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WINTER WHEAT VARIABILITY UNDER ETHYLMETHANSULFONATE ACTION

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Abstract

The object of research was the hereditary variability of a group of genotypes, selected in order to maximally characterize the existing biological diversity of winter wheat cultivated in Ukraine. Winter wheat dry seeds of eight varieties were acted with water (control) and EMS (ethylmethansulfonate) action in concentrations of 0.025%, 0.05%, 0.1%. An extremely high general mutation rates and variability in the spectra of changes was observed. The level of variability showed that the concentration of 0.05% was more promising in terms of the number of altered traits; it was also successful in inducing predominantly agronomical-valuable traits. (earliness, forms with long well-grained spike, short stem, semidwarf, with high photosynthetic ability, lines with high grain productive. New promising lines were identified with improved plant architecture, optimal ratio between grain and vegetative part of the plant (index of economic value). A further increase in the concentration of the mutagen to 0.1% only led to a decrease in variability and a smaller number of valuable forms. However, it can be used to obtain dwarf forms and forms with systemic changes in the spike.

Key words*: winter wheat, chemical mutagenesis, ethylmethansulfonate, mutation, plant improvement.*

INTRODUCTION

Winter wheat is a key food crop for Ukraine and the world (Andrusevich et al., 2018). Taking into account the problems of climate change, gradual migration and advancement of crops to the south in areas that previously did not guarantee stable high yields, the genetic improvement of this crop acquires a new strategic importance (Beiko and Nazarenko, 2022).

One of the options for stable improvement is induced biodiversity through the use of appropriate mutagenic factors (Yakymchuk et al., 2021; Shabani et al., 2022).

The utilization of factors that induce genetic diversity is associated with the effect of the so-called mutagenic depression (Nazarenko et al., 2022), which leads to significant problems with increasing the dose or concentration of this mutant agent, given the need to obtain a sufficient amount of viable fertile material for research (OlaOlorun et al., 2020).

One of the main ways of solving this problem are the use of less harmful agents (so-called supermutagens), which, with an increase in mutational activity in 20-60 times (Mangi et al., 2021), do not exceed more traditional factors such as gamma-rays or fast neutrons in terms of the level of physiological or genetical damages (Spencer-Lopes et al., 2018).

The use of site-specific mutagenesis with the establishment of the mechanism of specific factor nature action makes it possible to obtain new changes in forms with stable properties and traits useful from a breeding or genetic point of view (Nazarenko et al., 2020), in particular, valuable changes in the biochemical structure, in a short period (Nazarenko and Lykholat, 2018).

Chemical factors show significant site-specificity, relationship to certain areas of the hereditary substance, which leads to the predominant induction of only certain types of traits (Yali and Mitiku, 2022).

The investigation of the variety-specific activity of mutagens is carried out from the first generation, since the influence on the indicators of growth and development (Nazarenko et al., 2020), the formation of the plant organism occurs precisely at this stage (Ram et al., 2019).

The use of chemical mutagenesis to induce genetic diversity leads mainly to small changes in key traits, unlike physical mutagens, the changes are complex, and the lethal effects are significantly low (Shabani et al., 2022).

More useful in modern research is the possibility to get large samples of material with low damages without the use of substances with high

continuous activity (Beiko and Nazarenko, 2022), but for some agents the tendency may be the opposite, especially in the studies of mechanisms of some value traits genetic control (Anter, 2021).

The key is the process of interaction between the genotype of the variety and nature/concentration of mutagen, taking into account the threshold action of agent concentrations with lower damaging activity and site-specific capacity. Not only a decrease in the number of valuable changes may not occur, but they can be more likely if the optimal combinations of concentration, the nature of mutagen and the genotype of the initial form (le Roux et al., 2021).

The main purpose of our experiments was to characterize the genetic and phenotypic variability of winter wheat families after mutagen action with identification stable lines by key traits, evaluation of role genotype and mutagen interactions for new complex changes and new type of mutations. The first target was relations between initial material and nature of chemical substance, its concentrations. Second our aim was to identify mutagen effect and mutant lines suitability for future winter wheat improvement.

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 EMS (ethylmethansulfonate) 0.025%, 0.05%, 0.1% (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 (Shu et al., 2013; 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).

Sowing material were grown in rows with inter and intra-row spacing of 50 and 30 cm, respectively, for first generation of families. The control was sample of non-treated grains of parent varieties and one were also grown after ten rows for each variant as comparison with the first-generation plants and next generations families. Mutant lines and groups rows were planted at three replications with control-rows of parent variety for each twenty-row plot (Mangi et al., 2021).

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 2017-2022 years.

Statistic analyze of data was performed by ANOVA-analysis, grouping and estimation of data was provided by discriminant and cluster analysis (Euclidian distance, single linkage) (Statistic 10.0, multivariant module, TIBCO, Palo Alto, USA). The normality of the data distribution was examined using the Shapiro–Wilk W-test. Differences between samples were assessed by Tukey HSD test.

RESULTS AND DISCUSSIONS

In total, for control and material after mutagen action 15800 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. The

exception is the extreme concentration (EMS 0.1%), for some more susceptible varieties as Balaton, Niva Odeska, the sample can be 400 families.

Data about the general mutation rate for each variety and variant are presented at Tables 1 and 2 in such a way that the more susceptible varieties (subsequently it will be shown by grouping by clusters) were at Table 1, and significantly less variable at Table 2.

Table 1. General rate of mutations cases and families at second - third generations. First group (more sensitive to mutagen action) (x ± SD, n = 400-500)

Variety	Number of selecting families	Number of mutant families	Rate of mutations, %
Balaton	500	2	0.40 ± 0.10^{a}
Balaton, EMS 0.025%	500	34	6.80 ± 0.32^{b}
Balaton, EMS 0.05%	500	45	9.00 ± 0.45^{c}
Balaton, EMS 0.1%	400	60	15.00 ± 0.60^{d}
Zoloto Ukrainy	500	6	1.20 ± 0.24^{a}
Zoloto Ukrainy, EMS 0.025%	500	38	7.60 ± 0.52^{b}
Zoloto Ukrainy, EMS 0.05%	500	55	11.00 ± 0.80^{c}
Zoloto Ukrainy, EMS 0.1%	500	71	14.20 ± 0.90^{d}
Kalancha	500	5	1.00 ± 0.20^{a}
Kalancha, EMS 0.025%	500	31	6.20 ± 0.30^{b}
Kalancha, EMS 0.05%	500	41	8.20 ± 0.35^{c}
Kalancha, EMS 0.1%	500	65	13.00 ± 0.50^{d}
Niva Odeska	500	3	0.60 ± 0.18^{a}
Niva Odeska, EMS 0.025%	500	35	7.00 ± 0.35^{b}
Niva Odeska, EMS 0.05%	500	48	9.60 ± 0.48^{c}
Niva Odeska, EMS 0.1%	400	64	16.00 ± 0.75^{d}

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

As can be seen (in addition), the more variable varieties were also among those that showed higher depressive effects on growth and development in the first generation. There were varieties Balaton (general rate up to 15%), Zoloto Ukrainy (up to 14.2%), Kalancha (up to 13%), Niva Odeska (up to 16%).

As a result of factor analysis, a statistically significant difference from the varieties of the second group was established ($F = 12.17$; $F_{0.05} = 5.16$; $P = 0.01$), frequencies at the highest concentration vary from 13 to 16%, while in Table 2 the general rate of mutations at the same concentration was for the genotypes of the second group for the varieties Borovytsia (10.2%), Zeleny Gai (9.6%), Polyanka (8.8%) Pochayna (9.2%), that is at the level of 8.8-10.2%, which, firstly, is significantly lower than that for the varieties of the first group, and secondly, the variability within the group is also much lower.

Table 2. General rate of mutations cases and families at second - third generations. First group (more tolerance by genetic activity) (x ± SD, n = 400-500)

Variety	Number of selecting families	Number of mutant families	Rate of mutations, %
Borovytsia	500	4	0.80 ± 0.08^{a}
Borovytsia, EMS 0.025%	500	28	5.60 ± 0.30^{b}
Borovytsia, EMS 0.05%	500	37	7.40 ± 0.45^{c}
Borovytsia, EMS 0.1%	500	51	10.20 ± 0.68^{d}
Zeleny Gai	500	3	0.60 ± 0.06^{a}
Zeleny Gai, EMS 0.025%	500	27	5.40 ± 0.30^{b}
Zeleny Gai, EMS 0.05%	500	36	7.20 ± 0.45^{c}
Zeleny Gai, EMS 0.1%	500	48	9.60 ± 0.50^{d}
Polyanka	500	2	0.40 ± 0.12^{a}
Polyanka, EMS 0.025%	500	23	4.60 ± 0.40^{b}
Polyanka, EMS 0.05%	500	33	6.60 ± 0.50^{c}
Polyanka, EMS 0.1%	500	44	8.80 ± 0.55^{d}
Pochayna	500	2	0.40 ± 0.14^{a}
Pochayna, EMS 0.025%	500	24	4.80 ± 0.35^{b}
Pochayna, EMS 0.05%	500	32	6.40 ± 0.40^{c}
Pochayna, EMS 0.1%	500	46	9.20 ± 0.55^{d}

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

However, for both groups mutagen action was statistically significant both for the variance in the change in mutagen concentration ($F = 127.23$; $F_{0.05} = 3.86$; $P = 2.17*10^{-9}$) and for individual genotypes ($F = 55.16$; $F_{0.05} = 3.86$; $P = 1.19*10^{-5}$).

At all cases, the differences are statistically significant for all concentrations in all varieties, regardless of the group, both in relation to the control and in relation to the effect of the previous concentration (Tables 1 and 2, respectively).

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 two groups according to the genotype-mutagenic interaction.

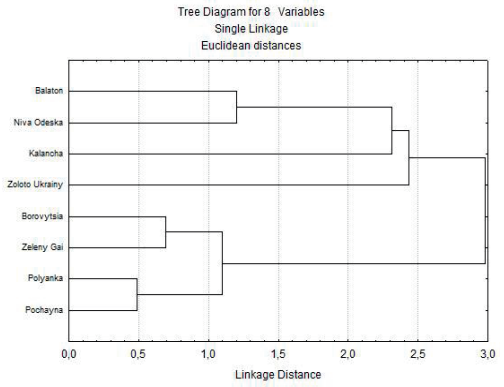

Figure 1. Results of cluster analysis by general mutation rate

Thus, at first group are more susceptible to EMS action varieties Balaton, Kalancha, Niva Odeska; at second group varieties Borovytsia, Zeleny Gai, Polyanka, Pochayna. Variety Zoloto Ukrainy stood out a little apart, which, although it belonged to the first group in the analysis of the date from tables, stood out in a special minor group after the cluster analysis. Apparently, this is due to the dynamics of the change in general rate depending on the concentration of the mutagen; there can be no other reasons.

More interesting was the investigation by the integrative parameter of the level of variability, which takes into account not only the general rate of variability under the mutagenic factor action, but also the width of the spectrum of changes, i.e., the number of traits that undergo changes under the action of a given concentration (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).

Based on the presented data, we find that there are also statistically significant changes with each concentration ($F = 179.13$; $F_{0.05} = 3.86$; $P = 3.02*10^{-12}$) and depending on the genotype of the initial material ($F = 73.18$; $F_{0.05} = 3.86$; $P = 2.07*10^{-7}$), we also again find differences between the two groups of varieties ($F = 18.34$; $F_{0.05} = 5.16$; $P = 0.002$).

At the same time, for the first group, the level of variability was at the highest concentration from 3.77 (сорт Kalancha) to 5.12 (сорт Niva Odeska), for second (more resistance group for action) group from 2.11 (variety Polyanka) to 2.69 (variety Zeleny Gai).

Table 3. Level of changeability, caused by mutation variability. First group (x ± SD, n = 400-500)

Variant	Level of variability	Changed traits
Balaton	0.01±0.01[a]	2
Balaton, EMS 0.025%	1.43±0.11[b]	21
Balaton, EMS 0.05%	2.34±0.23c	26
Balaton, EMS 0.1%	4.50±0.25[d]	30
Zoloto Ukrainy	0.07±0.02[a]	6
Zoloto Ukrainy, EMS 0.025%	1.37±0.14[b]	18
Zoloto Ukrainy, EMS 0.05%	2.86±0.22[c]	26
Zoloto Ukrainy, EMS 0,.1%	4.12±0.29[d]	29
Kalancha	0.05±0.01[a]	5
Kalancha, EMS 0.025%	1.12±0.09[b]	18
Kalancha, EMS 0.05%	1.89±0.21[c]	23
Kalancha, EMS 0.1%	3.77±0.27[d]	29
Niva Odeska	0.02±0.01[a]	3
Niva Odeska, EMS 0.025%	1.61±0.11[b]	23
Niva Odeska, EMS 0.05%	2.69±0.23[c]	28
Niva Odeska, EMS 0.1%	5.12±0.31[d]	32

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

Table 4. Level of changeability, caused by mutation variability. Second group (x ± SD, n = 400-500)

Variant	Level of variability	Changed traits
Borovytsia	0.03±0.01[a]	4
Borovytsia, EMS 0.025%	0.95±0.08[b]	17
Borovytsia, EMS 0.05%	1.63±0.17[c]	22
Borovytsia, EMS 0.1%	2.65±0.25[d]	26
Zeleny Gai	0.02±0.01[a]	3
Zeleny Gai, EMS 0.025%	1.03±0.11[b]	19
Zeleny Gai, EMS 0.05%	1.66±0.18[c]	23
Zeleny Gai, EMS 0.1%	2.69±0.22[d]	28
Polyanka	0.01±0.01[a]	2
Polyanka, EMS 0.025%	0.64±0.07[b]	18
Polyanka, EMS 0.05%	1.25±0.17[c]	23
Polyanka, EMS 0.1%	2.11±0.21[d]	29
Pochayna	0.01±0.01[a]	2
Pochayna, EMS 0.025%	0.91±0.09[b]	14
Pochayna, EMS 0.05%	1.41±0.12[c]	19
Pochayna, EMS 0.1%	2.39±0.19[d]	24

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

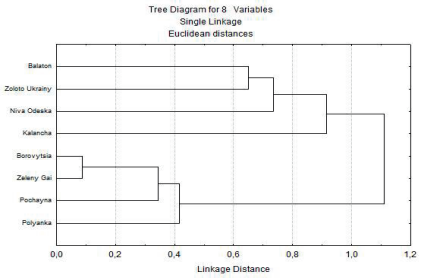

Figure 2. Results of cluster analysis by level of variability

Again, it finds that the variability within the second group is significantly lower, but the differences between the groups are very significant even at the initial assessment. Cluster analysis in this case showed a clearer division into two groups (Figure 1) without the presence of any minor options, while the factor space of the analysis is significantly smaller than in the first case. Thus, it can be considered mathematically justified that the estimate is more accurate in terms of the level of variability than in terms of the total rate of mutations.

In the spectrum of changes obtained, the traits were divided into 6 groups in accordance with the generally accepted classification. A classification analysis was also carried out both for individual characteristics of the mutation process (Tables 5 and 6) and for genotypes (varieties) (Table 7).

Table 5. Results of discriminant analyze

Variables at model	Wilks Lambda λ	Partial Lambda	F-remove (4,02)	p-level
Mutation rate	0.11	0.79	17.23	0.01
Level of variability	0.07	0.92	21.14	0.01
First group	0.14	0.70	8.92	0.01
Second group	0,57	0,31	1,99	0.13
Third group	0,32	0,55	3,69	0,06
Fourth group	0.11	0.78	16.17	0.01
Fifth group	0.19	0.61	5.14	0.03
Sixth group	0,39	0,47	2,93	0,09

The first group of mutations in plant architecture. These include the following signs of thick stem, thin stem, high stem, short stalk, semidwarf, dwarf, intensive waxy bloom, weak waxy bloom and the presence of a waxy bloom. On the whole, in the group, the most highly probable for EMS action are such forms as high stem, semidwarfs (at high concentrations) and forms with a weak waxy bloom. As can be seen, less pronounced mutations are more characteristic, with the exception of semi-dwarfs (but the latter is due to the fact that the initial material was predominantly short). The highest (up to 1.5%) probability of the appearance of high forms, which are present in almost every variant.

The second group includes changes in wheat grain. Such traits as barrel-shaped grain, coarse grain, fine grain was revealed. Only the large grain mutation occurs more or less often (and its probability increases slightly with increasing concentration in some varieties), other mutations are rare. The group is difficult to identify and has little weight in the factor space.

The third group includes changes in the spike structure (the most numerous, 15 different traits). These changes tend to occur more frequently as the concentration increases. Some varieties are characterized by the presence of a greater number of such changes as anthocyanin awns and a double spike. The mutation of the spike from awn spike to awnless form (almost three times) is more frequent than from awnless spike to with awn. Transitional forms (semi-awn spike) occur at the level of the second variant.

The fourth group (changes in the physiology of growth and development) is the most variable. Only 4 traits as sterility, early-maturing, late-maturing, disease tolerance. More frequent (for all variants) are changes in early maturity and disease tolerance. At the same time, for some variants, especially with an increase in concentration, late maturing may become. Sterility is more typical for high concentrations and practically does not occur at low concentrations. In general, all traits for this group were in model.

The fifth group includes systemic mutations that lead to extremely significant changes in the spike structure, going beyond the cultural form and leading to the phenotype of wild relatives. Such changes are most probable under the action of high concentrations of the mutagen and practically do not occur at low ones. More likely is the appearance of squarehead, which can form even at low concentrations. Other mutations are quite rare.

The sixth group consists of valuable forms by grain productivity and tillering. It occurs in most varieties, except for some varieties at a concentration of EMS 0.1%.

Modeling of individual parameters by groups was established for the mutation process in the course of discriminant analysis (Table 5). The model was the frequency, the level of variability, mutations in the first, fourth, fifth group.

Thus, it is possible to reliably predict for a given mutagen on a given material how to obtain sources for breeding for early maturity, disease resistance, plant height, but it is difficult to find

productive forms. As for the significance for the implementation of one or another trait depending on the genotype and concentration of the mutagen (Table 6), the value of the mutagen concentration is still of great importance, but only in the case of the last group there was no influence of the genotype.

Table 6. Factor loadings (varimax raw)

Parameter	Genotype	Concentration
Mutation rate	**0.616148**	**0.923626**
Level of variability	**0.783614**	**0.875313**
First group	**0.622911**	**0.776561**
Second group	0.357780	0.322229
Third group	**0.766511**	**0.728113**
Fourth group	**0.674513**	**0.796612**
Fifth group	**0.550213**	**0.901179**
Sixth group	0.321734	**-0.448767**
Expl.Var	2.935617	3.347610
Prp.Totl	0.453231	0.379119

Note: statistically significance in bold

Significantly, both the genotype of the initial material and the concentration of the mutagen affected on the mutation rate, the level of variability and the frequencies for the first and third-fifth groups of mutations, which somewhat contradicts the results of discriminant analysis, supplementing it with new significant parameters (the third group for mutations).

The classification by varieties in factor space showed that all genotypes are reliably identified by the obtained parameters, Zoloto Ukrainy variety is least of all. In general, varieties more susceptible to the mutagen are better classified (Table 7).

Table 7. Classification matrics - canonical roots

Genotype	Percent of classification
Balaton	100.0
Zoloto Ukrainy	75.0
Kalancha	100.0
Niva Odeska	100.0
Borovytsia	87.5
Zeleny Gai	100.0
Polyanka	87.5
Pochayna	87.5
Total	92.2

The obtained results make it possible to evaluate the possibilities of chemical mutagenesis and the use of highly active substances in terms of the induction of genetic changes to obtain new forms (Mangi et al., 2021; Hassine et al., 2022) or making adjustments to the initial material (variety or line), which generally meets to modern requirements, but needs to be improved for some parameters (Abaza et al., 2020).

This mutagen is quite actively used in practice to obtain new genetically- and breeding-value forms. (OlaOlorun et al., 2021), primarily due to the high variability in terms of changes in plant structure (plant architecture) (OlaOlorun et al., 2020; le Roux et al., 2021), the formation of plants that better meet to modern requirements for intensive varieties (Ram et al., 2019) through the transformation of local semi-intensive, but better adapted forms (under our conditions, local varieties of national breeding) (le Roux et al., 2021).

On the other hand, the investigated mutagen on this material showed a slightly different range of changes than in the case of foreign scientific programs (OlaOlorun et al., 2021). If the general mutation rate is quite significantly higher, primarily for the group of genotypes identified as less resistant to a factor of this nature, then the proportion of beneficial changes is less significant than expected (Hassine et al., 2022; Nazarenko et al., 2022). Perhaps, more thorough investigations of grain quality and various types of tolerance to unfavorable environmental factors for mutant lines of older generations will significantly correct the results (Chaudhary et al., 2019; Hong et al., 2022).

It should also be noted that there is a rather strong relationship between the frequency of chromosomal aberrations obtained for this material and the frequency of visually determined mutations. Although this effect has been noted in the past, but not always (Yakymchuk et al., 2021; Yali and Mitiku, 2022).

The use of integrative indicators, which, in addition to characterizing the total number of mutant cases, also includes the width of the spectrum of change by the number of individual traits affected by the genetic activity of the factor, was previously noted by us as more promising (Ram et al., 2019; Shabani et al., 2022; Nazarenko et al., 2022). This was confirmed primarily for this mutagen, even with greater justification through mathematical and statistical analysis than previously for gamma-rays (Nazarenko, 2017), nitrosoalkylureas, or other mutagenic factors (Nazarenko et al., 2022).

CONCLUSIONS

EMS as a mutagenic factor shows an extremely significant dependence on the characteristics of the genotype-mutagenic interaction, i.e. depends on the characteristics of the initial material genotype with a clear subdivision according to genetically determined reactivity to the action of this mutagenic factor.

When observing all types of effects of mutagenic action from the cellular to the whole plant level, there is a clear relationship in terms of the severity of the impact of this factor, which is not always the case. This indicates the nature of a rather direct (through a direct effect on the DNA structure), rather than an indirect effect on the hereditary apparatus of a plant cell.

The most important is a comprehensive assessment of the general rate and spectrum of the obtained changes, which shows the success of this mutagen at the induction of mutations (Spencer-Lopes et al., 2018). Consideration of only one component is meaningless and may lead to the loss of part of the data for the classification analysis (OlaOlorun et al., 2020).

Investigated mutagen is more valuable in terms of inducing a wide range of different changes than in terms of obtaining economically valuable forms and lines, at least at the level of visual identification of mutations (Yakymchuk et al., 2021). This allows us to consider it more promising for clarifying data on the control mechanisms of certain traits and obtaining a number of forms that can be used in the future not directly as commercial varieties, but as a source of some valuable key traits for the partial improvement of existing varieties and lines.

It is planned to conduct a study of the selected promising material on tolerance to adverse environmental factors (winter period, drought resistance, hot resistance), assess the technological qualities of grain (protein content, low- and high-molecular glutenins, gliadins), show possible changes in the content of valuable microelements in wheat grains.

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