

***EXPRESSION AND INHERITANCE OF PERFORMANCE CONSTITUENTS AND SEED COLOR IN WINTER RYE***

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Inheritance of morphological features of seed color and performance constituents in F<sub>1</sub> and F<sub>2</sub> winter rye hybrids from crossing (1.471 / 1.44) and (1.471 / 1.4351H.4-1-22) was investigated. Quantitative parameters of performance of F<sub>1</sub> hybrids, such as the number of productive shoots, the flower number per spike, the kernel number per spike, the kernel weight per spike, and the kernel weight per plant, exceeded the parents' averages. High degree of phenotypic dominance indicate that these traits are inherited by intermediate type, partially dominance and overdominance. In F<sub>2</sub> hybrids, dihybrid segregation by color was observed: purple pigments in the caryopsis coat (purple), aleurone layer pigmentation (intermediate) and wild-type color (white), with a quantitative prevalence of the wild-type color of the caryopsis. Regardless of the winter rye lines with colored and wild-type coloration involved in crossing, a monogenic recessive inheritance was revealed for the purple color of seeds.

**Key words:** *winter rye, genetic analysis, inheritance, seed color, morphological traits, lines*

**Introduction.** Despite significant achievements in genetics, the quantitative trait inheritance theory is always in the first stages of its development [1]. Current breeding programs require constant enrichment with new germplasm; this is a prerequisite for progress in breeding [2].

Today, on food markets in many countries worldwide (China, India, South Korea, Australia, Canada, the USA, Japan, Austria, the Czech Republic, etc.), the introduction of healthy foods based on unique varieties of cereals with colored (black, blue, purple) grain is gaining momentum. The authors of these studies called the appearance of cereals with colored grain on the market "the second green revolution", since colored grain as a way of its biofortification radically improves the nutritional (biological) status of grain and its products [3].

Therefore, recently, studies of pigmented seeds of cereals have become especially relevant, since anthocyanins and polyphenols, as powerful plant antioxidants, are able to scavenge (neutralize) free radicals, reducing risks of dangerous diseases associated with oxidative stress [4].

According to numerous clinical studies, foods that are rich in anthocyanins and polyphenols (referred to as micronutrients) are considered to prevent a whole set of pathologies, including cardiovascular diseases, cancer, diabetes, hypertension, inflammation, and obesity; they help to slow down aging, protect the body from destructive UV radiation, etc. [5].

Therefore, involvement of genetically valuable forms in hybridization raises probabilities of combining valuable economic traits, which increase yield, protein content in grain, bread-making properties, resistance to phytopathogens, and environmental plasticity, in one genotype [6].

Anyone working with biological materials is quickly confronted with the need to find a way to manage variability and inheritance. This refers to breeders whose main job is to create starting materials that are to be evaluated and/or compared with existing ones and between themselves in various aspects [7].

To create winter rye heterozygous varieties and hybrids consistently producing high yields under various environmental conditions, it is important to select crossing components with

colored grain (female and male forms), to explore the gene pool of this crop and to choose individual lines, basing on knowledge of genetic control and variability of grain color in this crop [8].

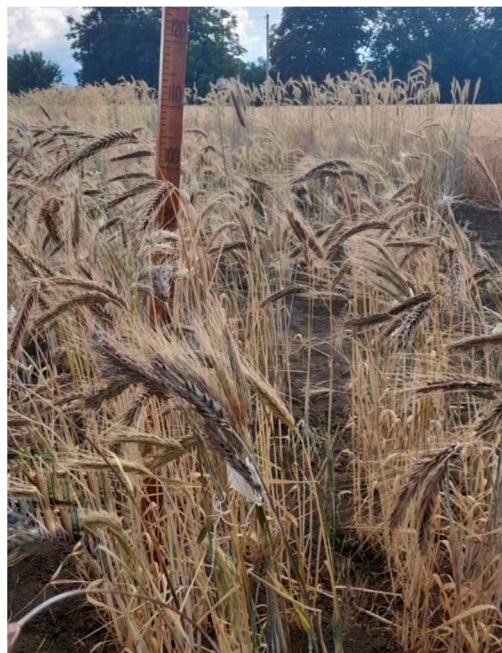
**Purpose.** To establish peculiarities of inheritance of major breeding traits in lines - parents of winter rye hybrids; to assess the effectiveness of creating starting material for heterotic winter rye breeding based on lines - donors of purple seed color and a set of valuable economic characteristics.

**Material and Methods.** We investigated F<sub>1</sub> and F<sub>2</sub> winter rye simple hybrids: 1.471 / 1.44 and 1.471 / 1.4351H.4-1-22.

From hybrid combination No. 16/17–743/18 created by selection and self-pollination in 2017–2020, we obtained a winter rye line with purple caryopses and spike glumes (hereinafter line 1.471) (Fig. 1).

In line 1.471, the plant height limit (Lim) is 100–131 cm; the spike length is 9.8–13.1 cm; , kernels are oval. Purple pigments are localized in the coat of the anthocyanin-colored aleurone layer of the caryopsis; the colored surface is with normal gloss and roughness that are not scraped off the caryopsis. Leaves are gray and densely coated with wax. Plants are V-shaped, or patulous. The thousand kernel weight is 36–51 g.

In line 1.44, the short stem is a dominant trait (plant height = 87–110 cm); the productive tillering capacity is high. The thousand kernel weight is 52.2 g, largely reflecting the big size of seeds. Big kernels are determined by two recessive non-allelic genes with a complementary or epistatic effect.



**Fig. 1.** Winter rye line 471 plants with purple spikelet glumes and seeds in a hybrid nursery.

Line 1.4351H.4-1-22 has short stems; plants are 80–88 cm tall; the leaf arrangement is erectoid, reducing the competition among plants for light and increasing the efficiency of photosynthesis; there is no wax coating on leaves. The kernel weights per spike and per plants as well as thousand kernel weight are high (the thousand kernel weight is 43.4 g).

The experiments were conducted in the fields of Verkhniachka Experimental Breeding Station of the Institute of Bioenergy Crops and Sugar Beet (Verkhniachka EBS IBCSB) of NAAS of Ukraine (Umanskyi District of Cherkassy Region) in 2021–2022. The soils in the experimental fields are variably podzolized and leached chernozems, occupying about 74% of the land area. The basic and pre-sowing tillage was traditional for the Forest-Steppe zone of Ukraine.

The climate is moderately continental and, according to the main weather parameters, corresponds to an unstably wetted zone. Accessions were sown within the optimal timeframe; the farming techniques were conventional for the Forest-Steppe. The above-described lines were studied in breeding nurseries, where their variability in major morphological features was demonstrated. The following F<sub>1</sub> and F<sub>2</sub> simple hybrids were studied: 1.471 x 1.44 and 1.471 x 1.4351H.4-1-22. F<sub>1</sub> hybrids were sown manually in single-row sections of 1.5 m long by wide-row method (interrow distance = 25–30 cm) to a depth of 3–4 cm in 2020. The sowing design was as follows: female form – F<sub>1</sub> – male form, using the female and male forms as references.

All fully ripened plants were harvested with roots. For structural analysis, 25 plants of the hybrids, female and male forms were taken; they were evaluated for the following morphological features: plant height (PH), the productive shoot number (PSN), and spike length (SL). We analyzed such performance constituents as the flower number per spike (FMS), kernel number per spike (KNP), kernel weight per spike (KWS), kernel weight per plant (KWP), and thousand kernel weight (TKW). During the growing period, the phases of plant growth and development were monitored.

To determine the inheritance of stem length and plant performance constituents by F<sub>1</sub>, we calculated the phenotypic dominance degree (hp) by Griffing's formula [9]. The data obtained were grouped in accordance with Bale and Atkins's classification [10].

$$hp = 2^{n-1}(F_n - MP)/(HP - MP),$$

where F<sub>n</sub> – hybrid mean in the n<sup>th</sup> generation obtained by self-pollination from F<sub>1</sub>;

MP – mean for both parents;

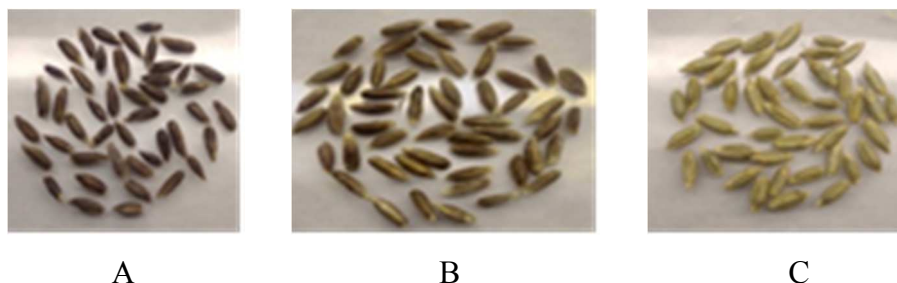
HP – mean of the better parent.

The dominance degree estimates were interpreted using Bale and Atkins's scale: from -∞ to +∞. -∞ < hp<sub>ref</sub> < -1 means hybrid depression; -1 ≤ hp<sub>ref</sub> < -0.5 – depression caused by negative dominance; -0.5 ≤ hp<sub>ref</sub> < +0.5 – intermediate inheritance caused by additive effects of genes; +0.5 < hp<sub>ref</sub> ≤ +1 – dominance; 1 ≤ hp<sub>ref</sub> ≤ +∞ – overdominance (true heterosis).

F<sub>2</sub> hybrids were sown in 2022, as a population in a breeding nursery in plots of 10 m<sup>2</sup>, thinned sowing. At the end of the winter rye growing period, plants with different combinations of phenotypic expression of spike morphology were evaluated in F<sub>2</sub> populations.

A total of 25 F<sub>1</sub> hybrids plants and 400–450 F<sub>2</sub> plants were analyzed. Significance of quantitative differences between the parents and F<sub>1</sub> was assessed using the Student's t-test [11]. Correspondence of the observed ratio of phenotypic classes to the theoretical one in F<sub>2</sub> was assessed by the χ<sup>2</sup> test [12].

**Results and Discussion.** F<sub>1</sub> and F<sub>2</sub> hybrids from two crossing combinations, 1.471 / 1.44 and 1.471 / 1.4351H.4-1-2, had identical morphological type, judging by performance constituents and purple coloration with pigments localized in the coat and aleurone layer of the caryopsis (Fig. 2).



**Fig. 2.** Samples of winter rye seeds: a – purple pigments are localized in the shell, b – purple pigments are localized in the aleurone layer of the grain, c – white color of the grain, purple pigments are absent, F<sub>1</sub> (2021) and F<sub>2</sub> (2022).

When studying the variability and inheritance of traits in F<sub>1</sub> plants, we found that some parameters were different from those in parents (Table 1).

Table 1

**Structural analysis of F<sub>1</sub> hybrids and dominance degrees of the performance constituents in the 1.471 / 1.44 and 1.471 / 1.4351H.4-1-22 crossing combinations, 2021**

Biometric parameters	Parent and combination				
	1. 471	1. 44	1. 4351 N.4-1-22	1. 471/	
				1. 44	1. 4351 N.4-1-22
Plant height, cm	116.9±3.4	90.6±2.5	65.4±1.1	<u>101.7±1.5*</u>	<u>84±4.0**</u>
Productive shoot number	8.9±0.3	7.1±0.8	8.3±0.5	<u>9.5±0.7</u>	<u>9.0±1.2</u>
Spike length, cm	9.2±0.2	8.9±0.2	10.4±0.2	<u>11.0±0.3</u>	<u>9.3±0.3**</u>
Flower number per spike	67.6±2.3	69.7±1.8	60± 3.4	<u>72±2.3</u>	<u>69.8±1.3**</u>
Kernel number per spike	44.1±3.4	53.4±1.9	45.8±3.5	<u>65.8±3.4</u>	<u>64.0±1.9</u>
Kernel weight per spike, g	1.6±0.2	2.3±0.2	1.7±0.1	<u>2.4±0.1</u>	<u>2.3±0.1</u>
Spike kernel weight per plant, g	17.9±1.7	20.6±3.2	11.1±1.5	<u>20.6±0.1</u>	<u>12.6±3.4</u>
Thousand kernel weight, g	4.4±0.2	4.6±0.2	2.9±0.1	<u>4.4±0.2</u>	<u>2.7±0.2</u>
				-1.0	-1.4

**Note:** numerator - absolute values; denominator - phenotypic dominance degree (hp);

\* statistically significant difference from a male form at  $p \leq 0.05$ ;

\*\* statistically significant difference from a male form at  $p \leq 0.05$ .

The 'plant height' trait was inherited by hybrid depression, with hp of -0.16 and -0.3 in the 1.471 / 1.44 and 1.471 / 1.4351H.4-1-22 hybrids, respectively. Burdeniuk-Tarasevych L.A. [13] reported that the plant height in F<sub>1</sub> winter bread wheat hybrids was mostly close to the mean values in the original forms. In F<sub>2</sub>, the plant height was widely variable, with segregation of biotypes that were taller than the tall parent. The authors [7, 13] noted that selection of semi-dwarf plants was possible from all crossing combinations between short-stemmed forms with tall-stemmed ones.

Productive tillering is one of the most important constituents of yield, which is influenced by hereditary and environmental factors. There were a lot of plants whose values were higher than the parents' mean, which meant the heterosis effect for productive tillering in F<sub>1</sub>. The phenotypic dominance degree (hp) in 1.471 / 1.44 and 1.471 / 1.4351H.4-1-22 was 1.7 and 1.3, respectively, indicating overdominance.

Compared to plant height, spike length is less dependent on environmental factors, which is taken into account when one selects genotypes among hybrid populations [7]. In 1.471 / 1.44, heterosis was seen (overdominance, hp = 19) for the 'spike length' trait. In 1.471 / 1.4351H.4-1-22, depression (negative heterosis, hp = -0.8) was observed. The expression index of the 'spike length' trait in F<sub>1</sub> was 9.3 cm, which was similar to the smallest value in the female form, 1.471, (9.2 cm), with 10.4 cm in the male form, 1.44.

As to the flower number per spike, F<sub>1</sub> hybrids showed heterosis compared to the parental forms. The trait was inherited by overdominance as true heterosis. The overdominance degree was 3.3 and 1.6 in the 1.471 / 1.44 and 1.471 / 1.4351H.4-1-22 combinations, respectively.

In F<sub>1</sub> hybrids, there were more kernels per spike than in the female form and the difference was significant in both crossing combinations. For the kernel number per spike, overdominance was inherent in 1.471 / 1.44 and 1.471 / 1.4351H.4-1-22 (hp = 3.6 and 23.8, respectively).

The main components of winter rye yield include kernel weight per spike. In 1.471 / 1.44 and 1.471 / 1.4351H.4-1-22, the 'kernel weight per spike' trait was inherited as true heterosis (overdominance). The dominance degree was 1.3 and 13, respectively. Having investigated interactions of winter rye genes that determine the 'kernel weight per spike' trait, V.V. Skoryk, N.V. Symonenko et al. [7] noted negative heterosis (depression).

Kernel weight per plant is the combined contribution of kernel weight per spike and productive tillering of individual plants. The "kernel weight per plant" trait in F<sub>1</sub> winter rye hybrids was 20.6 g in of 1.471 / 1.44 and 12.6 g in 1.471 / 1.4351H.4-1-22, which corresponded a 2.7-fold increase and a 5.3-fold decrease compared to the female forms of the hybrids. In 1.471 / 1.44), the kernel weight per plant was similar to that in the male form, amounting to 4.4 g, while in 1.471 / 1.4351H.4-1-22, this parameter was 1.5 g higher than in the male form. Taking into account the dominance degree (hp = 1.0 and hp = 0.6), we established that the 'kernel weight per spike' trait showed dominant inheritance.

Thousand kernel weight reflects kernel size and uniformity. In the F<sub>1</sub> hybrids, there was no significant difference in the thousand kernel weight compared to the parents. The dominance degree was -1.0 and -1.4 in 1.471 / 1.44 and 1.471 / 1.4351H.4-1-22, respectively, meaning negative heterosis (depression). Other researchers who studied the inheritance of kernel weight per plant, as one of the main indicators in selection of valuable hybrids, revealed that this trait was mainly inherited by overdominance and, to a lesser extent, by partial or incomplete dominance of a parent with a stronger or weaker expression of the trait, and by depression [1].

Our data on the performance constituents in the parental forms and their simple hybrids (1.471 / 1.44 and 1.471 / 1.4351H.4-1-22) demonstrate that the inheritance coefficients for the 'productive shoot number', 'flower number per spike', 'kernel number per spike', 'kernel weight per spike', and 'kernel weight per plant' traits are inherited by dominance and overdominance.

Analysis of segregation of F<sub>2</sub> hybrid plants divided the F<sub>2</sub> populations into phenotypic classes based on caryopsis color. The observed segregation by caryopsis color is shown in Table 2.

Table 2

**Segregation F<sub>2</sub> hybrids by caryopsis color in 1.471 / 1.44 and 1.471 / 1.4351H.4-1-22, 2022**

Segregation	Caryopsis color			Ratio	$\chi^2_{ob.}$
	Wild type	Intermediate	Purple		
<b>1.471 / 1.44</b>					
Observed	632	22	35	12:3:1	1.15
Expected	516.8	43.1	129.1		
<b>1.471 / 1.4351H.4-1-22</b>					
Observed	340	75	30	12:3:1	1.14
Expected	333.8	83.4	27.8		

**Note:** \*  $\chi^2_{\tau} = 5.99$ .

Of 689 analyzed F<sub>2</sub> plants in 1.471 / 1.44, there were 632 plants with wild-type (yellow-green) color of the aleurone layer, 35 plants with purple color and 22 with an intermediate color; of 445 studied plants in 1.471 / 1.4351H.4-1-22, there were 340 with wild-type, 30 with purple caryopses, and 75 with an intermediate color.

Hybridological analysis showed that the data obtained were a result of dihybrid segregation, which statistically significant close to the theoretical ratio of 12: 3: 1 ( $\chi^2_{ob.} = 1.15$  and 1.14, respectively, at  $\chi^2_{\tau} = 5.99$ ), indicating epistasis.

Thus, in F<sub>2</sub>, there were considerably more plants with wild-type color of the aleurone layer than plants with intermediate or purple color. Emergence of hybrid plants with intermediate color of caryopses, which was not seen in the parents, is noteworthy. The purple color of the caryopsis in the male lines, 1.44 and 1.4351H.4-1-22, turned out to be recessive relative to the wild type color, but dominant in 1.471 / 1.44 relative to intermediate color; however in 1.4351H.4-1-22, on the contrary, it was recessive relative to intermediate color. Perhaps there is epistasis in 1.471 / 1.44 in



the following sequence: wild-type > purple > intermediate, but in 1.471 / 1.4351H.4-1-22, the sequence is different: wild type > intermediate > purple. Morhun V.V. and Lohvynenko V.F. [14] studied different generations of winter wheat hybrids after individual selections and identified plants with purple seeds. Therefore, regardless of the genotypes of winter rye lines included in crossings, the 'purple color of seeds' trait showed a monogenic recessive inheritance. Figure 3 illustrates the segregation of the offspring from one plant by seed color.



**Fig. 3.** Splitting the offspring of one plant according to the color of winter rye seeds.

**Conclusions.** Having studied the inheritance of morphological characters in F<sub>1</sub> and F<sub>2</sub> hybrids from crossing winter rye lines with purple pigments in the caryopsis coat with lines with wild-type color of the caryopsis, we found that the purple color of seeds showed a recessive inheritance.

In F<sub>1</sub> hybrids, the quantitative constituents of the plant performance, such as the productive shoot number, flower number per spike, kernel number per spike, kernel weight per spike, and thousand kernel weight were higher than the parents' mean values.

In F<sub>2</sub> hybrids, dihybrid segregation into forms with different colors was observed: wild-type color of the caryopsis, purple color of the aleurone layer (intermediate), and pigments localized in the caryopsis coat (purple). The purple color turned out to be recessive relative to the wild-type color in both hybrid combinations, but at the same time it was dominant relative to the intermediate color in 1.471 / 1.44; however, in 1.471 / 1.4351H.4-1-22, on the contrary, the color of the aleurone layer (intermediate) showed a dominant inheritance over purple pigmentation, which may be a consequence of epistasis. Regardless of the genotypes of winter rye lines with wild-type and colored grain included in crossings, both combinations demonstrated a monogenic recessive inheritance.

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## **ОСОБЛИВОСТІ ПРОЯВУ ТА УСПАДКУВАННЯ ОЗНАК ЕЛЕМЕНТІВ ПРОДУКТИВНОСТІ ТА ЗАБАРВЛЕННЯ НАСІННЯ У ЖИТА ОЗИМОГО**

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**Мета.** Встановити особливості успадкування основних селекційних ознак у ліній – батьків гібридів озимого жита; оцінити ефективність створення вихідного матеріалу для гетеротичної селекції жита озимого на основі ліній – донорів пурпурового забарвлення насіння та комплексу господарсько-цінних ознак.

**Матеріали і методи.** Досліди проводили на полях Верхняцької ДСС ІБКіЦБ НААН України Уманського району, Черкаської області. Сівбу гібридів  $F_1$  здійснювали однорядковими ділянками довжиною 1,5 м вручну широкорядним способом (міжряддя 25-30 см.) на глибину 3–4 см. Схема сівби: материнська форма –  $F_1$ –батьківська форма, з використанням батьківських форм як стандартів. Всі рослини у фазі повної стиглості збирали з корінням. Для структурного аналізу брали по 25 рослин гібридів, материнських та батьківських форм, які досліджували за такими морфологічними показниками, як висота рослин (ВР), кількість продуктивних пагонів (КПП), довжина колосу (ДК). Аналізували такі елементи продуктивності, як кількість квіток в колосі (ККК), кількість зерен в колосі (КЗК), маса зерна з колоса (МЗК), маса зерна з рослини (МЗР), маса 1000 зерен (МТЗ). У процесі вегетації спостерігали фази росту та розвитку рослин. Відповідність фактичного співвідношення фенотипових класів теоретичному співвідношенню в  $F_2$  визначали за критерієм  $\chi^2$ .

**Результати та обговорення.** Досліджено характер успадкування морфологічних ознак забарвлення насіння та елементів продуктивності гібридів  $F_1$  і  $F_2$ , одержаних за схрещування (л.471 / л.44) та (л.471 / л.4351Н/4-1-22) жита озимого. Кількісні параметри елементів продуктивності гібридів  $F_1$ , як кількість продуктивних пагонів, кількість квіток в колосі, кількість зерен в колосі, маса зерна з колоса, маса зерна з рослини перевищували показники батьківських середніх значень. Високі показники ступеня фенотипового домінування вказують на успадкування цих ознак за проміжним, частково домінантним та наддомінантним типами. У гібридів  $F_2$  спостерігалася дигібридне розщеплення на форми із забарвленням: фіолетових пігментів локалізованих в оболонці зернівки (фіолетове), забарвлення алейронового шару зернівки (проміжне) та нормальне (біле), з кількісною перевагою нормального забарвлення зернівки.

**Висновки.** Незалежно від генотипів ліній жита озимого, включених у комбінації схрещування з кольоровим і нормальним забарвленням, за ознакою фіолетового забарвлення насіння виявлено моногенний рецесивний характер успадкування.

*Ключові слова:* жито озиме, генетичний аналіз, успадкування, забарвлення насіння, морфологічні ознаки, лінії



## ***FEATURES OF MANIFESTATION AND INHERITANCE OF TRAITS ELEMENTS OF PRODUCTIVITY AND SEED COLOR IN THE LIFE OF WINTER RYE***

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**Purpose.** To establish peculiarities of inheritance of major breeding traits in lines - parents of winter rye hybrids; to assess the effectiveness of creating starting material for heterotic winter rye breeding based on lines - donors of purple seed color and a set of valuable economic characteristics.

**Material and methods.** The experiments were conducted in the fields of Verkhniachka Experimental Breeding Station of the Institute of Bioenergy Crops and Sugar Beet (Verkhniachka EBS IBCSB) of NAAS of Ukraine (Umanskyi District of Cherkassy Region) in 2021-2022. The following F<sub>1</sub> and F<sub>2</sub> simple hybrids were studied: 1.471 x 1.44 and 1.471 x 1.4351H.4-1-22. F<sub>1</sub> hybrids were sown manually in single-row sections of 1.5 m long by wide-row method (interrow distance = 25–30 cm) to a depth of 3–4 cm in 2020. The sowing design was as follows: female form – F<sub>1</sub> – male form, using the female and male forms as references. All fully ripened plants were harvested with roots. For structural analysis, 25 plants of the hybrids, female and male forms were taken; they were evaluated for the following morphological features: plant height (PH), the productive shoot number (PSN), and spike length (SL). We analyzed such performance constituents as the flower number per spike (FMS), kernel number per spike (KNP), kernel weight per spike (KWS), kernel weight per plant (KWP), and thousand kernel weight (TKW). During the growing period, the phases of plant growth and development were monitored. Correspondence of the observed ratio of phenotypic classes to the theoretical one in F<sub>2</sub> was assessed by the  $\chi^2$  test.

**Results and discussion.** Various forms from a genetic collection, carriers of dominant and recessive alleles of one or several marker traits were used as the starting material to explore expression and inheritance of morphological traits. Hybrid combination No. 16/17–743/18 (line 471) with purple seeds, which had been created by selection and self-pollination (inbreeding), was crossed with donors of the dominant ‘short stem’ trait with wild-type (light) color of seeds, 1.44 and 1.4351H.4-1-22. In F<sub>1</sub> hybrids, the quantitative constituents of the plant performance, in particular the productive shoot number, flower number per spike, kernel number per spike, kernel weight per spike, and kernel weight per plant were higher than the parents’ mean values. High degree of dominance indicated that these traits were inherited by dominance and overdominance. In F<sub>2</sub> hybrids, dihybrid segregation into genotypes with different colors was observed: purple due to pigments in the caryopsis coat, intermediate and wild-type color of the aleurone layer, with greater number of wild-type plants. The purple color of the caryopsis in the parental lines, 1.44 and 1.4351H.4-1-22, turned out to be recessive relative to the wild-type color, but at the same time it was dominant in 1.471 / 1.44 relative to intermediate color, and in 1.4351H.4-1-22, on the contrary, it was recessive in relation to intermediate color. Epistasis in 1.471 / 1.44 was seen in the following sequence: wild-type > purple > intermediate, while in 1.471 / 1.4351H.4-1-22 the sequence differed: wild-type > intermediate > purple.

**Conclusions.** Regardless of the genotypes of winter rye lines with colored and wild-type seeds included in crossings, the purple color of seeds showed a monogenic recessive inheritance.

**Key words:** *winter rye, genetic analysis, inheritance, seed color, morphological traits, lines*