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A new spring barley variety 'In Memory of Dudin'

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Abstract. The purpose of the research is to conduct a comprehensive assessment of the new spring barley variety ‘in Memory of Dudin' on economically valuable signs. Studies were carried out in 2016-2019. The spring barley variety "in Memory of Dudin ' was created by the method of directional individual selection from the variant with treatment of spring barley variety ‘Bios 1’ with laser red light (wavelength 632.8 nm, continuous irradiation mode, beam power density 0.3 mW/cm^2) for 60 minutes, followed by soaking in a solution of sodium carbonate with a concentration of 0,1n for 12 hours. Ear of the plant has average length 8.9 cm, the number of spikelets and the grain in the ear are medium (25.6 pcs. and 24.3 pcs. respectively). The 1000-grain weight is high - 49.2 g. Culm has an average length of 72.4 cm. The average yield over the years of the study was 4.6 tons/ha, the maximum one - 7.02 tons/ha in 2019. Dry matter ash content 2.2%, fiber content 3.65%, starch content 52.61%, fat content 1.65%, protein content 10.19%; extractability 77.0%. The variety ripens 4-7 days earlier than the standard. Based on a comprehensive assessment, the spring barley variety of "in Memory of Dudin' was transferred to the State Variety Test in 2020.

1 Introduction

Recently, Russian agriculture has been experiencing an increasing need for domestic seed material. In this regard, there is a growing need for the creation and reproduction of new varieties and hybrids that will be adapted to changing climatic conditions, have a greater stable potential yield, resistance to diseases and pests, and processability [1].

The use of the new variety can be evaluated by many parameters: improving the quality of products (content of nutrients, proteins and others); improvement of product processing (grain size, fruit lying capacity, etc.); reducing the use of pesticides and fungicides through the introduction of disease and insect resistant varieties; new qualitative features (grain, leaf) and many others. Obtaining new properties and features of plants makes it possible to increase efficiency of agricultural industry [2]. Mutational breeding can give an improvement in the trait without significantly changing the genotype and phenotype of the plant, unlike traditional breeding.

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Over the past 80 years, more than 3,300 mutant varieties have been produced in the world. Of these, 60% are obtained after 1985, about 70% are mutant forms, and the remaining varieties are obtained by crossing with mutants [3].

From the mutant varieties Golden Promise and Diamant, more than 150 popular varieties of barley have been obtained, which are widely used around the world [4].

Spring barley is the leading grain fodder crop in the Kirov region [5]. Under the conditions of the Volga-Vyatka region, the breeding of spring barley is carried out by various methods, the main ones are crossing, biotechnology and mutagenesis with subsequent selection. Over the past twenty years, seventeen varieties of spring barley have been created in the region. Of these, 52.9% of varieties created by selection from hybrid combinations, 29.4% of varieties created by selection from mutant families and only 17.6% of varieties obtained using agricultural biotechnology methods [6].

Since the seventies of the XX century, within the walls of the Vyatka GSHA, under the guidance of Doctor of Biological Sciences, Professor G.P. Dudin, the study of photo- and chemomutagenesis was carried out in order to obtain a new source material of barley. Data were obtained on the mutagenic effectiveness of many drugs and substances: abscisic acid, albite, vincite, laser red light, urea, ethrel, ecloran, epin. As a result of the application of induced mutagenesis, six varieties of spring barley were created: Vyatsky, Gid, Izumrud, Kvant-2, Slobodskoy and Khlynovsky [7].

Currently, work is underway to search for mutagens with low toxicity and at the same time giving a high yield of valuable mutations in plants. Such factors are red radiation and sodium salts [8; 9]. In this regard, it is of scientific interest to study the complex mutagenic effect of sodium salts and red radiation on the output of mutant forms of spring barley.

2 Materials and methods

Field research was carried out in 2009... 2019 years on the training field of the Vyatka State Agricultural Academy in Kirov (Russian Federation). The soil is sod-medium-podzolic, loamy. The reaction of the soil solution is acidic and slightly acidic: pH ranged in years from 4.00 to 5.65. The availability of soils with mobile phosphorus is medium and high (91…190 mg / kg of soil), potassium is low and high (73... 202 mg / kg of soil), the sodium content in the soil is less than 0,001 mmol/100 g of soil. The content of humus ranged over the years from 1.35% to 2.59%.

The object of research is spring barley variety 'Bios 1' - the standard in the Kirov region. It was obtained at FIC Nemchinovka and ISA - a branch of FNAC VIM (Moscow, Russian Federation) by the method of agricultural biotechnology (haploidia) from a hybrid combination.

Sodium carbonate (Na_2CO_3) with a concentration of 0.01, 0.1 and 1 N was used with a purity of 99%. Soaking the seeds in water and sodium carbonate solution was carried out for 12 hours at room temperature.

Laser red light (LRL) was obtained on a 632.8 nm helium-neon OKG-12-1 unit. Far red light (FRL) with a wavelength of 754 ± 10 nm was obtained from an electric filament lamp through an interference filter using an OI-19 illuminator. Exposure of seeds irradiation is 60 minutes, mode of irradiation is continuous, beam power density is 0.3 mW/cm^2.

The study scheme included the following variants: 1 - Control (S.s. - seeds soaked in distilled water), 2 - 0.01 n Na_2CO_3; 3 - 0.1 n Na_2CO_3; 4 -1 n Na_2CO_3; 5 - S.s. + LRL; 6 – S.s. + FRL; 7 - 0.1 n Na_2CO_3 + LRL; 8 - LRL + 0.1 n Na_2CO_3; 9 - 0.1 n Na_2CO_3 + FRL, 10 - FRL + 0.1 n Na_2CO_3; 11 - LRL + 0.1 n Na_2CO_3 + FRL; 12 - FRL + 0.1 n Na_2CO_3 + LRL. In each variant, 500 grains were processed (125 pcs. per plot in 4 repetitions). Sowing was carried out manually, the distance between rows is 15 cm, between grains in a row is 4 cm, the plot area is 1 m^2.

In the first and subsequent generations, phenological observations were made, barley resistance to lodging was determined; field germination capacity of seeds, plant survival was taken into account; elements of productivity structure were analyzed; families with chlorophyll mutations were isolated according to the classification developed by Yu. Kalam, T. Orav [10], plants with visible morphological and physiological deviations from the original variety were selected.

In the second generation (M_2), seeds were sown by family from the main spike of M_1 plants. The analysis of the yield structure was carried out in all plants in the isolated family with changes; the spectrum of neoplasms and the frequency of families were determined with changes in percentage to the total number of seeded families in this variant.

In M_3 seeds were sowed by families order from the main ear of the changed M_2 plants. The inheritance of altered traits identified in the second generation was checked. New changes were taken into account. The percentage of heredity and mutation rate was calculated.

In M_4-M_{11}, isolated mutant forms with economic-valuable features, which are of interest for breeding, were studied according to the complete scheme of breeding process adopted for self-pollinators, in accordance with the methodology of the Russian Federation State Commission for variety testing of crops [11].

The standards used were varieties of spring barley recommended by the Russian State Commission for Variety Testing in the Kirov Region: 'Bios 1' (2009), 'Nur' (from 2010 to 2014) and 'Belgorodsky 100' (from 2015 to the present).

Statistical processing of experimental data was carried out to assess the variability of quantitative features.

The hordein formulas of the barley mutants were determined by electrophoresis in the laboratory of the genetic bases of plant identification of the Institute of General Genetics named after N.I. Vavilov RAS (Moscow). Determination of nutriency of new barley samples and content of ash, fiber, starch, fat and protein in dry matter was carried out on Spectra Star 2500 XL-R IR analyzer at Kirov regional veterinary laboratory. The determination of extractive activity was carried out according to the Pavlovsky method at the Federal Agricultural Research Center of the North-East named after N.V. Rudnitsky.

3 Results and discussion

In the first generation, after exposure to the considered factors, the effect of depression was established in all variants of the experiment. With increasing concentration of sodium carbonate, its depressing effect increases from -2.74 to -6.05% due to reduction of field germination ability of seeds, reduction of length of straw and ear, number of grains and mass of grain per ear. The most depressing effect had paired and complex variants with primary treatment with laser red light: LRL+ 0.1N Na_2CO_3 (D = -8.92%), LRL + 0.1N Na_2CO_3 + FRL (D = -7.63%). The return combination of factors had less depressing effect on M1 barley plants. This is due to the fact that when LRL affects seeds, the conformation of phytochrome changes, and the permeability of cell membranes for Na_2CO_3 increases. The increase in the cell Na+ concentration causes disruption of physiological processes in cell and has a negative effect on the plant growth and development.

In the M_2, 3,788 families were studied. Chlorophyll mutations were observed in all variants except for control. In total, 37 families with changes were selected. The largest number of families with chlorophyll mutations (10 families or 3.09% of total) was observed in the complex variant of FRL + 0.1N Na_2CO_3 + LRL.

In the experiment, 21 types of chlorophyll mutations were isolated. The most common mutation was *albina* (30.8%) - plants with white leaves. The part of chlorophyll mutations of the *viriduloalba* and *xanthovirescens* type was 7.7% each, *albotigrina*, *alboviridoterminalis*, *viridovirescens* - 5.1%; 15 types of chlorophyll mutations were observed with a frequency of 2.6%. In the variant with using 0.01N Na_2CO_3 and FRL + 0.1N Na_2CO_3 + LRL, 7 and 6 types of mutations were isolated, indicating that these factors produced a significant violation of the genetic program responsible for the chlorophyll formation. The identified chlorophyll mutations make it possible to judge that sodium carbonate, LRL and FRL are mutagens on barley culture.

In addition to chlorophyll mutations, families with morphological and physiological changes were isolated in the M_2. The largest number of families with changes (53) was observed in the variant with soaking of seeds in 0, 01n Na_2CO_3. The increase in the concentration of sodium carbonate did not significantly reduce the frequency of morphological and physiological changes (from 15.3% to 11.6%).

190 mutant barley families were selected in M_3. The inheritance of chlorophyll mutations was complex and depended on the type of mutations and mutagenic factor. The spectrum of chlorophyll mutations narrowed from 21 types (M_2) to 4 types in M_3.

A study of the nature of inheritance of morphological and physiological changes showed that some of them had a modifying nature and returned to the original phenotype in M_3. In the third generation, the mutant nature was confirmed in 60.7% of families selected in the second generation. In M_3, mutations of morphological and quantitative signs of barley prevailed among neoplasms. Differences in the spectral composition of mutations between variants were noted.

According to electrophoregram of component composition of storage proteins, conformity of genotypes to one or another variety or initial accession is established. Storage proteins of barley endosperm (hordein) in variety 'Bios 1' have a formula of component structure HrdA2 B8 F2. The obtained altered forms (mutants) of spring barley did not have deviations in the spectral composition of the electrophoretic formulas of hordeins from the initial variety, which is confirmation of their origin from it. The studied physical and chemical factors contributed to the appearance of morphological and physiological changes, but did not affect the basic part of the Bios 1 genome, including genes that determine the synthesis of endosperm storage proteins.

In M_4, eleven mutant forms of barley of the fourteen sowing samples showed a reliable yield increase compared to the standard by 17.1…51.7%. Barley mutant yields ranged from 3.6 to 5.3 tons/ha. Yield of standard variety "Nur" was 3.5 t/ha, initial variety "Bios-1" - 3.4 t/ha.

The highest yield was shown by sample 8-3-013 (obtained by irradiation with LRL followed by soaking in 0.1N Na_2CO_3). The high yield compared to the control is due to the increased productive plant stand, a large number of spikelets and grains per ear, and accordingly a larger mass of grain per ear.

In M_5, in 2013, the six most valuable barley samples were sown; three mutant samples: M 4-16-3, M 8-3-013, M 11-13-*Xa* provided a reliable increase in barley yield capacity; the data are given in tab. 1.

Table 1. Yield capacity of mutant forms of barley.

Variety, mutant	2013	2014	2015	Average for 3 years
Nur (standard)	2.99	4.66	3.48	3.71
Belgorodsky 100' (standard)	2.22	4.57	3.74*	3.51
M 2-37-6	3.37	4.92	4.06*	4.12
M 4-16-3	4.12*	6.20*	4.21*	4.84
M 6-7-x	3.34	4.80	3.24	3.79
M 9-5-3	2.92	5.39	3.91*	4.07
M 8-3-013	3.61*	5.90*	4.35*	4.62

M 11-13-*Xa*	3.41*	5.24	3.50	4.05

Note: * - significant difference at LSD_{05}

All studied samples of barley in the sixth generation (2014) showed an increase in yield capacity compared to standard varieties. The maximum yield capacity (6.2 tons/ha) was in mutant 4-16-3 (in the variety "Nur" - 4.7 tons/ha). In barley mutant 8-3-013, the yield was 5.9 tons/ha.

In 2015, the seventh generation mutants showed a reliable increase in yield capacity: M 2-37-6 (0.58 t/ha), M 4-16-3 (0.73 t/ha), M 9-5-3 (0.43 t/ha) and M 8-3-013 (0.87 t/ha). The highest average yield for the three years of study was in barley mutants 4-16-3 and 8-3-013, respectively 4.84 and 4.62 tons/ha. The best mutant barley samples $M_8 \ldots M_{11}$ were studied in 2016-2019 in competitive variety testing.

The average yield capacity of mutant samples for 5 years of study in the nursery of competitive variety testing is presented in tab. 2.

Table 2. Yield capacities of spring barley samples in competitive variety testing, t/ha.

Variety, mutant	2016	2017	2018	2019	Average for 4 years
Belgorodsky 100' (standard)	4.12	4.29	2.72	6.75	4.47
M 2-37-6	3.91	4.13	2.86	6.79	4.42
M 4-16-3	3.80	3.83	2.70	6.80	4.28
M 8-3-013	4.29	4.53•	2.89	7.02	4.73
M 9-5-3	3.72	3.89	2.74	6.57	4.23
M 11-13-*Xa*	3.84	3.74	2.09	6.59	4.07

Note: ● - significant difference at LSD_{05}

In 2016, when evaluating barley samples in a competitive variety test, all mutant samples showed yield capacities at the level of the standard variety "Belgorodsky 100" - 4.1 tons/ha. The greatest yield capacity in 2016 was noted in barley mutant M 8-3-013 - 4.3 t/ha.

According to the results of the ANOVA in 2017, out of five samples, only one mutant of barley (M 8-3-013 - 4.5 t/ha) showed a reliable increase in yield capacity compared to the standard variety 'Belgorodsky 100' (4.3 t/ha).

In 2018, yield capacities on variants ranged from 2.1 (M 11-13-Ha) to 2.9 tons/ha (M 8-3-013). The prevailing weather conditions of 2018 significantly affected the low yield capacity of barley on the training field of the Vyatka State Agricultural Academy.

The yield capacity in 2019, due to the prevailing weather conditions, was the maximum for the period of competitive variety testing and amounted to more than 6.5 tons/ha. In mutant sample M 9-5-3, it was at the standard level and was 6.6 t/ha. The greatest yield capacity of barley in 2019 was noted in the early mutant M 8-3-013 - 7.0 tons/ha. A longer, well-grained, productive ear provides the yield advantage of this mutant sample as well as a relatively high 1000-grain weight (see tab. 3). Sample M 4-16-3 is also promising for use in breeding programs for increase the parameters of the ear. By 1000-grain weight, sample M 9-5-3 was isolated in the study; the 1000-grain weight in the mutant changed from 47.5 g (2017) to 61.2 g (2015) and averaged 54.6 g, reliably exceeding the standard.

Table 3. Yield capacity and elements of its structure in mutant samples of barley, 2015-2019.

Variety, mutant	Plant height, cm	Ear			Grain weight, g	
		lenght, cm	number, pcs.		Per main ear	1000-grain
			spikelets	grain		

Belgorodsky 10 0' (standard)	69.6	7.5	22.1	21.0	1.17	51.6
M 2-37-6	70.2	8.1	23.2	22.0	1.12	48.3
M 4-16-3	68.1	8.2*	23.9*	22.8*	1.24	48.1
M 8-3-013	72.4	8.9*	25.6*	24.3*	1.31*	49.2
M 9-5-3	64.2	8.0	22.5	21.4	1.24	54.6*
M 11-13-*Xa*	58.9	7.1	21.3	20.1	1.02	49.0

Note: * - significantly differs from the standard at $p < 0.05$

The main disadvantage of the Bios 1 variety, when cultivated in the Volga-Vyatka region, were the long growing season and poor thrashing capacity in some years, which led to the complete rejection of the use of this variety in the farms of the Kirov region.

Unlike the Bios 1 variety, mutants were characterized by easily separable threshing awns. In addition, all isolated samples differed from the original form and standard by a shorter growing season. The early mutant M 11-13-Ha matured 11 days earlier than the Bios 1 variety and 7 days of the Belgorodsky 100 standard; M 8-3-013 - 8 and 5 days; M 2-37-6 and M 9-5-3 for 6 and 3 days, respectively. The duration of the growing season of mutant M 4-16-3 was at the level of the initial variety and 3 days shorter than that of the Belgorodsky 100 standard.

All selected samples differed in the use and combination of mutant factors. So, when soaking seeds in Na_2CO_3 solutions for 12 hours with concentrations: 1N, mutant M 4-16-3; 0. 01N - M 2-37-6 was obtained. In complex variants: 0.1N Na_2CO_3 + FRL sample M 9-5-3 was isolated; LRL + 0.1N Na_2CO_3 + FRL - M 11-13; LRL + 0.1N Na_2CO_3 - M 8-3-013. The results show the promise of using these mutagenic factors and combinations thereof to create a new initial material for barley breeding.

4 Conclusions

Thus, the efficiency of using the variety "Bios 1" to create a new barley initial material by mutagenesis with various concentrations of Na_2CO_3, laser red and far red light, together or separately, has been proved.

According to the results of the competitive test (2016-2019), the spring barley variety 'in Memory of Dudin' (M 8-3-013), characterized by high yield capacity, was transferred to the state variety test; the addition to the Belgorodsky 100 standard over the years of the variety test ranged from 0.3 to 0.8 tons/ha. For further breeding work, mutants having valuable features and properties were identified as the initial material. Promising for further breeding work are mutant samples M 4-16-3, M 9-5-3, and M 11-13-*Xa*.

Mutant sample 8-3-013 is obtained in the variant LRL + 0.1N Na_2CO_3. A variety is *nutans*. The ear is medium - 8.9 cm (in the initial form - 8.0 cm), the number of spikelets and grain per ear is medium (25.6 pcs., 24.3 pcs., respectively). The 1000-grain weight is high - 49.2 g. Culm has an average length of 72.4 cm. Yield capacity is 4.6 t/ha. Sample ripens 4-7 days earlier than the standard.

Using the methods developed by the author to create a new initial material increases the efficiency of obtaining a promising breeding material and breeding new high-yielding varieties of spring barley. Theoretical studies and experimental data served as the basis for creation of the barley variety 'in Memory of Dudin' (M 8-3-013, patent application No. 82980 / 7954589 of 30.11.2020) and 23 breeding-valuable mutant samples of barley.

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