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Mykola NAZARENKO[1], Vladislav HORSHCHAR [2]

PECULIARITIES OF THE SODIUM AZIDE ACTION AS A FACTOR OF VARIABILITY ON WINTER WHEAT

SUMMARY

The study of new agents for indicating practical biodiversity in local varieties of winter wheat is a promising area in terms of obtaining both new commercial varieties and components for recombination selection. The purpose of the study was to demonstrate the possibilities in this regard as a sodium azide mutagen, primarily in terms of optimizing the yield of mutant forms based on local varietal resources. Winter wheat dry seeds of eight varieties were acted with water (control) and SA (sodium azide) action in concentrations of 0.01%, 0.025%, 0.05%, 0.1%. Successful use for all the varieties is primarily the effect of SA concentrations of 0.01%, 0.025%, for individual, less variable genotypes, the use of SA 0.05% can also be optimal in terms of the yield of valuable forms. Herewith, only three of the varieties demonstrated a significant genotype-mutagenic interaction in terms of significant changes in the parameters of variability, and only one of them in a positive sense. This agent is promising in obtaining short-stemmed, early-ripening, disease-resistant forms and lines with large grains and heads. Herewith, the number of negative changes is also quite high and their specific weight in the spectrum is higher in comparison with the previously studied factors of variability. Only one of the studied genotypes demonstrated the possibility of using it to create a mutation induction system with an increased yield of valuable forms. In the future, it is planned to study the variability in biochemical parameters of grain and resistance to abiotic environmental factors.

Keywords: winter wheat, chemical mutagenesis, sodium azide, mutation, plant improvement.

[1] Mykola Nazarenko (corresponding author: nazarenko.m.m@dsau.dp.ua), Department of Plant Breeding and Seeds Production, Dnipro State Agrarian and Economic University, 49600, Dnipro, UKRAINE.

[2]Vladislav Horshchar, Department of Plant Production, Dnipro State Agrarian and Economic University, 49600, Dnipro, UKRAINE.

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INTRODUCTION

Bread winter wheat continues to be an extremely valuable grain food crop, especially for areas of risky farming, which include the entire territory of Ukraine (Nazarenko et al., 2021). Problems with global warming and climate change lead, on the one hand, to the advancement of more heat-loving crops to the north, mitigation of overwintering conditions, which is especially important for winter crops, but also with an increase in the likelihood and severity of droughts during critical periods of development of some crops (Hongjie, et al.,2019; Harkness, et al., 2020).

One of the actively used and promising ways is the genetic improvement of varieties to better match the growing conditions (Cann et al., 2022) or improve local forms and bring them up to the level of world samples in terms of yield and quality traits is the use of mutation induction, not least through chemical mutagenesis (Hassine, et al., 2023).

Chemical mutagenesis is of interest primarily in terms of a higher interaction with a specific genotype, site specificity with respect to native, original DNA, which is much less typical for physical mutagens (Yali & Mitiku, 2022). Herewith, several extremely promising opportunities open up at once for the genetic improvement of agricultural crops, which are less typical when using other factors of variability (Abdel-Hamed et al., 2021).

Firstly, the use of new factors on local material (in this case, sodium azide) allows us to hope for significant changes in terms of the spectrum and frequency of the changes obtained (Cann et al., 2022), a significant predominance of certain types of rare valuable mutations in the case of identifying a more susceptible genotype (Spencer-Lopes et al., 2018).

Secondly, the presence of complex small changes, without additional negative mutant traits, is more typical for chemical factors, primarily in terms of biochemical mutations, which can significantly improve the nutritional characteristics of traditional crops, which by no means always correspond in their compositions of biologically active substances and microelements to the needs of consumers (Kumar et al., 2018).

Last but not least, the use of a certain chemical, usually at a moderate concentration, can lead to an optimal source material-mutagen system in terms of mutation yield, which, under certain conditions can increase the yield of valuable forms, as well as the overall mutational variability, up to 60 – 80% and it is not a fact that such values can be limiting, especially for some classes of previously unused or limitedly used chemicals (Abdel-Hamed et al., 2021). In particular, the use of sodium azide on some cereal crops also demonstrates certain hopes in this area (Hassine et al., 2023).

Herewith, many chemical agents, especially those with a high damaging effect, can vary within one concentration in terms of the degree of inhibition of a trait up to 10-12% of its absolute value, which makes the study of the genotype-mutagenic component of the interaction even more important (Mangi et al., 2021).

Clearly, with an increase in the genetic activity of the factor of variability, this kind of interaction also decreases, but not always. It is possible to identify forms for which the rate of interaction decrease is significantly lower or even absent. (Chaudhary et al., 2019; Abdel-Hamed et al., 2021).

Qualitative differences in the genotype-mutagenic interaction led to the need to conduct appropriate studies on the frequency and spectrum of emerging changes in the widest possible range of genotypes in order to compare the source material in variability and the most diverse set of mutagens (Chakraborty et al., 2023). Herewith, the issue of limitation in the doses or concentrations used is raised each time, which is solved by monitoring studies in the first generation and by limiting the sample to establish the objective patterns of the mutation process, its key criteria (Chaudhary et al., 2019).

The purpose of the study was to demonstrate hereditary variability based on visual and biometric analysis of phenotypic changes in the second generation of mutant families, to identify new lines with inherited altered traits in the third and subsequent generations, to identify the boundaries of possible, primarily practically valuable, variability for the source material in comparison of individual varieties, including in connection with the already established depressive effects in the first generation, to identify possible optimal compositions in the system of source material – mutagen concentration.

MATERIAL AND METHODS

The experiments were carried out under the conditions of the research fields station of the Science-Education Center of the Dnipro State Agrarian Economic University during 2017–2021 (48°51′10″ n. l. 35°25′31″ e. l.).

Winter wheat seeds (1000 grains for each concentration and water) were acted with a SA (sodium azide) 0.01%, 0.025%, 0.05%, 0.1% (Sigma-Aldrich, Germany) at water solution. Seed material was acted with an exposition of 24 hours by the generally recommended protocols for chemical mutagens action. Concentrations were trivial for this type of mutagens. The control was soaked in water (Spencer-Lopes et al., 2018).

Seeds material has been sown by 40 variants (in total) (2-rows plots for second generation, 5-rows for third generations and 10-rows plots for next generations, initial variety as control, interrows were 0.15 m, 1.5 m length of row). Eight varieties with difference at ecotype were used (in brackets FS – forest-steppe ecotype, all for all zones, S – steppe by state examination classification) Balaton (FS for Ukraine, only one variety of West-Europe ecotype), Borovytsia (all), Zeleny Gai (S), Zoloto Ukrainy (FS), Kalancha (all), Niva Odeska (all), Polyanka (all), Pochayna (all). The genotypes were identified by general national breeding classification as for Steppe conditions semi-intensive Niva Odeska, Zoloto Ukrainy; intensive West-Europe ecotype Balaton, semi-intensive Forest-Steppe ecotype (all other) (Spencer-Lopes et al., 2018).

The crop cultivation is trivial for the Steppe zone. The control was non-treated initial and one were also grown after ten plots for each variant as

comparison with the mutant families at second generation. 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, 2 rows for sample with control-row of initial variety samples. Mutant lines 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 mutations were identified though visual evaluation and biometrical analyze of yield structure. At second generation preliminary evolution by visible changes, at second identification as mutation by traits heredity. Estimation was conducted during 2018 – 2019 years for second-third generations and during for 2020 –2022 years for next generation in collection of genetic-value samples and lines grain production exam.

Level of changeability was calculated as $P\nu = \alpha*\gamma$, where $P\nu$ – level of changeability of variant; α – number of mutations for general number of families at variant; γ – number of types changed traits at variant.

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 DISCUSSION

In total, for control and material after mutagen action 19400 families at second generation and 1692 mutant lines at third generation were investigated. Mutagen has been used in recommended concentrations for cereal breeding practice. The number of families at M2 generations on average is about 500 per variant, except extreme concentration SA 0.1% for six varieties (not for Polyanka and Pochayna).

Data about the general rates of change at second-third generation for each variety and concentration of mutagen factor are presented at tables 1 and 2 for two groups of genotypes respectively (with higher level of mutagen depression at first generation varieties for first group and with less depression effects at the same generation for second group (subsequently it will be shown by cluster analyze). As for previous investigations level of depression has significantly relation with mutation variability at next generation. Its main reason for this type classification of plant material.

However, when analyzing the first group, we do not find a significant high dependence, which is confirmed primarily due to the variability of the varieties of the first group. Thus, varieties of the first group showed the following mutation rates Balaton (general rate up to 21 %), Zoloto Ukrainy (up to 19,5 %), Zeleny Gai (up to 19.25 %), Niva Odeska (up to 20.5 %). This is approximately the same value as in the varieties of the second group. The exception was the varieties Polyanka and Pochayna, where the variability is much lower than in

other genotypes. Thus, it makes sense to talk about the response of each genotype separately, but not by groups of mutagenic depression.

Table 1. General rate of hereditary changes for winter wheat samples 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, SA 0.01%	500	34	6.80 ± 0.30^{b}
Balaton, SA 0.025%	500	49	9.80 ± 0.43^{c}
Balaton, SA 0.05%	500	69	13.80 ± 0.55^{d}
Balaton, SA 0.1%	400	84	21.00 ± 0.62^{e}
Zoloto Ukrainy	500	6	1.20 ± 0.24^{a}
Zoloto Ukrainy, SA 0.01%	500	31	6.20 ± 0.28^{b}
Zoloto Ukrainy, SA 0.025%	500	39	7.80 ± 0.40^{c}
Zoloto Ukrainy, SA 0.05%	500	64	12.80 ± 0.44^{d}
Zoloto Ukrainy, SA 0.1%	400	78	19.50 ± 0.57^{e}
Zeleny Gai	500	5	1.00 ± 0.20^{a}
Zeleny Gai, SA 0.01%	500	27	5.40 ± 0.32^{b}
Zeleny Gai, SA 0.025%	500	41	8.20 ± 0.37^{c}
Zeleny Gai, SA 0.05%	500	58	11.60 ± 0.51^{d}
Zeleny Gai, SA 0.1%	400	77	19.25 ± 0.65^{e}
Niva Odeska	500	3	0.60 ± 0.18^{a}
Niva Odeska, SA 0.01%	500	39	7.80 ± 0.39^{b}
Niva Odeska, SA 0.025%	500	53	10.60 ± 0.44^{c}
Niva Odeska, SA 0.05%	500	73	14.60 ± 0.74^{d}
Niva Odeska, SA 0.1%	400	82	20.50 ± 0.69^{e}

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

For first group mutagen action was statistically significant for the variance in the change in mutagen concentration ($F = 146.23$; $F_{0.05} = 3.48$; $P = 3.15*10^{-9}$) and for genotype-mutagen interaction ($F = 6.10$; $F_{0.05} = 2.72$; $P = 0.01$), but not for genotypes ($F = 3.16$; $F_{0.05} = 3.86$; $P = 0.07$).

For second group mutagen action was statistically significant not only for the variance in the change in mutagen concentration ($F = 158.23$; $F_{0.05} = 3.48$; $P = 4.09*10^{-10}$) and for genotype-mutagen interaction ($F = 28.77$; $F_{0.05} = 2.72$; $P = 6.17*10^{-4}$), but for genotypes ($F = 9.90$; $F_{0.05} = 3.86$; $P = 0.003$) too.

Table 2. General rate of hereditary changes for winter wheat samples at second – third generations. Second 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, SA 0.01%	500	29	5,80±0.27[b]
Borovytsia, SA 0.025%	500	40	8,00±0.41[c]
Borovytsia, SA 0.05%	500	60	12,00±0.52[d]
Borovytsia, SA 0.1%	400	79	19.75±0.65[e]
Kalancha	500	3	0,60 ± 0.06[a]
Kalancha, SA 0.01%	500	31	6,20±0.31[b]
Kalancha, SA 0.025%	500	39	7.80±0.42[c]
Kalancha, SA 0.05%	500	65	13.00±0.52[d]
Kalancha, SA 0.1%	400	85	21.25±0.68[e]
Polyanka	500	2	0,40 ± 0.12[a]
Polyanka, SA 0.01%	500	22	4,40±0.32[b]
Polyanka, SA 0.025%	500	33	6,60±0.51[c]
Polyanka, SA 0.05%	500	53	10,60±0.50[d]
Polyanka, SA 0.1%	500	62	12.40±0.63[e]
Pochayna	500	2	0,40 ± 0.14[a]
Pochayna, SA 0.01%	500	23	4,60±0.32[b]
Pochayna, SA 0.025%	500	34	6,80±0.41[c]
Pochayna, SA 0.05%	500	51	10,20±0.51[d]
Pochayna, SA 0.1%	500	61	12.20±0.66[e]

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

However, for all varieties in two groups supermutagen action was statistically significant in all cases for the variability in the change in mutagen concentration ($F = 131.23$; $F_{0.05} = 3.10$; $P = 1.92*10^{-8}$), by varieties ($F = 4.92$; $F_{0.05} = 3.59$; $P = 0.03$) and for genotype-mutagen interaction ($F = 11.98$; $F_{0.05} = 2.54$; $P = 0.002$).

For all cases, the differences were statistically significant for all concentrations of mutagens 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).

To finally establish the differentiating ability of the overall mutation frequency as an indicator, a cluster analysis was carried out (Figure 1), which demonstrated that, in general, all the presented varieties are divided into three main groups when exposed to SA

Thus, at first group are varieties Balaton, Niva Odeska from the first group, which are characterized by somewhat different variability when acted to moderate concentrations of the mutagen. At second group varieties Borovytsia,

Zeleny Gai, Zoloto Ukrainy, Kalancha with the similar variability as for the first group. At the third group are varieties Polyanka and Pochayna with lowest variability under SA action, first of all for higher concentrations (third and fourth). It is noticeable that SA in its action on individual varieties differs significantly from previously studied substances with a high damaging ability. It can also be concluded that the frequency of mutations increases progressively and sequentially with increasing concentration, especially high at the last, highest, concentration.

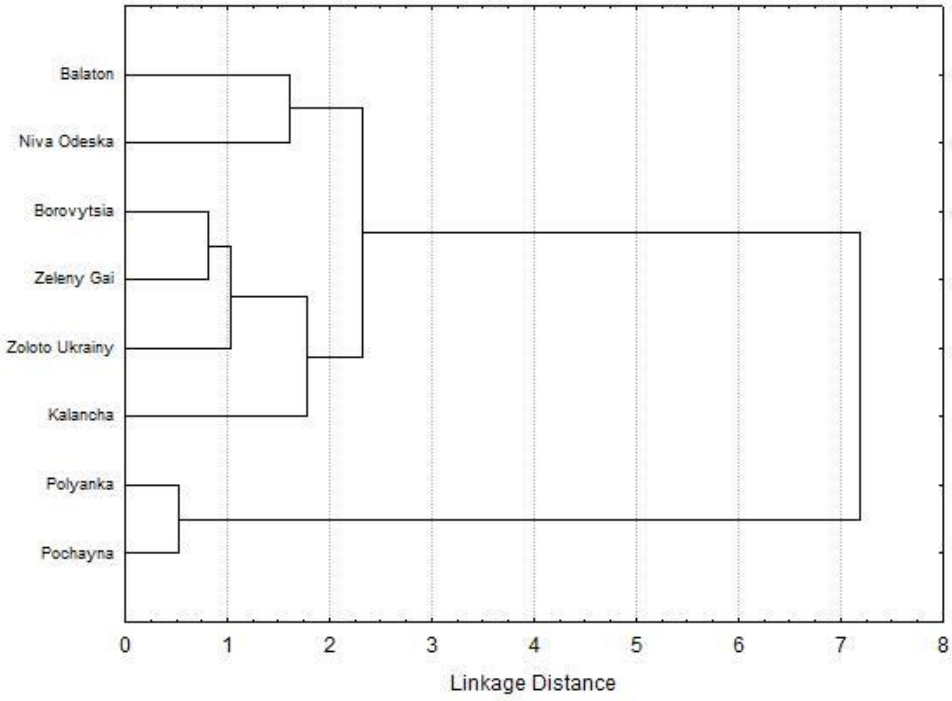

Figure 1. Results of cluster analysis by general mutation rate.

No less interesting, however, than variability in general is such a basic characteristic as the number of features that have undergone changes. Thus, an increase in the overall frequency does not always mean an increase in the diversity of the mutant material for selection, and, in some situations, it can, on the contrary, significantly reduce this indicator. Therefore, the level of variability is used, calculated as the ratio between the number of mutant cases and the number of traits that have undergone changes (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).

Varieties of the first group showed the next level of changeability Balaton (level up to 6.72), Zoloto Ukrainy (up to 6.05), Zeleny Gai (up to 6.35), Niva Odeska (up to 6.15). Herewith, a significant decrease in the number of traits for which mutations have passed with an increase in concentration to the maximum

is observed only in the Niva Odeska variety. And for this trait, in comparison with the varieties of the second group, only Polyanka and Pochayna differ in a low level, in which the dynamics in the number of traits, however, remains the same.

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

Variant	Level of changeability	Changed traits
Balaton	0.01 ± 0.01^{a}	2
Balaton, SA 0.01%	1.56 ± 0.23^{b}	23
Balaton, SA 0.025%	3.14 ± 0.29^{c}	32
Balaton, SA 0.05%	4.55 ± 0.34^{d}	33
Balaton, SA 0.1%	6.72 ± 0.41^{e}	32
Zoloto Ukrainy	0.07 ± 0.01^{a}	6
Zoloto Ukrainy, SA 0.01%	1.12 ± 0.19^{b}	18
Zoloto Ukrainy, SA 0.025%	1.56 ± 0.21^{c}	20
Zoloto Ukrainy, SA 0.05%	3.46 ± 0.27^{d}	27
Zoloto Ukrainy, SA 0.1%	6.05 ± 0.34^{e}	31
Zeleny Gai	0.02 ± 0.02^{a}	3
Zeleny Gai, SA 0.01%	1.03 ± 0.21^{b}	19
Zeleny Gai, SA 0.025%	2.05 ± 0.31^{c}	25
Zeleny Gai, SA 0.05%	3.83 ± 0.34^{d}	33
Zeleny Gai, SA 0.1%	6.35 ± 0.41^{e}	33
Niva Odeska	0.02 ± 0.01^{a}	3
Niva Odeska, SA 0.01%	1.95 ± 0.01^{b}	25
Niva Odeska, SA 0.025%	3.29 ± 0.11^{c}	31
Niva Odeska, SA 0.05%	4.96 ± 0.23^{d}	34
Niva Odeska, SA 0.1%	6.15 ± 0.31^{e}	30

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

For first group mutagen action was statistically significant for the variance in the change in mutagen concentration ($F = 111.23$; $F_{0.05} = 3.48$; $P = 2.93*10^{-7}$) and for genotype-mutagen interaction ($F = 5.15$; $F_{0.05} = 2.72$; $P = 0.02$), but not for genotypes ($F = 3.01$; $F_{0.05} = 3.86$; $P = 0.08$).

For second group SA action was statistically significant not only for the variance in the change in mutagen concentration ($F = 142.93$; $F_{0.05} = 3.48$; $P = 1.22*10^{-9}$) and for genotype-mutagen interaction ($F = 19.34$; $F_{0.05} = 2.72$; $P = 2.98*10^{-3}$), but for genotypes ($F = 8.17$; $F_{0.05} = 3.86$; $P = 0.007$) too.

At Table 4 the level of changeability at the highest concentration were for the varieties of the second group Borovytsia (5.93), Kalancha (6.16), Polyanka (3.47) Pochayna (3.90), that is significantly lower for the varieties Polyanka and

Pochayna ($F = 22.17$; $F_{0.05} = 4.74$; $P = 5.15*10^{-5}$). The variability within the group is significantly higher than for first.

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

Variant	Level of changeability	Changed traits
Borovytsia	0.03±0.01[a]	4
Borovytsia, SA 0.01%	1.16±0.08[b]	20
Borovytsia, SA 0.025%	2.40±0.18[c]	30
Borovytsia, SA 0.05%	3.48±0.28[d]	29
Borovytsia, SA 0.1%	5.93±0.37[e]	30
Kalancha	0.05±0.02[a]	5
Kalancha, SA 0.01%	1.36±0.15[b]	22
Kalancha, SA 0.025%	1.79±0.28[b]	23
Kalancha, SA 0.05%	3.90±0.43[c]	30
Kalancha, SA 0.1%	6.16±0.51[d]	29
Polyanka	0.01±0.01[a]	2
Polyanka, SA 0.01%	0.66±0.13[b]	15
Polyanka, SA 0.025%	1.45±0.21[c]	22
Polyanka, SA 0.05%	2.86±0.29[d]	27
Polyanka, SA 0.1%	3.47±0.37[e]	28
Pochayna	0.01±0.01[a]	2
Pochayna, SA 0.01%	0.83±0.13[b]	18
Pochayna, SA 0.025%	1.43±0.19[c]	21
Pochayna, SA 0.05%	2.96±0.25[d]	29
Pochayna, SA 0.1%	3.90±0.29[e]	32

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

Although, for all genotypes in these two groups SA action was statistically significant in all variants for the changeability with the change in mutagen concentration ($F = 93.17$; $F_{0.05} = 3.10$; $P = 3.47*10^{-6}$), by genotypes ($F = 4.17$; $F_{0.05} = 3.59$; $P = 0.04$) and for genotype-mutagen interaction ($F = 10.02$; $F_{0.05} = 2.54$; $P = 0.004$).

For all cases, the differences were statistically significant for all concentrations of mutagens in all varieties, regardless of the group, both in relation to the control and in relation to the effect of the previous concentration with one exception. There was no difference between Kalancha, SA 0.01% and Kalancha, SA 0.025% ($F = 4.08$; $F_{0.05} = 4.82$; $P = 0.07$).

If the cluster analysis of other chemical mutagens demonstrated some differences in the classification of genotypes and a greater systemic nature of the second indicator, then in this case its results did not differ significantly(Fig. 2),

again all the varieties presented under the influence of SA are divided into three of the same groups.

Thus, at first group are varieties Balaton, Niva Odeska from the first group, which are characterized by somewhat different variability when acted to moderate concentrations of the mutagen. At second group varieties Borovytsia, Zeleny Gai, Zoloto Ukrainy, Kalancha with the similar variability as for the first group. At the third group are varieties Polyanka and Pochayna with lowest variability under SA action, first of all for higher concentrations (third and fourth).

Cluster analysis for the level of changeability demonstrated a similar division into three groups like as previous parameter (Fig. 2). In spite of previous cases with other mutagens, it cannot be considered mathematically justified that the estimate is more accurate in terms of the level of changeability than in terms of the general rate of mutations. Only for one case, variety Niva Odeska, number of changed traits lower with statistically significance for SA 0.1% action.

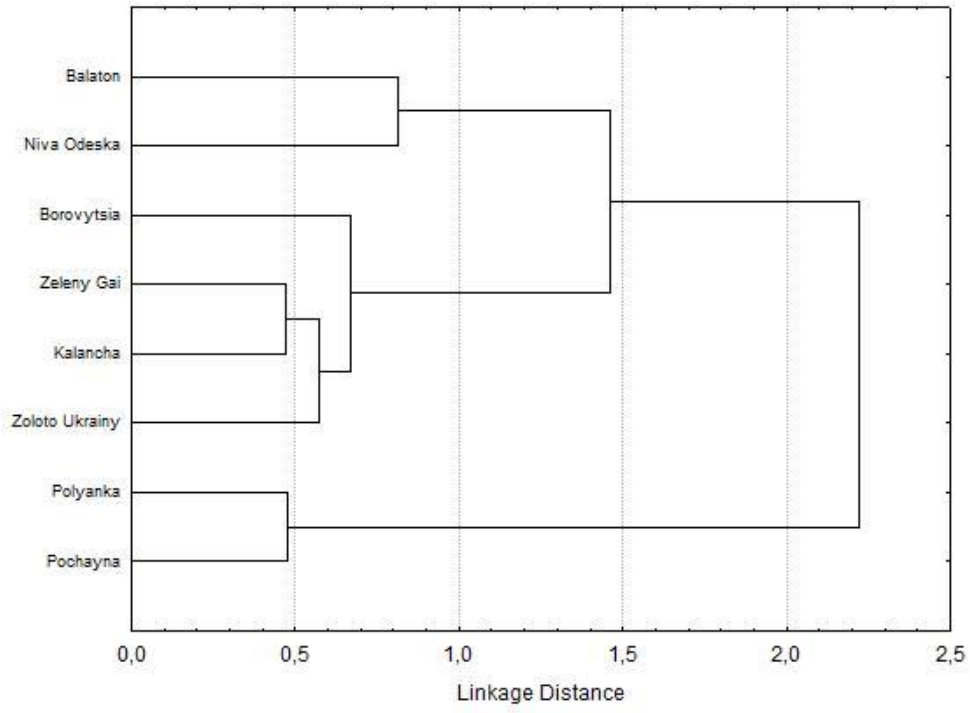

Figure 2. Results of cluster analysis by level of variability.

As for the spectrum of obtained changes, the traits can be divided into 6 groups due to the generally accepted classification for mutation breeding practice.

The first group was mutations by traits plant structure. Thise group includes such traits as thick, thin, high and short stem, semidwarf and dwarf

forms, plants with intensive, weak and the presence or absence (regarding parent variety trait) of a waxy bloom. As for the group, new fact was the high probability for SA action induction rare mutation by stem thickness (up to 0.25 - 0.50%) and dwarf or semi-dwarf forms at high concentration (up to 0.75 – 1.00%). SA as a mutagen very effective in induction any type of mutations by first group for only one exception forms without waxy bloom. The highest (up to 1.50 -1.75%) probability of the appearance of high and short-stem forms, weak waxy bloom, which are present in every variant.

The second group consists of mutants by grains size and shape. Its traits like as barrel-shaped grain, coarse grain, large grain. Only the large grain mutations occur more or less probably (up to 0.20%, its probability decreases with increasing concentration for some varieties), other mutations are rare. Mutants with large grain were more often for varieties of first group ($F = 7.01$; $F_{0.05} = 4.32$; $P = 0.02$).

The third group consists of mutations by spike structure (the most numerous, 15 different types). The most part of these mutations tends to occur more frequently as the concentration increases, except large spike and some other seldom mutations. Varieties of first group and two more variable genotypes from second group Borovytsia and Kalancha are characterized by the presence of a greater number of such mutations as long and large spike, for less variable varieties are more inherent anthocyanin awns and a semi-awn spike. The forms with the changes in spike from awn to awnless is more frequent (almost four times) than from awnless to with awn. SA as a mutagen effective in induction awnless, long, small and dense spikes form (0.20 – 0.40% at average).

The fourth group consists of mutant forms with changes in the physiology of plant growth and development is one of the most variable, in spite of only four traits such as sterility, early-maturing, late-maturing, disease tolerance. More frequent (for all varieties, preferable for fist-second concentrations) are mutations by early maturity and disease tolerance (up to 1.75%). For some varieties (for third-fourth concentrations) late maturing may become more often (up to 1.75% for variety Zoloto Ukrainy at SA 0.1%, no more 1.0% for other genotypes). Sterility is more typical to for SA 0.05% - 0.1% concentrations and practically does not occur at low concentrations. In general, three (early-maturing, late-maturing, disease tolerance) traits for this group are in model for mutation process.

The fifth is the group of systemic mutations (extremely changes in the spike structure, going beyond the cultural form and leading to the phenotype of wild wheat relatives). Such traits are most probable under the action of SA 0.05% - SA 0.1% concentrations and low doses cannot act by the same way. More likely is the appearance of squareheads (up to 1.0% for some varieties) and sometimes speltoids (especially for Borovytsia and Zoloto Ukrainy at SA 0.1%), sometimes it can be form even at low concentrations. Other mutations are quite rare. This type of mutations isn’t interesting for breeding practice.

The sixth group includes agriculture-value forms with high grain yield or tillering ability. It occurs in most varieties, preferably for SA 0.01% and SA 0.025% concentrations. Its occurrence decreases with increasing concentration for all varieties. This type of mutations is not very common.

In addition to establishing the variability of individual parameters and their groups, it is quite important to demonstrate the model variability (especially for common parameters and a group of valuable mutations), which was done through the discriminant analysis of individual variables (Table 5, Fig. 3). The model was the general rate of mutations, the level of changeability, mutations in the first, third, fourth groups.

Table 5. Results of discriminant analyze

Variables at model	Wilks Lambda λ	Partial Lambda	F_{remove} (4,34)	p-level
Mutation rate	0.08	0.59	5.76	0.02
Level of changeability	0.07	0.47	9.34	0.01
First group	0.05	0.41	11.12	0.01
Second group	0.52	0.77	2.59	0.09
Third group	0.10	0,57	6,99	0,01
Fourth group	0.06	0.43	10.27	0.01
Fifth group	0.17	0.63	4.14	0.06
Sixth group	0.24	0.69	2.03	0.11

The data obtained demonstrate that the applied supermutagen is quite effective in terms of both the induction of general variability and the impact on individual traits. The range of variable features is wide, in fact, the maximum. Herewith, it should be noted that the effectiveness of this agent lies primarily in changes in plant height (induction of low-growing and semi-dwarf forms as a necessary part of an intensive ecotype), early maturity, and resistance to diseases. However, the sixth group is not in the model.

In addition, the discriminant analysis (Fig. 3) once again demonstrated a clear difference in the effects of individual concentrations of mutagens, while the effects of the first and second concentrations are mixed, however, the date cloud in this case is far from the case with water treatment. The second and third concentrations may partially associate, but not the first. Stands completely apart with a high value of the last concentration. The data obtained demonstrate that the first-third concentration may be more effective and partially (depending on the genotype) in action. Summing up with the previous analysis – the first and second, only for individual genotypes – the third (Polyanka and Pochayna).

Classification in the factor space of the obtained data demonstrated that such varieties as Niva Odeska, Polyanka and Pochayna demonstrated themselves most effectively in terms of genotype-mutagenic interaction. And the last two in terms of reducing variability (Table 6).

The data obtained demonstrate that the use of this substance as a mutagenic factor should be used primarily to obtain forms for use as components for the subsequent improvement of existing varieties through recombinant breeding (Ahumada-Flores et al., 2021; le Roux et al., 2021). It is much less

likely to produce lines that can be used directly as commercial varieties (Shimelis et al., 2019).

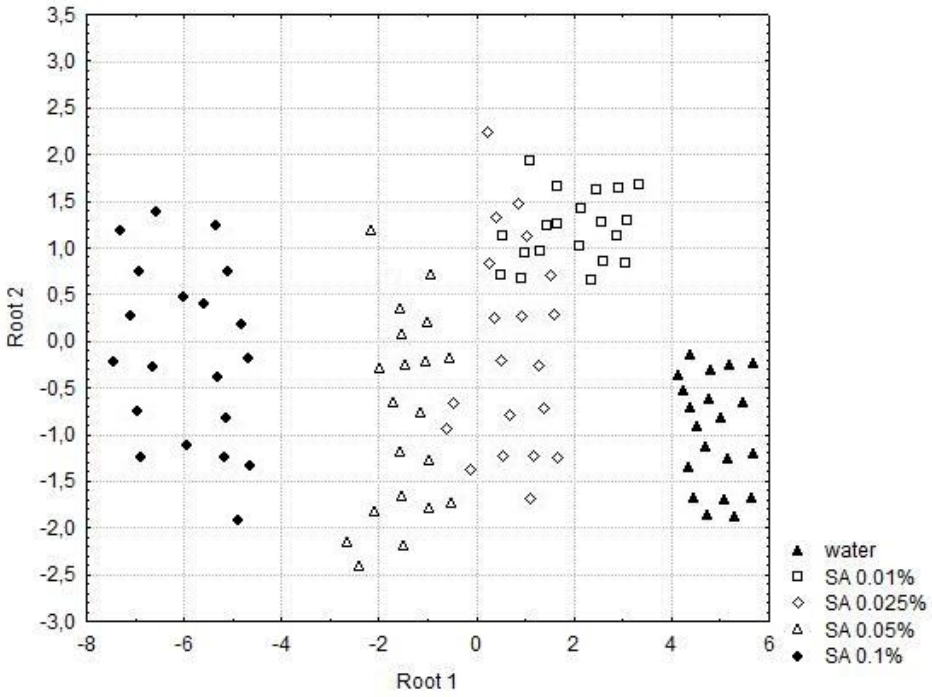

Figure 3. Results of discriminant analysis for model parameters

Table 6. Classification matrics - canonical roots.

Genotype	Percent of classification
Balaton	72.5
Zoloto Ukrainy	75.0
Kalancha	70.0
Niva Odeska	100.0
Borovytsia	72.5
Zeleny Gai	70.0
Polyanka	100.0
Pochayna	100.0
Total	86.3

It is more optimal to use this mutagen as a factor for the induction of short-stemmed forms with a long ear, early ripening lines, which, due to this feature, can avoid the typical May-June drought that is typical for our region (Nazarenko et al., 2019 Nazarenko et al., 2022). The use of sodium azide in selection for resistance to diseases is promising, however, in general, a fairly wide range of substances have demonstrated their effectiveness for this kind of improvement (Anter, 2021).

In contrast to previous studies (Shimelis et al., 2019; le Roux et al., 2021), although the given concentrations approached semi-lethal depression in the first generation, they did not demonstrate a significant decrease in mutational activity (OlaOlorun et al., 2020; Cann et al., 2022). Thus, even a higher concentration of the mutagen only led to a significant increase in biodiversity induction (OlaOlorun et al., 2021). Thus, the dose range used in principle is applicable without major problems to obtain genetically valuable forms when needed (Ariraman et al, 2018).

However, the shift towards greater complexity and an increase in the proportion of adverse changes leads to the conclusion that in this case, it is also more optimal to use optimal and low concentrations (Ahumada-Flores et al., 2021), primarily in the range of 0.01% - 0.05%. Herewith, the first and second (0.025%), according to the results of the analysis, should be used without taking into account the genotype of the initial material, the latter only for some initial forms (Yali & Mitiku, 2022)., especially those that demonstrated little variability (two varieties in our studies).

The genotype-mutagenic interaction of this substance is clearly significantly lower compared to the previous ones, especially in a positive way (Abdel-Hamed et al., 2021; Horshchar & Nazarenko, 2022). The low suitability of two varieties for the treatment of these agents was especially pronounced, and only one genotype can be recognized as promising.

Herewith, the use of higher concentrations is quite acceptable when obtaining genetically-valuable forms (Lal et al., 2020; Anter, 2021), especially for dwarfs and semi-dwarfs. The yield lines obtained under the action of the third or fourth concentration are late-ripening (Nazarenko et al., 2019), which is not very suitable for the country's agriculture and leads to increased vulnerability to droughts in the summer.

CONCLUSIONS

The studied chemical supermutagen demonstrated a fairly high overall level of variability and demonstrated extremely high activity in obtaining new forms for all essential features of winter wheat plants. However, the activity of this agent is predominantly focused on the induction of such types of mutations as changes in plant height, culm thickness, which sharply distinguishes the factor from a number of related ones, induction of changes in the length and width of the ear and grain, both positive and negative, changes in ripeness, before the entire production of a significant number of early ripening forms, the production of mutants with squarehead and speltoid head, disease-resistant mutants. Particular attention should be paid to the possibility of obtaining undersized, primarily semi-dwarf forms, mutants with a large ear and full grain, early maturing lines and disease-resistant lines. Only one of the studied varieties demonstrated a sufficiently high genotype-mutagenic interaction in a positive sense. It can be concluded that the chances of obtaining an optimal scheme for the release of valuable mutations under the action of this substance are

significantly lower than those previously studied for these varieties. Further plans to study the effectiveness of this mutagen include an analysis of the variability in biochemical parameters of the obtained forms, in particular, the content of protein and gluten in grain, the quality of protein components and their ratio, the presence of biologically active substances and valuable trace elements. It will also be interesting to analyze the obtained forms for winter hardiness and drought resistance.

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