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WINTER WHEAT MUTATION VARIABILITY UNDER LOW-DAMAGE ABILITY MUTAGEN ACTION

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Abstract

The purpose of our study was to show the possibilities of a chemical supermutagen in the regular induction of certain types of mutations. Winter wheat dry seeds of eight varieties were acted with water (control) and DAB (1.4-bisdiazoacetylbutane) action in concentrations of 0.1%, 0.1%, 0.3%. Studies have shown that the use of this mutagen is expedient from the point of view of obtaining valuable forms of low-growing, early-ripening, late-ripening, high-yielding lines, forms with high productive tillering. The mutagen by its nature has a rather low affinity, at least for this group of genotypes, however, this factor still affects the frequency of some types of mutations. Even extreme concentrations of this mutagenic agent did not lead to a significant decrease in the number of mutations; however, they reached a maximum in the spectrum of possible changes. Further studies are needed on changes in photosynthetic activity and associated persistence.

Key words: *winter wheat; mutation; mutagen; DAB (1.4-bisdiazoacetylbutane).*

INTRODUCTION

The use of mutagenesis to improve crop plants follows three main strategies (Venske et al., 2019). Firstly, this is the obtaining of radically new forms, which, first of all, have a fundamentally new plant architecture (Zulfiqar et al., 2021), which allows not only introducing new parameters into a promising variety model, but also significantly improving the basic parameters of existing varieties (Mitura et al., 2023). One example of this is the production of high-intensity short-stemmed forms in winter wheat (Nazarenko et al., 2020).

The second very promising direction is the use of the so-called small changes or micromutations, primarily of the biochemical (but not only) plan (Di Pane et al., 2018; Ogutu & Charimbu, 2020). These mutations are promising primarily in the sense of a synergistic effect on economically valuable traits and properties in combination with the absence of negative additional points (Baye et al., 2020; OlaOlorun et al., 2020).

The third promising direction is the use of new genetically active factors for the induction of genetically and selectively valuable mutations (Henry, 2019). In this case, both new chemical agents and types of radiation are used. In addition, adjacent to this direction is the use of various substances and the physiological state of the source material to reduce the damaging ability of the mutagenic factor while maintaining the level of mutational activity (Munda et al., 2022). Also a promising direction in this case is the use of epimutagens (Beiko & Nazarenko, 2022).

The use of mutagens fundamentally encounters a barrier both in terms of reducing the number of mutations due to lethal and sublethal damage at the DNA level and due to the physiological consequences of exposure to a mutagenic factor. It is possible, however, to significantly shift this barrier by using new mutagenic factors, as well as by varying the source material. The presence of at least two different mechanisms of genetic resistance to the effects of a mutagen has been repeatedly noted earlier, and a decrease in the number of actual mutations is not necessarily among the consequences (Spencer-Lopes et al., 2018; Balkan et al., 2021).

In the context of the above main directions and general practices in improving the economic properties of cultivated plants, these studies are aimed at determining the optimal combinations of a mutagen with structurally determined low damaging characteristics, the genotype of the initial material of both local and world origin, and individual concentrations of the active factor in terms of obtaining the maximum yield of valuable both directly and in terms of participation in the interbreeding program (Balkan, 2018).

MATERIALS AND METHODS

The experiment has been conducted under the conditions of the experimental fields station of the Science-Education Center of the Dnipro State Agrarian Economic University during 2017-2022.

Winter wheat seeds (1000 grains for each concentration and water) were acted with a DAB (1,4-bisdiazoacetylbutane) 0.1%, 0.2%, 0.3% (Sigma-Aldrich, Germany). Seeds has been treated with an exposition of 24 hours in order to with the generally recommended method for chemical mutagens actions protocol. These concentrations are trivial for mutagens (chemical supermutagens) of this group. The control was soaked in water (Spencer-Lopes et al., 2018).

Seeds samples were sown for 32 variants (in total) (10-rows plots for every variant, in water as control, interrow-spacing was 0.15 m, length of row was 1.5 m) by varieties (ecotypes in brackets FS - forest-steppe, all for all zones, S - steppe) Balaton (FS), Borovytsia (all), Zeleny Gai (S), Zoloto Ukrainy (FS), Kalancha (all), Niva Odeska (all), Polyanka (all), Pochayna (all). The genotypes were identified according to characterize winter wheat varieties variability for North Steppe subzone (Dnipro region) (Spencer-Lopes et al., 2018).

The agrotechnology of crop cultivation is trivial for the Steppe zone (semi-arid area).

At M_2-M_3 generations mutation families have been selected via visual evaluation. The sowing was done by hand, at the end of September, at a depth of 4-5 cm and with a rate of 100 viable seeds to a row (length 1.5 m), interrow was 15 cm, between samples 30 cm, 2 rows for sample with control-row of initial variety samples after every 20 variants.

Field experiment was conducted at the Science-Education Center of Dnipro State Agrarian and Economic University (48°51′10″ n. l. 35°25′31″ e. l.). Trivial for zone agricultural practices including fertilization were provided. Estimation was conducted during 2018-2022 years.

Statistic analyze of data was performed by ANOVA-analysis with Bonferroni amendment, grouping and estimation of data was provided by discriminant and cluster analysis (Statistic 10.0, multivariant module Differences between samples were assessed by Tukey HSD test.

RESULTS AND DISCUSSIONS

In total, for control and material after mutagen action 16000 families at second-third generation were investigated. The concentrations of mutagen characteristic for breeding practice have been used. The number of families on average was about 500 per variant, with no exception. Even the highest concentration of DAB 0.3 did not lead to such a significant decrease in the viability of the plant organism that it would have such a critical effect on the volume of the obtained material, which once again indicates a low damaging ability from a physiological point of view.

However, we divided the material into two groups - the first is the varieties with high sensitivity to mutagenic effects, which resulted in higher depression in the first generation, and the second - more resistant group of genotypes (respectively, according to the total mutation frequency of Tables 1 and 2).

Significantly, the activity of the mutagen in terms of the induction of the overall rate of mutations was affected both by an increase in the concentration of the mutagen ($F = 96.70$; $F_{0.05} = 3.86$; $P = 2.17*10^{-6}$) and by differences in the genotype of the initial material ($F = 35.44$; $F_{0.05} = 4.11$; $P = 2.34*10^{-3}$). As you can see, in the case of varieties more sensitive to the action of the mutagen, the effect of all concentrations of this mutagen significantly differs from each other and from the control within the same variety (Table 1). At the same time, only Niva Odeska (up to 11.6%) and Balaton (up to 8.2%) differ significantly from the second group (Table 2) in a higher overall mutation rate ($F = 17.12$; $F_{0.05} = 4.99$; $P = 0.001$). The varieties Zoloto Ukrainy (up to 7.0%) and Kalancha (up to 7.0%) do not differ significantly from the second group of varieties more resistant to the mutagen ($F = 3.92$; $F_{0.05} = 4.99$; $P = 0.07$).

As for the varieties that were more resistant in terms of mutagenic depression, their total variability under the action of the maximum concentration of the mutagen was 6.6-7.8%, while the group was significantly more homogeneous, no statistical differences were found between the varieties ($F = 2.34$; $F_{0.05} = 3.86$; $P = 0.09$), but an increase in the concentration of the mutagen led to statistically significant increase in the general rate of mutations ($F = 23.17$; $F_{0.05} = 3.86$; $P = 0.004$).

Table 1. General rate of mutations cases and families at second - third generations. First group (more sensitive to mutagen action) (x ± SD, n = 500)

Variety	Number of selecting families	Number of mutant families	Rate of mutations, %
Balaton	500	2	0.40 ± 0.09^{a}
Balaton, DAB 0.1%	500	23	4.60 ± 0.14^{b}
Balaton, DAB 0.2%	500	32	6.40± 0.23^{c}
Balaton, DAB 0.3%	400	41	8.20±0.29^{d}
Zoloto Ukrainy	500	6	1.20 ± 0.24^{a}
Zoloto Ukrainy, DAB 0.1%	500	17	3.40± 0.28^{b}
Zoloto Ukrainy, DAB 0.2%	500	25	5.00±0.41^{c}
Zoloto Ukrainy, DAB 0.3%	500	35	7.00±0.44^{d}
Kalancha	500	5	1.00 ± 0.20^{a}
Kalancha, DAB 0.1%	500	20	4.00±0.16^{b}
Kalancha, DAB 0.2%	500	29	5.80±0.21^{c}
Kalancha, DAB 0.3%	500	35	7.00±0.35^{d}
Niva Odeska	500	3	0.60 ± 0.18^{a}
Niva Odeska, DAB 0.1%	500	25	5.00±0.22^{b}
Niva Odeska, DAB 0.2%	500	39	7.80±0.26^{c}
Niva Odeska, DAB 0.3%	500	58	11.60±0.35^{d}

Note: indicate significant differences at $P < 0.05$ by ANOVA-analyse with Bonferroni amendment. Comparison in terms of one variety

At the same time, in all cases, the differences between the effects of individual concentrations within the same variety in all variants, including in contrast to the control. In general, the variability of this group is somewhat lower.

In general, all varieties belong to stable genotypes and the level of spontaneous variability is low, moreover, as for modern varieties, for which a significantly lower genome stability is noted, it is even low.

The cluster analysis carried out by the total mutation frequency showed (Figure 1) that, in general, varieties are quite clearly divided into three groups according to the genotype-mutagenic interaction.

At the same time, the first group includes such varieties as Balaton, Borovytsia, Kalancha, Zeleny Gai, that is, varieties belonging to both the first and second groups, which are differentiated by the strength of mutagenic depression in the first generation.

Table 2. General rate of mutations cases and families at second - third generations. Second group (more tolerance to mutagen action) (x ± SD, n = 500)

Variety	Number of selecting families	Number of mutant families	Rate of mutations, %
Borovytsia	500	4	0.80 ± 0.08^{a}
Borovytsia, DAB 0.1%	500	21	4.20±0.25^{b}
Borovytsia, DAB 0.2%	500	28	5.60±0.31^{c}
Borovytsia, DAB 0.3%	500	37	7.40±0.34^{d}
Zeleny Gai	500	3	0.60 ± 0.06^{a}
Zeleny Gai, DAB 0.1%	500	22	4.40±0.22^{b}
Zeleny Gai, DAB 0.2%	500	30	6.00±0.31^{c}
Zeleny Gai, DAB 0.3%	500	39	7.80±0.39^{d}
Polyanka	500	2	0.40 ± 0.12^{a}
Polyanka, DAB 0.1%	500	18	3.60±0.25^{b}
Polyanka, DAB 0.2%	500	25	5.00±0.30^{c}
Polyanka, DAB 0.3%	500	34	6.80±0.42^{d}
Pochayna	500	2	0.40 ± 0.14^{a}
Pochayna, DAB 0.1%	500	19	3.80±0.31^{b}
Pochayna, DAB 0.2%	500	26	5.20±0.30^{c}
Pochayna, DAB 0.3%	500	33	6.60±0.41^{d}

Note: indicate significant differences at $P < 0.05$ by ANOVA-analyse with Bonferroni amendment. Comparison in terms of one variety

Thus, it is confirmed that in our case (with the action of DAB) a higher mutation rate in subsequent generations will not necessarily be a consequence of higher depression.

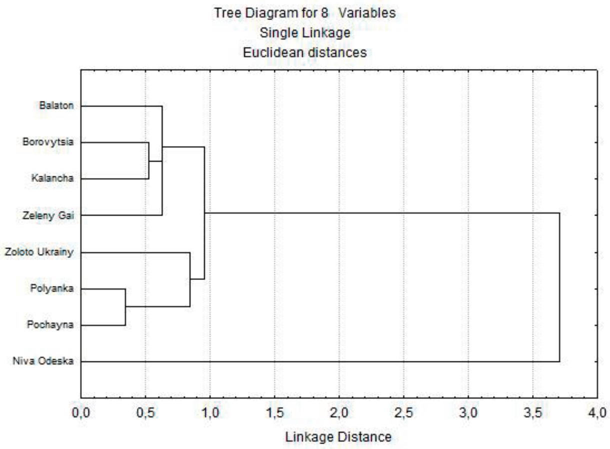

Figure 1. Results of cluster analysis by general mutation rate

The second group included the genotypes of the varieties Zoloto Ukrainy, Polyanka, Pochayna, which also belong to different groups in terms of the manifestation of depression and fell into one group in cluster analysis due to a significantly lower total frequency at DAB concentrations of 0.1% and DAB 0.2%, without taking into account the rate at maximum concentration. Separately, the variety Niva Odeska stood out in the third minor group due to its extremely high responsiveness to the action of this mutagen. The highest level of depression was also noted in the same variety.

Perhaps a somewhat more detailed picture will be provided by the level of variability, which, in addition to the total number of altered families, also takes into account the number of characters for which these changes have passed, i.e., integratively evaluates the picture taking into account the spectrum. It should be noted that, in general, as a result of the action of this mutagen, the number of such features is significantly less than for other chemical supermutagens (Table 3 for the first group of varieties and Table 4 for the second group, the cluster analysis data for this parameter are presented in Figure 2).

Table 3. Level of changeability, caused by mutation variability. First group (x ± SD, n = 500)

Variant	Level of variability	Changed traits
Balaton	0.01±0.01^{a}	2
Balaton, DAB 0.1%	0.78±0.10^{b}	17
Balaton, DAB 0.2%	1.34±0.07c	21
Balaton, DAB 0.3%	1.56±0.09^{c}	19
Zoloto Ukrainy	0.07±0.02^{a}	6
Zoloto Ukrainy, DAB 0.1%	0.44 ±0.13^{b}	13
Zoloto Ukrainy, DAB 0.2%	0.80±0.15^{c}	16
Zoloto Ukrainy, DAB 0.3%	1.54 ±0.17^{d}	22
Kalancha	0.05±0.01^{a}	5
Kalancha, DAB 0.1%	0.60±0.09^{b}	15
Kalancha, DAB 0.2%	1,22 ±0.15^{c}	21
Kalancha, DAB 0.3%	1.47±0.16^{c}	21
Niva Odeska	0.02±0.01^{a}	3
Niva Odeska, DAB 0.1%	0.70±0.05^{b}	14
Niva Odeska, DAB 0.2%	1.40±0.13^{c}	18
Niva Odeska, DAB 0.3%	2.44±0.16^{d}	21

Note: indicate significant differences at $P < 0.05$ by ANOVA-analyse with Bonferroni amendment. Comparison in terms of one variety

It is the first of the tables that shows that by no means always, due to a decrease or remaining at the same level in the number of signs for which changes have taken place, the action of the subsequent concentration differs from the previous one. In two cases, for variety Balaton, DAB 0.3% and variety Kalancha, DAB 0.3%, a higher level of variability a higher level of variability is not statistically significantly different from DAB 0.2% ($F = 3.02$; $F_{0.05} = 5.17$; $P = 0.10$).

At the same time, in general, an increase in the concentration of the mutagen had a statistically significant effect on the increase in the level of variability ($F = 45.15$; $F_{0.05} = 3.86$; $P = 1.17 * 10^{-4}$), while at the same time, the differences in the genotype were generally not significant ($F = 4.04$; $F_{0.05} = 4.11$; $P = 0.06$), and in general, the genotypes of Balaton, Zoloto Ukrainy, Kalancha did not differ significantly within the group in pairwise comparison (the level of variability was about the mark 1.5 differed from the variety Niva Odeska ($F = 8.17$; $F_{0.05} = 5.43$; $P = 0.04$), the level of variability up to 2.44, the parameter under the action of DAB 0.2% already corresponds to the action of DAB 0.3% in other varieties.

As for the second group of varieties (Table 4), here again the level of variability depends on the concentration of the mutagen ($F = 32.13$; $F_{0.05} = 3.86$; $P = 2.03 * 10^{-3}$), while there is no difference in genotype ($F = 2.17$; $F_{0.05} = 4.11$; $P = 0.10$), it makes no sense to compare both groups, due to their heterogeneity. In general, the level of variability in all varieties, except for the Niva Odeska variety, is the same.

Table 4. Level of changeability, caused by mutation variability. Second group (x ± SD, n = 500)

Borovytsia	Level of variability	Changed traits
Borovytsia	0.03±0.01^{a}	
Borovytsia, DAB 0.1%	0.59±0.05^{b}	4
Borovytsia, DAB 0.2%	0.95±0.07^{c}	14
Borovytsia, DAB 0.3%	1.41±0.19^{d}	17
Zeleny Gai	0.02±0.01^{a}	19
Zeleny Gai, DAB 0.1%	0.66±0.10^{b}	3
Zeleny Gai, DAB 0.2%	0.96±0.14^{c}	15
Zeleny Gai, DAB 0.3%	1.56±0.19^{d}	16
Polyanka	0.01±0.01^{a}	2
Polyanka, DAB 0.1%	0.40±0.05^{b}	11
Polyanka, DAB 0.2%	0.75±0.11^{c}	15
Polyanka, DAB 0.3%	1.43±0.19^{d}	21
Pochayna	0.01±0.01^{a}	2
Pochayna, DAB 0.1%	0.49±0.08^{b}	13
Pochayna, DAB 0.2%	0.94±0.12^{c}	18
Pochayna, DAB 0.3%	1.25±0.19^{c}	19

Note: indicate significant differences at $P < 0.05$ by ANOVA-analyse with Bonferroni amendment. Comparison in terms of one variety

For the level of variability, in turn, cluster analysis showed a division already into 4 groups (Figure 2). At the same time, in this case too, a minor group with the variety Niva Odeska was also distinguished, as in the analysis by the total frequency of mutations, however, for other varieties, the picture was already somewhat different.

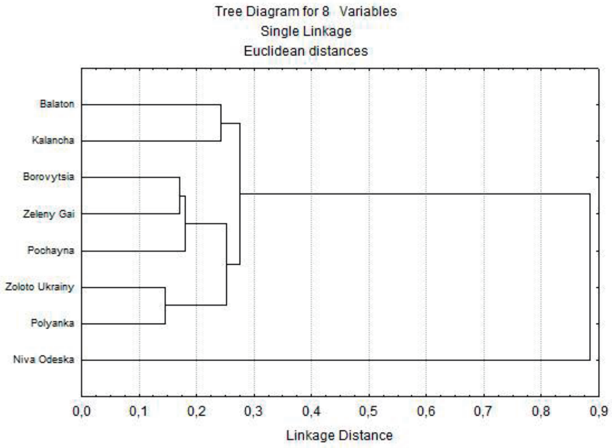

Figure 2. Results of cluster analysis by level of variability

Thus, the varieties Balaton and Kalancha belonged to the first group, which apparently differed in the nature of the transition from the concentration of 0.2 to 0.3; there are no other variants. Varieties Borovytsia, Zeleny Gai, Pochayna belong to the second group, varieties Zoloto Ukrainy and Polyanka belong to the third group. Differences between groups are in the dynamics of increasing variability depending on the growth of mutagen concentration.

In the spectrum of changes in traits, only 33 were noted for which hereditary changes were recorded during visual observation, structural analysis or through pattern analysis.

In accordance with common practice, they were classified into 6 groups. Factorial (Tables 5 and 6) and discriminant analyzes (Tables 5 and 7) were performed to determine the significance of individual groups, as well as such parameters as the overall mutation rate and the level of variability and their dependence on factors, mutagen concentration and genotype of the starting material.

The first group includes changes in the structure of the plant shoot. Groupe includes the following traits of thick stem, thin stem, high stem, short stem, semidwarf, weak waxy bloom and the presence of a waxy bloom. The number and variety of mutations in this group as a whole increase with increasing mutagen concentration. At the same time, mutations with a high stem (up to 1%) are the most frequent in this group, and low-stem forms can also be quite probable. The rest of the mutations are single at best, there are no sharp, extreme variants - for example, there are no forms with a pronounced waxy bloom or dwarf forms, which are quite typical for chemical mutagenesis, especially at high concentrations.

The second group includes changes in the shape and size of the grain. In total, three traits were taken into account, the most common being mutations with small grains. However, even this type of change cannot be considered regular and its significance for the mutation process is small (up to 0.6% in the maximum variant, but no more than 0.15% on average).

The third group is the largest and includes mutations to change the structure of the ear, its color and its parts (except for systemic mutations, which are included in the fifth group). In total, the group includes 15 different traits, however, such changes as a long spike (up to 0.6% maximum, up to 0.19 on average, but regularly according to variants, unlike the second group), a dense spike, a large spike, and a semi-awned spike (also up to 0.6%, on average the rate is less, but they are regular) are significant for the mutation process. Their rate somewhat increases depending on the increase in the concentration of the mutagen, but more so on the genotype of the source material, primarily for semi-awned forms.

The fourth group included 4 traits, changes in which are associated with the physiological processes of growth and development in plants. These are such signs as sterility, earlymaturing, latematuring, resistance to diseases. More frequent and regular for all variants are such mutations as early- and latematuring (up to 1% on average 0.39 and 0.36%, respectively). A higher frequency (up to 1.2%) is for the sterility sign, however, it is associated only with variants with a high concentration, which makes it less regular. Thus, the group is characteristic of the mutation process, but determines more the concentration than the genotype of the initial material.

The next group is usually the least applicable from a practical point of view, but the most interesting from the point of view of modeling the mutation process and genetic control. These are called systemic mutations, in which the phenotype of a cultivated plant changes so dramatically that it is more characteristic of the phenotype of wild relatives. However, for this mutagen, only a speltoid-type mutation is more or less regular (up to 0.6%). There are also some number of squareheads, but only at high concentrations. That is, mutations of this type are not typical for this mutagen.

Table 5. Results of discriminant analyze

Variables at model	Wilks Lambda λ	Partial Lambda	F-remove (4.02)	p-level
Mutation rate	0.13	0.84	18.12	0.01
Level of variability	0.14	0.77	16.02	0.01
First group	0.19	0.65	6.17	0.02
Second group	0,52	0.34	1,32	0.17
Third group	0,30	0.50	3,99	0,06
Fourth group	0.09	0.82	19.24	0.01
Fifth group	0.39	0.21	1.99	0.15
Sixth group	0.14	0.75	8.87	0.03

The sixth group includes the most interesting forms from an economic point of view, which are more likely to be used directly as varieties in the future. These are more productive and bushy families, which were re-selected for the same trait in the next generation, i.e., confirmed their inheritance. For this mutagen, the number of such forms is small; however, they are regular, which is not usually seen.

Regularity and patterning for individual parameters were established through discriminant analysis (Table 5). At the same time, the mutation frequency, the level of variability, mutations of the first, fourth and, which is especially interesting, the sixth group were included in the model. This is usually uncharacteristic of mutagens.

At the same time, during the factor analysis, it was established (Table 6) that the factor of the genotype of the initial material was significant for the level of variability, the first and third groups of mutations. The concentration factor was significant for the frequency of mutations and the level of variability, the first, third, fourth, sixth groups. Thus, the effect of this mutagen is less dependent on the genotype of the source material and is less site specific. The results of discriminant analysis and factor analysis are consistent and complementary, it is interesting to confirm the reliable confirmation of the fact that this mutagen is effective in obtaining a stable number of selection-valuable mutations with no dependence on the genotype of the source material.

Table 6. Factor loadings (varimax raw)

Parameter	Genotype	Concentration
Mutation rate	0.414144	**0.873611**
Level of variability	**0.593224**	**0.815111**
First group	**0.522511**	**0.756111**
Second group	0.257280	0.132129
Third group	**0.611711**	**0.511413**
Fourth group	0.321713	**0.811412**
Fifth group	0.220193	0.317279
Sixth group	0.231994	**-0.678117**
Expl.Var	1.335517	2.247220
Prp.Totl	1.773271	1.213119

Note: statistically significance in bold

The classification of genotypes in the factor space showed that the most significant genotype-mutagenic interaction was for the Niva Odeska variety (Table 7). The varieties Balaton and Kalancha are also determined with rather high reliability. Other varieties showed themselves to a much weaker degree.

Table 7. Classification matrics - canonical roots

Genotype	Percent of classification
Balaton	87.5
Zoloto Ukrainy	75.0
Kalancha	87.5
Niva Odeska	100.0
Borovytsia	75.0
Zeleny Gai	75.0
Polyanka	75.0
Pochayna	75.0
Total	81.3

This mutagen has a significantly lower damaging ability than other previously studied types of chemicals supermutagens (OlaOlorun et al., 2021), which led to both a lower overall mutation rate and a lower level of variability (Nazarenko, 2017; Di Pane et al., 2018).

However, this mutagen showed that, firstly, it is significantly less dependent in its initiation of mutations on the source material, at least for this set of varieties (Horshchar & Nazarenko, 2022).

This mutagen can be valuable, firstly, due to the model character and regularity of obtaining economically valuable families in the mutation process, which we discovered for the first time (OlaOlorun et al., 2020). It is believed that modified families with stable traits in inheritance are the most likely to be successful in the future directly as commercial varieties (Nazarenko & Lykholat, 2018; Nazarenko et al., 2020).

Secondly, this mutagen is quite successful in inducing mutations in terms of ripeness, and it may be interesting to further study the processes of photosynthesis and remobilization in these mutant plants (Henry, 2019; Balkan et al., 2021). As a number of studies have shown, quite often mutations under the action of chemical supermutagens led to the appearance of forms with a different version of the implementation of photosynthetic activity (Nazarenko et al., 2022).

In turn, the presence of a significant number of early-ripening lines is also significant for our region, since the presence of a certain part of such varieties in the structure of sown areas for winter wheat can significantly increase the resistance in grain production to May droughts (Aly et al., 2018; Nazarenko et al., 2022).

CONCLUSIONS

For the success of any modern program for the improvement of cultivated plants through mutagenesis, the problem of the compatibility of this mutagen with the starting material in principle is of key importance, which makes it possible to increase its efficiency up to 60-80% and the problem of achieving the regular appearance of a significant number of the required forms.

Unfortunately, given rather significant number of families in the second generation, it is more promising to work with those forms that have already shown any changes in the phenotype. A different approach leads only to a significant multiple increase in costs. At the same time, there is often a connection with the effects of mutagenic depression, activity at the cellular level, and mutation frequency, which also needs to be taken into account.

Further studies to obtain the necessary confirmation of the mutant nature of the obtained material are already focused on the study of possible micro-changes in terms of changes in photosynthetic activity, drought and winter resistance, micro-changes in grain quality and the ratio of protein-gluten complex components. Thus, in the future, a comprehensive biochemical analysis is planned. Also not the last issue are the possibilities for the accumulation of useful microelements and the peculiarities of the metabolism of heavy metals (which is of particular importance for areas under significant anthropogenic load).

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