peonidins [12, 13].

Unlike vegetables and fruits, cereal crops are not a typical source of anthocyanins. Since cereal grains and products made from them are a source of daily mass consumption for the world's population, colored grains such as rice, wheat, and barley, given their high biological value, have recently become particularly attractive to consumers, grain processors, functional food manufacturers, and breeders in many countries around the world, especially China, India, the United States, Canada, Australia, Austria, the Czech Republic, Japan, South Korea, and others [14,15]. The growing demand for healthy foods made from anthocyanin-fortified colored wheat has led to a new global trend in scientific research on colored grains. Given the number and origin of publications in this field, at least 60 scientific institutions from 16 countries are currently involved [14].

There are currently no varieties of wheat or barley with colored grains in Ukrainian fields. Accordingly, there are no widely available everyday products made from colored wheat or barley grains on the Ukrainian food market. The people of Ukraine, as well as the scientific community, is not well informed about the existence of wheat and barley with colored grains, and little is known about the high biological value of functional products made from colored grains.

Our research team was the first in Ukraine to initiate a large-scale program for the development and study of wheat and hulless barley varieties with colored grains. We were the first in Ukraine to release two varieties of winter wheat with purple grains, Chornobrova (originator: Plant Breeding and Genetics Institute—National Center of Seed and Cultivar Investigation, National Academy of Agricultural Sciences of Ukraine (PBGI—NCSCI NAASU)) and Chornozerna (originator: Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine (IPPG NASU)), and for the first time in the world, we developed spelt wheat with dark purple grains, Bilberry (in collaboration with the seed company Biligrain). These varieties are listed in the State Register of Plant Varieties Suitable for Distribution in Ukraine.

The biosynthesis of anthocyanin pigments is localized in the peripheral anatomical layers of the grain — the husk and aleurone layer, while they are completely absent in the endosperm of the grain. Black grains, combining blue (aleurone layer) and purple (pericarp) colors, contain the highest content of anthocyanins (~140 mg/kg), while blue grains contain ~80 mg/kg, purple grains ~40 mg/kg, and ordinary red grains only ~5 mg/kg of anthocyanins [14]. Therefore, the target genotypes in our program are genotypes with black grains and the highest anthocyanin content.

Over the past two years, we have expanded our research program on biofortification of colored wheat with anthocyanin pigments by adding genetic material with a high content of yellow carotenoid pigments in the endosperm of the grain [16]. The physiological role of carotenoids in the human body is as important as that of anthocyanins [17, 18].

Thus, our breeding program aims to biofortify wheat with a combination of anthocyanins and carotenoids with high antioxidant activity in the grain. In order to maximize the preservation of nutritional benefits, wheat grain biofortified in this way should be used exclusively and without alternative as whole grain (unrefined) flour for the production of whole grain products only. Traditional technologies aimed at using white refined flour for the production of food products are completely unacceptable for varieties with biofortified grain in terms of anthocyanin pigment content, as they negate all the nutritional benefits of this grain. In connection with this focus of colored grain processing technology, the breeding program for developing varieties with colored grains has a number of features related not only to the combination of pigments, but also to the baking and technological quality of the grain and flour of wheat varieties with colored grains, which must be taken into account.

Therefore, this article is devoted to highlighting the features of the breeding program for isolating varieties with grain biofortified with anthocyanins and carotenoids.

Materials and methods

Aiming for the fastest and most effective results, our breeding program involves a fairly large amount of initial genetic and breeding material. Table 1 lists the winter wheat varieties of different origins that we used in crosses with grain color donors as the maternal component.

The breeding program involves winter bread wheat varieties selected by various foreign companies, breeding material from the IPPG NASU, and the PBGI—NCSCI NAASU. The material also includes the Zymoyarka variety, which is unique in its agronomic characteristics, spring-winter (facultative) growth type, and two varieties of durum wheat. Table 2 lists the donors of different grain colors and donors of yellow endosperm (as pollen sources) used in the crossbreeding.

As evidenced by the origin of the samples, the genetic material of donors of different colors and shades of colored grain has been collected by us from virtually all collections in the world where colored grain material is available. A significant part of our collection of colored wheat samples was obtained from the renowned Czech breeder Petr Martinek (Agricultural Research Institute Kromeriz, Ltd. Czech Republic), to whom we are sincerely grateful for providing us with this valuable genetic resource. Currently, our collection of colored wheat grain samples is

TABLE 1. List of winter (alternative) wheat varieties and breeding lines in the crossing program

No.	Variety	Originator	Origin	Notes
1	Emerick	KWS	Germany	winter
2	Imperium	KWS	Germany	winter
3	Keitum	KWS	Germany	winter
4	Spencer	KWS	Austria	winter
5	Sphere	KWS	Germany	winter
6	Ultim	KWS	France	winter
7	Solindo	Lidea	France	winter
8	Somtuoso	Lidea	France	winter
9	Sorrial	Lidea	France	winter
10	Sophie	Lidea	France	winter
11	Sofru	Lidea	France	winter
12	Bodycek	RAGT	Czech Republic	winter
13	Depot	RAGT	Germany	winter
14	Provision	RAGT	Germany	winter
15	Rebell	RAGT	Germany	winter
16	Specialist	RAGT	Germany	winter
17	Telemark	RAGT	Czech Republic	winter
18	Tacitus	SAATBAU	Austria	winter
19	Lemmy	Saaten Union	Germany	winter
20	Aventinus	Strube	Germany	winter
21	Admont	Strube	Germany	winter
22	Berwald	Strube	Germany	winter
23	Lennox	Strube	Germany	winter
24	Mangold	Strube	Germany	winter
25	Matthus	Strube	Germany	spring-winter (facultative)
26	Rotax	Strube	Germany	winter
27	Salasar	Strube	Germany	winter
28	Katarina	Vitera seeds	Croatia	winter
29	Papillon	Vitera seeds	France	winter
30	Python	Vitera seeds	France	winter
31	Tika Taka	Vitera seeds	Croatia	winter
32	Trublion	Vitera seeds	France	winter
33	Felix	Vitera seeds	Croatia	winter
34	Frisky	LG (Limagrain)	Germany	winter
35	Adora	Durum Seeds LLC	Serbia	winter
36	Duriamo	Durum Seeds LLC	Serbia	facultative, durum
37	Ikona	Durum Seeds LLC	Serbia	winter
38	Klimatika	Durum Seeds LLC	Serbia	winter
39	Logika	Durum Seeds LLC	Serbia	winter
40	Unigold	Durum Seeds LLC	Bulgaria	winter
41	Flexadur	Durum Seeds LLC	Serbia	facultative, durum
42	Manitoba	Seed Grain	Canada	winter
43	Zymoyarka	IPPG NASU	Ukraine	facultative
44	Donor Kyivsky	IPPG NASU	Ukraine	winter
45	UK 3836	IPPG NASU	Ukraine	winter, line
46	UK 53/19	IPPG NASU	Ukraine	winter, line
47	UK 55/19	IPPG NASU	Ukraine	winter, line
48	UK 171/19	IPPG NASU	Ukraine	winter, line
49	UK 1496/16	IPPG NASU	Ukraine	winter, line

TABLE 1 (End)

No.	Variety	Originator	Origin	Notes
50	Kujalnik	PBGI—NCSCI NAASU	Ukraine	winter
51	SL4029 Gpc-B1 × KUL	PBGI—NCSCI NAASU	Ukraine	winter, line
52	SL4030 Gpc-B1 × KUL	PBGI—NCSCI NAASU	Ukraine	winter, line
53	SL4032 Gpc-B1 × KUL	PBGI—NCSCI NAASU	Ukraine	winter, line
54	SL4051 Gpc-B1 × KUL	PBGI—NCSCI NAASU	Ukraine	winter, line
55	SL4053 Gpc-B1 × KUL	PBGI—NCSCI NAASU	Ukraine	winter, line
56	SL4054 Gpc-B1 × KUL	PBGI—NCSCI NAASU	Ukraine	winter, line

Note. Here and in Table 2: KUL — Kujalnik variety; ALB — Albatros variety; HAW — high amylose wheat.

TABLE 2. Wheat samples as donors of different grain colors and yellow endosperm

No.	Sample	Color of pericarp/endosperm	Country of origin	Notes
1	Hedong Wumai	dark blue	China	facultative
2	BGW 76	black	China	spring
3	Donghei 10	black	China	winter
4	CZ-16-19	dark blue	Czech Republic	winter
5	CZ-19-19	dark blue	Czech Republic	winter
6	CZ-20-19	dark blue	Czech Republic	winter
7	CZ-41-19 BD	dark blue	Czech Republic	winter
8	CZ-5-19	blue	Czech Republic	winter
9	CZ-394-19	purple	Czech Republic	winter
10	CZ-356-19	purple	Czech Republic	winter
11	CZ-372-19	dark purple	Czech Republic	winter
12	CZ-330-19	purple	Czech Republic	winter
13	CZ-369-19	dark purple	Czech Republic	winter
14	KM 15-17	black	Czech Republic	winter
15	CZ-400-19	dark purple	Czech Republic	winter
16	CZ-377-19	dark purple	Czech Republic	winter
17	AF Jumiko	purple	Czech Republic	winter
18	Can 25 02 20p	purple	Canada	winter
19	Lx 958 Cp	purple	Canada	winter
20	Lx 949 Cb	black	Canada	winter
21	Can b	blue	Canada	winter
22	Can p	purple	Canada	winter
23	GF Aggia PI 534333	purple	Canada	winter
24	GF b	blue	Canada	winter
25	GF p	purple	Canada	winter
26	GF H-86-701	black	Canada	winter
27	GF Sindi	purple	Canada	winter
28	CZ-11-21 AF Zora	purple	Czech Republic	Ba2+Pp
29	CZ-4-21 AF Jumiko	purple	Czech Republic	Pp (purple pericarp)
30	CZ-9-21 AF Oxana	blue	Czech Republic	Ba2 (blue aleurone)
31	CZ-2-21 Anthograin	purple	Canada	Pp (purple pericarp)
32	CZ-8-21 Skorpion	blue	Czech Republic	Ba2 (blue aleurone)
33	Xiao Yan*	blue	China	Ba3 (new gene for blue colour) *heterogeneous in grain colour
34	CZ-178-21 Pannonia/Aoi Yu	blue	Czech Republic	Ba3?, awned
35	CZ-175-21 Forhand/Aoi Yu	dark blue	Czech Republic	Ba3?, awned

TABLE 2 (End)

No.	Sample	Color of pericarp/endosperm	Country of origin	Notes
36	CZ-50-21 Darker blue Skorpion	dark blue	Czech Republic	Ba1 + Ba2
37	CZ-186-21 RU 440-6/UC66409//SG-S1590-08	dark blue	Czech Republic	probably Ba2 + Ba1
38	CZ-14-21 RU 440-6/UC 66049//Hybrid F_2 seeds (ENOLA/Fla-10-401)	dark blue	Czech Republic	probably Ba2 + Ba1
39	CZ-23-21 UC 66049/Skorpion BC_6F_2	dark blue	Czech Republic	Ba1 + Ba2
40	Aoi Yu	blue	Japan	Ba3?
41	CZ-13-21 Bona Vita	yellow	Slovakia	Ye (yellow endosperm)
42	Citrus	yellow	Germany	Ye (yellow endosperm)
43	SL4088 ALB × Amph#4	yellow	PBGI—NCSCI NAASU	Ye (yellow endosperm)
44	SL4116 ALB × Amph#4	yellow	—"—	Ye (yellow endosperm)
45	SL4172 ALB × Amph#4	yellow	—"—	Ye (yellow endosperm)
46	SL4200 ALB × Amph#4	yellow	—"—	Ye (yellow endosperm)
47	SL4310 ES56 × KUL	yellow	—"—	Ye (yellow endosperm)
48	SL4338 ES56 × KUL	yellow	—"—	Ye (yellow endosperm)
49	SL4401 KUL × HAW	yellow	—"—	Ye (yellow endosperm)
50	16 Winter Black Population 1-20 — grain from individual spikes	black	Czech Republic	heterogeneous in grain colour
51	17 Winter Black Population 1-20 — grain from individual spikes	black	Czech Republic	heterogeneous in grain colour
52	18 Winter Black Population 1-20 — grain from individual spikes	black	Czech Republic	heterogeneous in grain colour
53	19 Winter Black Population 1-20 — grain from individual spikes	black	Czech Republic	heterogeneous in grain colour
54	20 Winter Black Population 1-20 — grain from individual spikes	black	Czech Republic	heterogeneous in grain colour
55	2022-87 var. *viridiferrugineum*	dark blue	russia	winter
56	2003-87 var. *vavilovii*	blue	russia	winter
57	KM 186/2 IR16413 UA0108445	blue	Slovakia	winter
58	? lue × red IR16410 UA0108477	light brown	Slovakia	winter
59	Chornozerna IR16498 UA0122885	dark purple	Ukraine	winter
60	*T. compactum* UA0300280 var. *viridicterinum*	dark blue	russia	winter
61	*T. aestivum* SHL19-1 (syn. SH Haval-200-1) var. *erviol*	dark purple	Ukraine	spring
62	*T. aethiopicum* UA0300158 var. *arraseita*	purple	Ethiopia	spring
63	*T. aethiopicum* UA0300378 var. *decoloratum*	purple	Ethiopia	spring
64	*T. aestivum* IR00416 var. *violacea*	dark purple	Czech Republic	spring
65	*T. aestivum* IR070634 NOU 59/11 var. *viriderinaceum*	blue	belarus	spring

unique in Ukraine and has already been used to the maximum extent in crossbreeding to create colored wheat varieties.

In order to improve the quality of colored wheat grain and flour, we have also incorporated our latest genetic material with high baking and technological potential from distant crosses, including promising breeding lines that are sources of the *Gpc-B1* gene from *T. turgidum* var. *dicoccoides*, which increases the protein content in grain [19, 20]. For the 2025 harvest, 160 F_1 cross combinations and 320 $F_{2\text{-}5}$ populations were sown. A total of 480 combinations from different crosses.

When developing Ukrainian first winter wheat variety, Chornobrova, the winter wheat variety Kujalnik and the black-grain donor Donghei 10 were used in crossbreeding. When isolating spelt wheat with dark purple grains, the original Schwabencorn spelt (Germany) and the Chornobrova winter wheat variety with purple grains were used. The Chornozerna variety was created by crossing the Chornobrova variety with a wheat line with a chromosomally engineered rye-wheat translocation 1RSm.1BL from Prof. Adam J. Lukaszewski (University of California, Riverside, U.S.A.), in which the *Sec-1* rye locus, which is negative for baking quality, is replaced by the positive wheat cluster *Gli-B1*(*Glu-B3*).

Two varieties, Bona Vita and Citrus, and seven breeding lines with yellow endosperm, obtained by us from distant crosses with hexaploid synthetic amphiploids with the genome formula AABBDD (2n=6x=42), were used as donors of yellow endosperm.

The anthocyanin pigment content in grain was determined using the method [21], and the carotenoid content was determined using the standard method [22]. The baking quality characteristics of wheat varieties as components of crosses were determined using the following methods [23]; grain hardness on the Inframatic 8611 infrared analyzer, protein content in grain (flour) on dry matter (N×5.7) %; wet gluten content (%) according to [24]; gluten index according to [24]; Zeleny test ml according to ISO 5529:2007; Zeleny test with preliminary incubation of 120 min (ml) based on ISO 5529:2007; rheological characteristics of dough (*W*, *P*, *L*, *P*/*L*, *Ie* %) on a Chopin alveograph according to ISO 27971:2015.

Results and discussion

For successful color combinations, it is important to understand the genetic control and inheritance of blue and purple pigmentation. Anatomically, the biosynthesis of purple pigments is localized in the pericarp of the grain, while blue pigments are synthesized in the cells of the aleurone layer, located directly under the grain pericarp. Genetically, the biosynthesis of purple and blue pigments are independent systems and can therefore be easily combined in one genotype. Genes encoding blue color are designated by the symbol *Ba* (*blue aleurone*), while genes for purple color are designated by the symbol *Pp* (*purple pericarp*). The cells of the grain coat have a diploid set of chromosomes (2n=42) and, accordingly, the inheritance of purple color is carried out according to the maternal type: the color of *PpPp* homozygotes or heterozygotes is determined by the genotype of the maternal plant, while the inheritance of blue color has a more complex manifestation, since it depends on the effect of the triploid set of chromosomes (3n=63) of the aleurone layer cells. And a heterozygote for the *Ba* genes will exhibit xenia effect: both blue and red (or white) colors are present on one plant and in the same ear, depending on the parental gene sets.

The aforementioned wheat grain colors are completely absent in modern varieties developed using traditional breeding programs. Colored grains are an attribute of some wild species and have been transferred to the genome of cultivated wheat through distant (introgressive) hybridization of cultivated wheat with the corresponding donor species.

Thus, the purple color of the grain was transferred to cultivated wheat from wild tetraploid Abyssinian wheat originating in Ethiopia (*Triticum aethiopicum*). Genetic control of purple coloration is encoded by at least three currently known dominant genes. The *Pp-1* gene is located on chromosome 7BL and is designated *Pp-B1* (7B *T. durum*, 7S *Aegilops speltoides*). The *Pp-D1* gene is located on chromosome 7D (*T. aestivum*) and the *Pp3* gene is located on chromosome 2A. Thus, two complementary factors in genomes A and B, or A and D, are necessary for the manifestation of purple coloration. Even if the two genes *Pp-B1* and *Pp-D1* are dominant in the presence of the recessive allele *Pp3*, the purple color of the grain will not manifest itself. Therefore, the purple pericarp trait is expressed only at the hexaploid level of ploidy and not at the tetraploid level. The blue color of the grain is introgressed into the genome of cultivated wheat from wild species such as wild einkorn wheat, *T. boeoticum*, and species of wheatgrass *Agropyron trichophorum*, *Ag. glaucum*, and *Ag. elongatum*. The dominant gene *Ba1* is localized in chromosome 4AgL *Ag. elongatum*. The gene with incomplete dominance *Ba2* is located on chromosome 4A of *T. monococcum* / *T. boeoticum*. The dominant gene *Ba(c)* is concentrated on chromosome 4J of *Thinopyrum bessarabicum*, and the gene *Ba(d)* on chromosome 4E of *Th. elongatum* / *Th. ponticum*. However, the molecular mechanism of blue aleurone formation has not been fully studied yet. As can be seen, genetic control and manifestation of purple and blue grain traits appear to be quite complex, which complicates the selection process itself [14, 25].

Our first breeding programs to create the Chornobrova and Chornozerna wheat varieties with colored grains began with a combination of crossing the (♀) Kujalnik variety (PBGI—NCSCI NAASU) with the Donghei 10 variety with black grains. As a result, a productive breeding line with dark purple grains was obtained from the population, which gave rise to the Chornobrova variety, which was entered into the State Register of Plant Varieties Suitable for Distribution in Ukraine in 2014. From the population resulting from the crossing of the (♀) Chornobrova variety with a wheat line bearing chromosomal translocation 1RSm.1BL, a promising breeding line was selected, which became the Chornozerna variety, entered in the State Register of Plant Varieties Suitable for Distribution in Ukraine in 2018. The anthocyanin pigment content in the grain of these varieties is within the range of 50—60 mg/kg. These varieties are characterized by average baking quality indicators (protein content in grain 12—13 % and flour strength *W* within 180—200 u. a.), which is quite sufficient for baking bread from white refined flour, but this is the minimum quality level for baking products from whole grain flour. It should be noted right away that any products made from colored wheat varieties must be produced

TABLE 3. Baking quality indicators of wheat varieties as crossing components (2023 harvest):
GI — gluten index; Ie % — dough elasticity index; W — dough strength; P — dough tenacity; L — dough extensibility; P/L — alveogram configuration index

Variety	Variety origin	Test weight, g/l	Grain hardness	Protein, %	Gluten, %	GI	Zeleny test		Gluten elasticity (alveograph)				
							regular	120 min	Ie %	W	P	L	P/L
Tacitus	Aust.	768^{b}	62^{bc}	14.1^{bc}	23.0^{d}	99.7^{a}	55^{c}	69^{b}	61^{cd}	229^{e}	78^{d}	78^{fg}	1.00^{d}
Matthus	Germ.	761^{b}	68^{b}	14.9^{b}	26.5^{c}	99.8^{a}	67^{a}	72^{a}	64^{bc}	309^{bc}	99^{a}	81^{f}	1.22^{c}
Lennox	Germ.	780^{b}	87^{a}	15.2^{a}	28.2^{b}	99.7^{a}	67^{a}	73^{a}	59^{d}	375^{a}	92^{b}	118^{a}	0.77^{e}
Zymoyarka	Ukr.	763^{b}	54^{c}	12.3^{e}	22.5^{d}	99.6^{a}	56^{c}	66^{bc}	60^{c}	194^{f}	53^{f}	104^{c}	0.50^{f}
Rebell	Germ.	772^{b}	57^{c}	13.9^{c}	28.1^{bc}	91.7^{b}	45^{d}	65^{c}	50^{e}	178^{f}	56^{f}	106^{c}	0.52^{f}
Felix	Germ.	824^{a}	68^{b}	14.4^{bc}	29.6^{b}	98.0^{a}	55^{c}	73^{a}	62^{cd}	324^{b}	100^{a}	88^{e}	1.13^{c}
Athlon	Fran.	755^{b}	67^{b}	15.9^{a}	33.9^{a}	97.6^{a}	66^{a}	73^{a}	63^{c}	325^{b}	81^{cd}	112^{b}	0.72^{e}
Reform	Germ.	768^{b}	30^{d}	13.6^{cd}	26.3^{c}	99.9^{a}	68^{a}	71^{a}	64^{bc}	247^{de}	68^{e}	96^{d}	0.70^{e}
Veteran	Ukr.	766^{b}	35^{d}	13.2^{cd}	23.6^{d}	99.8^{a}	45^{d}	55^{e}	71^{a}	266^{d}	102^{a}	59^{i}	1.73^{a}
Hoduvalnytsa	Ukr.	765^{b}	2^{e}	13.0^{d}	24.3^{d}	99.6^{a}	62^{b}	60^{d}	64^{bc}	233^{e}	86^{c}	67^{h}	1.28^{bc}
Kujalnik	Ukr.	763^{b}	34^{d}	13.1^{cd}	23.8^{d}	99.7^{a}	42^{d}	65^{c}	67^{b}	300^{c}	100^{a}	75^{g}	1.33^{b}
LSD_{05}		34	7	0.8	1.8	3.7	3	3	3	20	5	5	0.09

Different letters indicate significant differences at $p \leq 0.05$.

exclusively and without exception from whole grain flour in order to maximize the high nutritional value of such grain, since anthocyanin pigments with high antioxidant activity are localized precisely in the shell (pericarp) and aleurone layer of the grain, and when colored grains are ground into white refined flour, they are almost completely lost and end up in the bran. Precisely because the requirements for the quality of gluten in whole grain flour are higher than the requirements for the quality of gluten in white flour, wheat varieties with colored grains must have the highest possible baking quality characteristics. Taking this into account, our breeding program with colored grain sources, in addition to the varieties mentioned above (Table 1), also involves various genetic potentials of high and extra-high baking quality that we have previously characterized [20]. Table 3 shows the baking quality characteristics of some winter wheat varieties used in our breeding program with colored grain donors listed in Table 2.

The crossbreeding also includes material with extra-high baking quality, the level of which is determined by the extra-expression of individual alleles, especially the *Glu-1* locus, which encodes the biosynthesis of high-molecular-weight glutenins. An important source of high quality in our crossing program is material with the *Gpc-B1* gene originating from *T. turgidum* var. *dicoccoides*, which is capable of significantly (up to 2 %) increasing the protein content in grain (Table 1, samples No. 51—56).

It is no coincidence that we chose mainly foreign varieties for cross-breeding. In addition to high productivity and disease resistance, these varieties are also characterized by high baking quality (e.g., Matthus, Lennox, Felix, Athlon). In our tests, the early-maturing Felix variety also proved to be quite drought-resistant, had a high level of baking quality, and was the record holder among the varieties studied for such an important indicator of milling quality as «test weight» with a value of 824 g/L. All of this source material is currently present in 480 populations from crosses with sources of grain pericarp and endosperm color.

However, in addition to sources with high baking quality, our cross-breeding program also includes material with low baking characteristics, low grain hardness, and low WAC % (water absorption capacity), which are precisely the indicators that meet the requirements of biscuit baking technology. After all, colored wheat varieties should be used not only for baking bread, but also for a range of high-quality flour-based biscuit products.

The next stage of our breeding program was to create spelt wheat with dark brown (black) grains [26]. Spelt wheat (*Triticum aestivum* ssp. *spelta* L.) is a filmy type of bread wheat (*T. aestivum* L.) and has the same hexaploid chromosome set (AABBDD, 6x=2n=42). Spelt belongs to the group of so-called ancient wheats, and its grain is tightly enveloped by a hard film, the morphological features of which are controlled by mutations in two loci [27]. Spelt has a number of attractive nutritional characteristics and surpasses modern bread wheat varieties in terms of nutritional value [28]. Bread made from spelt flour has earned such epithets as healthy and more natural than bread made from modern wheat varieties. Spelt grain contains more total and soluble (digestible) protein, more valuable lipids, more valuable minerals (Mg, P, Fe, Cu, Zn), and more soluble dietary fiber than modern wheat varieties. Spelt has 8—10 times higher content of resistant starch similar to the properties of soluble dietary fiber [29, 30]. Spelt is characterized by a fairly high adaptive potential to growing conditions and is particularly well suited for organic farming. Therefore, it is no coincidence that the area under spelt cultivation has increased significantly in recent years [31].

At the same time, spelt is a natural wild wheat that has not been affected by modern breeding, and the issue of improving it through breeding is quite problematic. We have not yet found an answer to this question in the numerous scientific literature on spelt wheat available to us. Among the varieties of spelt wheat known today, we have not found any evidence of the existence of varieties with colored grains. And our task was precisely to create such spelt.

The main prerequisite for achieving our goal was not to violate the fundamental status of spelt as wild wheat, because otherwise it would negate its unique nutritional value. Specifically, our task of improving the nutritional value of spelt boiled down to transferring only one target trait, «purple grain,» into its genome from a donor of this trait, the bread wheat variety Chornobrova, which we had designed earlier, while leaving the original status of spelt as untouched as possible. We managed to accomplish this task through conventional traditional breeding using several interrupted backcrosses with systematic careful control of the morphological traits of the original spelt and the target trait «purple grain».

F_1 seeds from crossing spelt (♀) Schwalbenkorn × Chornobrova (♂) were obtained in 2009. In 2010, Schwalbenkorn spelt (♀) was pollinated with pollen from the F_1 hybrid (♀) Schwalbenkorn × Chornobrova (♂) and seeds were obtained from the first BC_1 backcross. In 2011, BC_1/F_2 seeds were obtained and, in parallel, (♀) Schwalbenkorn was pollinated with pollen from BC_1/F_2 plants (randomized), and plants with morphological characteristics of spelt and purple grain were selected from among the BC_1/F_2 plants. In the following years, from 2012 to 2019, a total of six interrupted backcrosses were performed on the original spelt (♀) Schwalbenkorn from the initial cross. In each cross, the original spelt was used as the maternal plant in order to preserve the cytoplasm of the original spelt. After the second interrupted backcross, a recombinant short plant with dark purple grain, almost 30 cm shorter than the original spelt, appeared in the offspring, and it was this plant that was used in subsequent backcrosses with the original spelt (♀) Schwalbenkorn, invariably as the mother plant.

In 2020—2021, selected elite short-stemmed plants that were morphologically as similar as possible to the original spelt were studied for morphological stability, grain productivity, resistance to plant diseases, resistance to lodging, and baking quality characteristics, and were used for further propagation. In close cooperation with Biligrain (Agrokray LLC, Khmelnytskyi region), the Bilberry spelt variety was submitted for state variety testing and registered in 2023 (reg. no. 230792, patent no. 230659). The average yield of Bilberry spelt grain under optimal high agricultural conditions in the fields of Biligrain was 4.8 t/ha, with a maximum yield of 6 t/ha.

The plant, ear, and grain of Bilberry spelt are shown in Fig. 1. Samples of grain of the common wheat variety Admiral with red grain, the purple grain variety Chornobrova, and the dark purple grain variety Bilberry spelt are shown in Fig. 2. Comparing the color of the Bilberry variety with that of the original Chornobrova variety, it can be seen that the Bilberry grain is significantly darker than the purple grain of the Chornobrova variety and is even close to black. The increase in the intensity of the color of Bilberry spelt grains compared to the donor variety of the «purple grain» trait indicates the influence of the genetic environment on the intensity of the color, which is associated with the increased activity of anthocyanin pigment biosynthesis. This is confirmed by data on the pigment content in the grain. Thus, under the same growing conditions, the anthocyanin content in the Chornobrova variety is ~60 mg/kg, while in spelt it is almost 80 mg/kg.

Thus, we have created the first innovative product in Ukraine (and probably the first in the world) — spelt wheat with dark purple grains and a high content of anthocyanin antioxidant pigments [26]. The baking quality characteristics of Bilberry spelt are presented in Table 4. It can be seen

Fig. 1. Plant, ear, and grain of a spelt variety Bilberry

Fig. 2. Grain samples (from left to right): Admiral, Chornobrova, Bilberry

that Bilberry spelt (like the original Schwalbenkorn spelt) has a significantly higher content of crude protein (16.8 %) and crude gluten (45 %) in the grain compared to the Admiral variety (13 % and 20.1 %, respectively). However, baking quality characteristics such as the Zeleni test, SDS-30K, and flour strength *W* in Bilberry spelt (as in the original spelt) are significantly lower.

Fig. 3 shows the alveograms of Admiral wheat flour, a high-protein wheat line with the *Gpc-B1* gene, and Bilberry spelt. There is a striking difference between the alveograms of Bilberry spelt versus the alveograms of high-quality samples of Admiral wheat flour and wheat with the *Gpc-B1* gene.

TABLE 4. Comparative characteristics of the baking quality of Bilberry spelt

Sample	Whole wheat flour				White flour (70 %)		
	Moisture, %	Protein, %	Hardness Inframatic	Gluten, %	Zeleny, ml	SDS-30K, ml	*W*
Admiral	11.9^{a}	13.0^{b}	34^{a}	20.1^{c}	38^{a}	47^{a}	283^{a}
Bilberry	12.2^{a}	16.8^{a}	6^{c}	45.0^{b}	21^{b}	15^{b}	75^{b}
Schwalbenkorn	11.8^{a}	17.4^{a}	10^{b}	49.0^{a}	22^{b}	17^{b}	74^{b}
LSD_{05}	0.6	0.8	3	2.8	3	4	25

Different letters indicate significant differences at $p \le 0.05$.

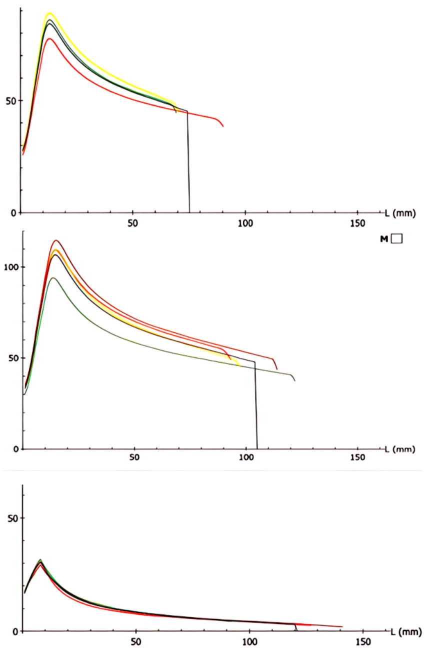

Fig. 3. Flour alveograms (from top to bottom): wheat variety Admiral (*W*-283, *L*-74 mm, *P*/*L*-1.26, *Ie*-68.3 %); wheat line with the *Gpc-B1* gene (*W*-454, *L*-104 mm, *P*/*L*-1.13, *Ie*-68.4 %); spelt variety Bilberry (*W*-75, *L*-119 mm, *P*/*L*-0.29, *Ie*-31.3 %)

As mentioned earlier, Bilberry spelt bread must be made exclusively and without exception from wholemeal flour (close to 100 % output) because the main nutritional factor of Bilberry spelt is anthocyanin pigments concentrated in the grain shell, which are almost completely lost when milled with bran.

Therefore, in order to make bread from whole Bilberry spelt flour, it was necessary to develop a special technology, starting with high-quality grinding of grain into whole flour and ending with baking recipes and dough kneading and baking technology. However, these technological difficulties apply exclusively to bread. As for the production of biscuit products such as cookies and crackers, Bilberry whole spelt flour is even more suitable than flour from traditional bread-baking wheat varieties.

The technology for grinding high-quality wholemeal flour from Bilberry spelt grain was developed by millers at Dar Mlyn Zakarpattia LLC (Fig. 4). The private bakery brand Mamyn Khlib (Khmelnytskyi region) has developed original recipes and technology for kneading flour and produces Ukrainian first bread and other products from whole Bilberry spelt flour with a 100 % natural dark brown color without any colorants (see Fig. 5).

As mentioned earlier, our breeding program for creating wheat varieties with colored grains currently involves 480 cross combinations, mainly of the following type: (♀) traditional bread wheat variety or promising breeding line × sample with colored grains. The ultimate target genotype of our breeding program, as emphasized earlier, is a productive genotype (95 % or at the level of the standard variety) with black grain, which com-

Fig. 4. Whole grain flour and flakes made from Bilberry spelt produced by Dar Mlyn Zakarpattia LLC and Biligrain

Fig. 5. Bread products of the Mamyn Khlib brand made from whole spelt flour of the variety Bilberry

bines genes for blue coloration (of various shades) in the aleurone layer and genes for purple coloration (of various shades) in the pericarp with valuable agronomic and technological characteristics, plus yellow endosperm color as an indicator of carotenoid content from the donors available in our collection (Table 5). The traditional variety (line) serves as a donor of valuable agronomic and technological traits in crosses, and the sample with colored grain is a donor of the desired color type.

Thus, the emergence of wheat varieties with colored grains is becoming an innovative global trend that is actively developing. We currently know from reliable sources that China, one of the world leaders in the selection of colored wheat varieties, has formed a consortium dedicated to breeding and promotion of colored wheat varieties and products made from them. Densely populated India occupies a prominent place among the world leaders in the development of colored wheat varieties, where local experts declare that soon all wheat varieties in India will be colored [14].

Our breeding program for promoting wheat varieties with colored grains is currently the only one of its kind in Ukraine and is quite effective despite the lack of modern laboratory tools for researching specific breeding material with colored grains. In addition, there are several entirely objective factors that influence the effectiveness of our work. Among them are the following:

1. As evidenced by global breeding practices, existing varieties of colored wheat often lag behind the best commercial wheat varieties in terms of grain yield (Petr Martinek, oral communication). This is explained by

TABLE 5. Donor samples of wheat with high carotenoid content in grain (mg/kg)

Sample	Carotenoid content, mg/kg	Sample	Carotenoid content, mg/kg
Admiral	3.61^{f}	4172	5.50^{e}
Citrus	8.48^{b}	4310	6.66^{d}
Bona Vita	9.58^{a}	4338	6.16^{de}
4200	9.14^{a}	4401	7.44^{c}
4088	5.89^{e}	*T. durum*	6.16^{de}
4116	5.66^{e}	Carrots	255.80
LSD_{05}		0.47	

Different letters indicate significant differences at $p \le 0.05$.

the presence in the genome of varieties with colored grains of foreign chromosomal translocations carrying *Pp* or *Ba* genes, which cause a partial depression of grain productivity [14]. This indicates the particular importance of selecting plants in populations based on a complex of elements of high grain productivity.

2. Our genetic collection of colored samples does not include such crossbreeding components as wheat varieties with target black grain (yellow endosperm) and sufficiently high (at the level of standard varieties) grain productivity. Such material still needs to be created.

Therefore, work with the breeding material of our program is divided into four main stages: 1) obtaining productive selections with target blue grains of various shades; 2) obtaining productive selections with target purple grains of various shades; 3) combining blue and purple grain colors with yellow endosperm; 4) hybridization and selection of promising material with target black grain from the previous three stages.

Laboratory work on evaluating color grain genotypes is complicated by the fact that we do not yet have laboratory tools for machine rating and separation of grain colors and shades, which we are forced to do manually. Breeding work with colored grain wheat samples is incomparably more difficult than breeding work with wheat with traditional (red) grain color. It is especially difficult to work with early (F_2, F_3) plant populations from crossing samples with blue and purple grain colors. Figure 6 shows a heterogeneous seed population by grain color as offspring from only one F_2 plant. From the crossing of two genotypes with blue and purple grains on a single F_2 plant, the resulting seed population includes white, red, blue, purple, and black grains. Selection by grain color is also complicated by the fact that the purple color of the grain is inherited from the mother, while the blue color, due to the triploidy of the aleurone layer where the blue pigments are localized, exhibits a pronounced xenia effect. And if we add to the task of combining grain shell colors with yellow endosperm, plus the agronomic and technological characteristics of plants and grains, the reader can imagine the peculiarities and complexity of breeding wheat varieties with colored grains.

However, despite the difficulties, our program is being successfully implemented. Currently, 7,300 individual selections have been obtained (4,500 with black grains, 1,600 with blue grains, and 1,200 with purple

Fig. 6. Population of seeds by color (white, red, blue, purple, black) on one F_2 plant

grains), as well as several hundred promising breeding lines with different grain colors and shades (Fig. 7). All the material survived the rather difficult and snowless winter of 2025. We have quite realistic prospects for the successful development of our breeding program to create wheat varieties with colored grains and significantly improved nutritional status due to the high content of antioxidant pigments anthocyanins and carotenoids in the grain.

Since our breeding program is aimed at implementing a unique innovative solution — wheat varieties with colored grains and processed products from colored grains, which have never been seen before in Ukraine — we are faced with the extremely important issue of introducing colored grain varieties into production and promoting innovative colored grain processing products in the retail network. There is the need to invest in pilot production of new products, to advertise colored varieties and their processing products, to inform potential consumers about the benefits and advantages of colored grain products over traditional products, etc. Moreover, an innovative product of this type, which is beneficial to health and has added functional value, cannot be cheap. Therefore, without fruitful cooperation with interested private companies, it is practically impossible to promote such products into production. We are currently working closely with the seed company Biligrain (Biligrain Food) in the Khmelnytskyi region, which has its own fields, a seed plant, grain processing facilities, bakeries, and its own retail chain. As a result of our fruitful cooperation with Biligrain, the first Ukrainian products made from colored wheat varieties have already appeared in the retail network (Fig. 5), and there are prospects for a real increase in the production of these products and their promotion in the retail network both in Ukraine and abroad.

Conclusions. The outlined features are characteristic of the only large-scale breeding program in Ukraine aimed at developing wheat varieties with colored (blue, purple, and black) grains biofortified with antioxidant pigments anthocyanins and carotenoids. The breeding of varieties with colored grains is technologically much more complex than the breeding of traditional wheat varieties, as it aims to combine complex target traits in grain color with agronomic parameters of plants and technological characteristics of grain and flour. Successful work on the development of wheat varieties with colored grains and high grain productivity and quality requires the involvement of a large amount of diverse genetic material in terms of grain color, agronomic and technological characteristics.

Fig. 7. Sample selections by grain color, from left to right: blue; purple + black; black

The first innovative varieties of winter wheat with purple grains, Chornobrova and Chornozerna, as well as a variety of spelt wheat with dark purple (black) grains have been released in Ukraine. As a result of the implementation of the breeding program to develop wheat varieties with colored grains, 480 crossbreeding combinations were processed, 7,300 individual selections were obtained (4,500 with black grains, 1,600 with blue grains, and 1,200 with purple grains) as well as several hundred promising breeding lines with different grain colors and shades.

In cooperation with the private company Biligrain, Ukrainian first whole grain functional food products from colored grains (bread, pasta, crackers, biscuits) have been designed with 100 % natural dark brown color without the addition of any colorants. Dar Mlyn Zakarpattya LLC has established the production of wholemeal flour from wheat and spelt varieties with colored grains. Innovative food products made from colored grains have already appeared in the Ukrainian retail network, and the flour is mainly intended for export.

Since food products made from colored wheat grains must be produced exclusively and without exception from wholemeal flour, they have dual nutritional functionality due to biofortification with antioxidant pigments and bioactive food components of the husk, aleurone layer, and germ that are valuable for health.

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Compliance with ethical principles. This article does not contain the results of any research in which humans or animals are the subjects of study.

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ПШЕНИЦЯ З КОЛЬОРОВИМ ЗЕРНОМ: ОСОБЛИВОСТІ СЕЛЕКЦІЇ

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Створення сортів пшениці з кольоровим зерном — це новітній напрям досліджень, спрямований на істотне поліпшення харчових характеристик зерна шляхом біофортифікації біоактивними пігментами антоціанінами з високим рівнем антиоксидантної активності. Метою роботи було виявити особливості селекції сортів пшениці з кольоровим (фіолетовим, синім, чорним) зерном на основі вмісту біоактивних компонентів кольорових пігментів-антиоксидантів антоціанів і каротиноїдів з високою антиоксидантною активністю, залучити різні джерела біоактивних пігментів до схрещування, відібрати елітні рослини в популяціях, оцінити перспективний матеріал з кольоровим зерном та створити нові сорти. Здійснено схрещування джерел біоактивних компонентів з серією сортів пшениці, відбір елітного матеріалу за цільовими характеристиками кольору зерна, поєднанням цільових генів, що визначають пігментацію зерна та його технологічні й хлібопекарські властивості. В Україні вироблено перші сорти м'якої пшениці та спельти з кольоровим зерном. Вперше на українському продовольчому ринку отримано функціональні цільнозернові продукти з кольорової пшениці та борошна зі спельти з 100 %-м натуральним темно-коричневим кольором. Шляхом схрещування джерел біоактивних пігментів антоціанів і каротиноїдів отримано кілька сотень популяцій, а також відібрано перспективний селекційний матеріал для створення сортів пшениці з чорним (синім+фіолетовим) зерном. Селекція сортів пшениці з кольоровим зерном є досить складною технологією та передбачає низку важливих особливостей отримання й відбору матеріалу з різними кольорами і відтінками зерна. Успішна робота зі створення сортів пшениці з кольоровим зерном і високою продуктивністю та якістю зерна потребує залучення великої кількості різноманітного генетичного матеріалу за кольором зерна, агрономічними й технологічними характеристиками.

Ключові слова: пшениця, кольорове (синє, фіолетове, чорне) зерно, антоціани, каротиноїди, біофортифікація.

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