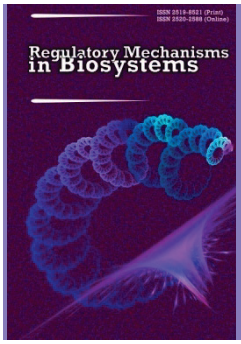


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# Winter wheat cytogenetic variability under the action of a chemical supermutagen

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The analysis of cytogenetic activity is a key component in determining prospects of future hereditary variability after, subject to a certain mutagenic factor, primarily identifying the significance of the genotype-mutagenic interaction, the correctness of the selected concentrations for more thorough screening of some development parameters. Winter wheat seeds of eight varieties (Balaton, Borovytsia, Zeleny Gai, Zoloto Ukrainy, Kalancha, Niva Odeska, Polyanka, Pochayna) were subjected to EMS (ethylmethansulfonate) at the concentrations of 0.025%, 0.05%, 0.10% The exposure lasted for 24 hours. Cytogenetic analysis was carried out for pollen fertility; we also examined the rates and spectras of chromosomal rebuildings in proper cell devision phases in relation to plant gcnotype and concentration of the mutagen. The experiment was aimed at identification of interrection between geotype, concentration of mutagen and mutagen nature, determining genome response to mutagen action. Such indicators of cytogenetic activity as the total rate of chromosomal abnormalities, fragments and double fragments, single and double bridges, micronucleus and lagging chromosomes were studied. The selected concentrations of the mutagen significantly influenced all the analyzed parameters, they can be attributed to the optimal and high range of concentrations according to the nature of the impact on bread wheat. We determined that in the case of the mutagenic action, the genotype had a significantly lesser effect on the nature and rate of individual aberrations than an increase in the concentration, while having a significant effect on the rate of increase in pollen sterility. The mutagen was characterized by a significantly lower site-specificity at the cellular level than other chemical supermutagens, manifesting only in the correlation between individual types of aberrations, but not in the character of the increase in their number. The key parameter to identify the activity of this agent was the frequency of fragments and double fragments, their ratio with bridges.

*Keywords*: cereals; chromosomal aberrations; ethylmethansulfonat; pollen fertility; bridges; fragments; chemical mutagenesis.

## Introduction

Analysis of the consequences of the mutagenic action towards the chromosomal level makes it possible to monitor the genetic activity of a substance, to show its capabilities in terms of induced variability, which will subsequently result in changes in the hereditary nature of economically and genetically valuable traits (Chaudhary et al., 2019; Bezie et al., 2020; Dwinanda et al., 2020; Udage, 2020; Hassine et al., 2022). At the same time, chemical supermutagens demonstrate an increased affinity with certain DNA regions, which further leads to an increase in the variability (Bezie et al., 2020). It is also necessary to monitor the changes directly influencing the plant viability (Shabani et al., 2022).

The role of the chromosomal aberrations is the fixation of mutational changes at the level of the cell chromosomal apparatus, leading not only to inherited changes, but also to problems in the development of cultivated plants. Also, such evidence depends on genetically determined mechanisms of increasing the resistance to genetically active substances. This type of genetic mechanism (mainly recessive) has been studied for a great number of cultivated plants, primarily within the framework of radiobiological and radioecological researches, and at least two such possible loci have been identified, there could be other loci. However, the mechanism of genetic tolerance to the action of chemical supermutagens is much less understood, although it is already clear that those mechanisms are more specific and characterized by a significantly greater diversity in plant response than sensitivity to ionizing radiation (Abdelsalam et al., 2019; Hasan et al., 2022).

There is now underway a search for possibilities of adapting the classic methods of using mutagenic action in the realities of today's restrictions, elimination of the negative aftermaths of this activity or fully taking them into account in programs for the genetic improvement of winter wheat, regulation of the interaction within the framework of using a specific anthropic system and the need for maximum involvement of local resources for increasing the stability of the obtained results (Abaza et al., 2020). All those aspects are the main problem areas that modern ecological genetics must solve. The phenomenon of mutation, as a rule, is associated with changes in the structure of DNA. At the current level of understanding, the main cause of spontaneous mutagenesis is repair errors and the mobility of the genome of a cultivated plant. Other reasons are rare and unlikely (Hasan et al., 2022). The ecological-genetic approach in experimental mutagenesis begins with complex studies: first of all, recording radiobiological effects of a mutagenic action, then identifying specific DNA-disruption at the molecular level, determining the relationship between phenological variability and specific changes in DNA (Bondarenko & Nazarenko, 2020), and seeking for variations of a resulting change and identifying the limits of variability in the manifestation of a changed trait or group of key traits (Bhat & Wani, 2017; Spencer-Lopes et al., 2018).

The beginning of our century was marked by the synthesis of the use of the mutation in plant improvement through the use of reverse genetics methods, which require large populations with high induced genetic variability to obtain the necessary numbers for data analysis with a large number of molecular variants of changes in each DNA fragment and DNA associations (Badr et al., 2014; Ram et al., 2019). Understanding the genetic basis of the occurrence of mutations (through transformation and hereditary changes in DNA) has transformed the induction of biodiversity from a method of randomly obtaining possible beneficial changes to a concrete technique (Udage, 2020).

The effects of mutagenic action on seeds can lead to many types of chromosomal changes. As an example of winter wheat varieties, there occur chromosomal abnormalities such as centromere breaks, terminal deletions of chromosomes, deletions of one chromatid arm, and chromosome fragments. We recorded all changes during the metaphases of the meristematic cells of the root. In meiosis of the first generation, mutagen action can cause the appearance of lagging chromosomes as a ring formation. In the mitosis of the second generation, some of the root meristematic cells maintained a normal euploid (2n) chromosome set, but some of them had aneuploid (Hase et al., 2020). At the same time, chromosome segmentation, terminal deletions, lagging chromosomes, chromosome bridges and other abnormalities are observed in some metaphase divisions (Amri-Tiliouine et al., 2018; Ram et al., 2019). Cytological abnormalities caused by mutagen action can be corrected by chromosomal manipulation techniques. The biological consequences of mutagen action (abnormal cell division, cell death, mutations, impaired development of tissues and organs, reduced plant growth) can occur at various stages of ontogenesis. The effect depends on the type and level of mutagen action, physiological state and genetic composition of the treated material (Shu et al., 2013; Bhat & Wani, 2017).

The main objective of study was to show the peculiarities of the cytogenetic activity of ethylmethansulfonate as a chemical supermutagen, its specificity in inducing the rate and spectrum of chromosome aberrations depending on the object of mutagenic action, the effect on the fertility of the plant in first generation after mutagen action, and determining possible prognostic elements for identification of the genome stability of a particular genotype (variety).

## Materials and methods

The experiment was conducted in 2017–2021 in the conditions of the experimental fields of the Science-Education Center of the Dnipro State Agrarian Economic University.

Winter wheat seeds (1,000 grains for each concentration and water) were coated by EMS (ethylmethansulfonate) in 0.025%, 0.05%, 0.10% concentrations (Sigma-Aldrich, Germany). The exposure was 24 hours, according to the generally recommended method for chemical mutagens. Those concentrations are trivial for mutagens (chemical supermutagens) of this group. The control was soaked in water (Spencer-Lopes et al., 2018).

The experiment consisted of 32 variants (in total) with 8 winter wheat varieties: Balaton, Borovytsia, Zeleny Hai, Zoloto Ukrainy, Kalancha, Nyva Odeska, Polianka, Pochaina. The genotypes were identified to characterize winter wheat varieties' variability for the North Steppe subzone (Dnipro region) (Shu et al., 2013; Spencer-Lopes et al., 2018). The agrotechnology of crop cultivation is crucial for the Steppe zone (semi-arid area). Laboratory studies were carried out to determine the degree of fertility of pollen grains. We also performed a cytological analysis of chromosome aberrations. Pollen sterility was determined by acetocarmine staining and its intensity. Pollen for the analysis was collected from properly developed main spikes (during the flowering phase, with yellow anthers in the medium part of spike) of first-generation plants. A total of 25 samples were gathered and analyzed (Shu et al., 2013).

Using light microscopy, we conducted cytogenetic analyses of chromosomal abnormalities on mitosis preparations of primary root tips of winter wheat (during the last period of metaphase and initial anaphase for all types abnormalities). After EMS action, the samples of root tips were cultivated in Petri dishes on a filter paper with distilled water in a thermostat at the temperature of + 22 °C. Then the tip of every root (20–25 samples) with the length of 0.8–1.0 cm was cut and fixed in the Clark fixer, consisting of 3 parts of 96% ethyl alcohol and 1 part of ocular acid, for 24 hours. Preservation of samples was provided in 70% ethyl alcohol solution at the temperature of +2 °C in the refrigerator. For such a variant, about 25–30 roots were prepared. Cytological study was carried out on temporary pressure preparations stained with acetocarmine. For the samples that had problems with pressure, the tips were coated by 45% acetic acid. The samples were prepared according to the generally accepted method. This method can reveal such abnormalities as single pairs of fragments, dicentric chromosomes, micronuclei and mixed chromosomes. The samples of root tips were evaluated on a Micromed XS-3330 (Micromed, Poltava, Ukraine) light microscope (multiply in 600 times) with a 5 M camera. In every variant, there were about 1,000 plants cells in the proper stages of cell division for each concentration of the mutagen (Spencer-Lopes et al., 2018; Oney-Birol & Balkan, 2019).

Statistical analysis of the results was conducted in Statistica 10.0 (TIBCO, Palo Alto, USA). Values in the tables are given as x ± SD (mean ± standard deviation) (Chernysky & Gumentyk, 2020). The differences between the selections were determined using the single-factor dispersion analysis (ANOVA) and were considered significant at $P < 0.05$. The normality of the data distribution was examined using the Shapiro–Wilk W-test. The differences between samples were assessed by the Tukey HSD test.

## Results

Pollen fertility is an important indicator for studying mutational variability. Under the action of EMS as a mutagen, sterility increased gradually, without sharp peaks. The Tukey HSD test revealed that, according to significant differences, varieties as objects of EMS action can be divided into two groups – the first group with a smaller decrease in the fertility, comprising the varieties Borovytsia, Zeleny Hai, Zoloto Ukrainy, Kalancha, Polianka, Pochaina ($F = 12.34$; $F_{0.05} = 2.97$; $P = 0.0017$); the second group included more sensitive varieties Balaton, Nyva Odeska ($F = 5.92$; $F_{0.05} = 2.56$; $P = 0.01$). Moreover, this effect of all the concentrations of chemical supermutagen caused no significant differences within the groups. It should be noted that those varieties demonstrated a significantly higher inhibition of the mutagenic effect in terms of other parameters of ontogenesis, so this effect was among the expected ones.

In general, the fertility changed in the range of 98.9–99.4% in control, and 79.1% (variety Niva Odeska) – 86.0% (variety Zoloto Ukrainy) under the action of EMS 0.025%, 74.3% (variety Balaton) – 81.0% (variety Zeleny Hai) after the treatment with 0.05% EMS and from 69.5% (variety Nyva Odeska) to 75.0% (varieties Zeleny Hai, Kalancha, Polianka) in 0.1% EMS variant.

**Table 1**
Pollen fertility of winter wheat plants under the mutagen action (x ± SD, n = 25)

| Variety | Control | EMS 0.025% | EMS 0.05% | EMS 0.10% |
|---|---|---|---|---|
| Balaton | $99.15 \pm 1.14^{a}$ | $80.14 \pm 0.98^{b}$ | $74.33 \pm 1.12^{c}$ | $70.17 \pm 2.27^{d}$ |
| Borovytsia | $99.44 \pm 0.97^{a}$ | $84.03 \pm 1.19^{b}$ | $78.83 \pm 1.67^{c}$ | $74.19 \pm 1.58^{d}$ |
| Zeleny Hai | $99.21 \pm 0.99^{a}$ | $85.60 \pm 1.56^{b}$ | $81.02 \pm 1.80^{c}$ | $75.36 \pm 2.08^{d}$ |
| Zoloto Ukrainy | $98.97 \pm 0.98^{a}$ | $86.01 \pm 1.18^{b}$ | $79.52 \pm 2.04^{c}$ | $74.10 \pm 2.11^{d}$ |
| Kalancha | $99.24 \pm 1.25^{a}$ | $84.12 \pm 1.52^{b}$ | $79.42 \pm 2.19^{c}$ | $75.17 \pm 2.14^{d}$ |
| Nyva Odeska | $98.99 \pm 1.32^{a}$ | $79.09 \pm 1.12^{b}$ | $73.14 \pm 2.06^{c}$ | $69.53 \pm 2.29^{d}$ |
| Polianka | $99.04 \pm 1.11^{a}$ | $85.17 \pm 1.12^{b}$ | $79.12 \pm 2.19^{c}$ | $75.00 \pm 2.14^{d}$ |
| Pochaina | $99.07 \pm 1.06^{a}$ | $85.01 \pm 1.42^{b}$ | $78.44 \pm 2.26^{c}$ | $73.19 \pm 2.39^{d}$ |

*Note*: different letters indicate significant differences at $P < 0.05$ by Tukey HSD test with Bonferroni correction; comparison in terms of one variety in a line.

The rate of chromosomal aberrations was not subject to genotype influence ($F = 2.47$; $F_{0.05} = 2.48$; $P = 0.06$), but only increased after increases in concentration ($F = 542.48$; $F_{0.05} = 3.07$; $P = 1.25*10^{-18}$). The general rate of chromosomal aberrations varied 9.8% (variety Kalancha) to 11.2% (variety Borovytsia) under the action of EMS 0.025%. At the EMS concentration of 0.05%, it ranged 13.6% (variety Nyva Odeska) to 18.1% (variety Zeleny Hai); under the action of EMS 0.1%, the range was from 19.9% (variety Kalancha) to 25.7% (variety Borovytsia). During the initial analysis, only two genotypes more or less stood out. However, the Tukey HSD test showed that the variety Kalancha ($F = 4.18$; $F_{0.05} = 2.48$; $P = 0.012$) was the most vulnerable to the action of this mutagen. Moreover, the cytogenetic test depends much more on the characteristics of a particular variety genome than on phenologically manifested adaptability. At the same time, the variability of the selected components for this type of analysis was significantly lower than in the case of ontogenesis parameters, which allows us to make conclusions about the predominantly external causation of inhibition at the level of the organism as a whole.

With increasing mutagen concentration, the general rate of chromosomal aberrations decreased statistically significantly in each variety, with the exception of the Polyanka variety when switching from the EMS

0.025% concentration to the EMS 0.05% variant (F = 1.01; $F_{0.05}$ = 2.08; P = 0.09), where there was no statistically significant difference. In all the cases, already the first concentration of the mutagen caused a sharp increase in the abnormalities. The Tukey HSD test confirmed the results in a pairwise comparison. In general, the mutagen for those concentrations showed high cytogenetic activity. Unlike the previous parameters, this one was extremely sensitive to the action of the mutagen, but the genotype-mutagenic interaction was significantly lower, practically absent, and, as a result, the response of varieties was uniform.

As with the spectrum of chromosomal abnormalities, we identified and calculated such types of abnormalities as fragments (single and double), bridges (chromatids and chromosomes), micronucleus, lagging chromosomes, number of cells at the stages of mitosis with two or more aberrations (Table 3 and 4). Each abnormality was taken into account as a separate case; the frequency was calculated as a relation of number of this aberration type to the total number of abnormalities for this variant, expressed as percentage.

As in the case of the general rate of chromosome aberrations, the frequency of fragments and double fragments did not depend on variety characteristics (F = 2.01; $F_{0.05}$ = 2.48; P = 0.11), but was increased by heightened concentrations of the chemical agent (F = 214.36; $F_{0.05}$ = 3.07; P = $3.17*10^{-10}$). The Tukey HSD test showed that when this type of aberration was induced, there were no statistically significant differences in terms of varieties in any of the cases.

In general, under the action of EMS 0.025% concentration, fragments and double fragments varied from 51.8% (variety Borovytsia) to 62.0% (variety Zeleny Pai); for EMS 0.05%, from 53.9% (variety Pochayna) to 62.0% (variety Zeleny Hai), and for concentration EMS 0.1% from 44.7% (variety Pochaina) to 52.6% level (variety Nyva Odeska). Significantly, according to the genotype factor, we saw differences in the behaviour of the varieties Pochayna (F = 3.11; $F_{0.05}$ = 2.48; P = 0.003) and Zeleny Hai (F = 2.74; $F_{0.05}$ = 2.48; P = 0.008).

For aberrations of the bridge type (single and double), the genotype factor also did not have a determining value (F = 2.32; $F_{0.05}$ = 2.48; P = 0.08), but an increase in concentration significantly changed the rate of this type of aberrations (F = 98.36; $F_{0.05}$ = 3.07; P = $5.14*10^{-5}$).

**Table 2**
General rates of chromosomal aberrations for winter wheat mitotic cells

| Variety | Variant | Mitosis, number | Chromosomal aberrations | |
|---|---|---|---|---|
| | | | number | x ± SD, % |
| Balaton | water | 1,002 | 9 | $1.00 \pm 0.12^{a}$ |
| | EMS 0.025% | 1,010 | 102 | $10.10 \pm 0.43^{b}$ |
| | EMS 0.05% | 1,006 | 161 | $17.99 \pm 0.25^{c}$ |
| | EMS 0.10% | 871 | 211 | $24.23 \pm 0.10^{d}$ |
| Zeleny Hai | water | 1,008 | 8 | $0.89 \pm 0.32^{a}$ |
| | EMS 0.025% | 1,009 | 108 | $10.70 \pm 0.30^{b}$ |
| | EMS 0.05% | 1,001 | 179 | $18.08 \pm 0.60^{c}$ |
| | EMS 0.10% | 817 | 201 | $24.48 \pm 0.39^{d}$ |
| Zoloto Ukrainy | water | 1,001 | 8 | $0.80 \pm 0.21^{a}$ |
| | EMS 0.025% | 1,004 | 99 | $10.86 \pm 0.29^{b}$ |
| | EMS 0.05% | 1,010 | 158 | $17.62 \pm 0.30^{c}$ |
| | EMS 0.10% | 908 | 209 | $23.02 \pm 0.60^{d}$ |
| Nyva Odeska | water | 1,009 | 9 | $0.79 \pm 0.23^{a}$ |
| | EMS 0.025% | 1,002 | 101 | $10.08 \pm 0.12^{b}$ |
| | EMS 0.05% | 1,001 | 147 | $13.59 \pm 0.29^{c}$ |
| | EMS 0.10% | 879 | 213 | $24.23 \pm 0.22^{d}$ |
| Borovytsia | water | 1,001 | 7 | $0.70 \pm 0.20^{a}$ |
| | EMS 0.025% | 1,001 | 112 | $11.19 \pm 0.12^{b}$ |
| | EMS 0.05% | 1,010 | 161 | $15.94 \pm 0.35^{c}$ |
| | EMS 0.10% | 911 | 234 | $25.69 \pm 0.39^{d}$ |
| Kalancha | water | 1,000 | 10 | $1.00 \pm 0.15^{a}$ |
| | EMS 0.025% | 1,005 | 98 | $9.75 \pm 0.21^{b}$ |
| | EMS 0.05% | 1,003 | 143 | $14.26 \pm 0.29^{c}$ |
| | EMS 0.10% | 992 | 201 | $19.86 \pm 0.45^{d}$ |
| Polianka | water | 1,007 | 6 | $0.60 \pm 0.26^{a}$ |
| | EMS 0.025% | 1,006 | 111 | $14.02 \pm 0.16^{b}$ |
| | EMS 0.05% | 1,001 | 153 | $15.28 \pm 0.36^{b}$ |
| | EMS 0.10% | 912 | 221 | $24.23 \pm 0.44^{c}$ |
| Pochaina | water | 1,005 | 8 | $0.80 \pm 0.06^{a}$ |
| | EMS 0.025% | 1,009 | 105 | $10.14 \pm 0.30^{b}$ |
| | EMS 0.05% | 1,003 | 143 | $14.26 \pm 0.21^{c}$ |
| | EMS 0.10% | 869 | 217 | $24.97 \pm 0.33^{d}$ |

*Note*: different letters significant differences at P < 0.05 by Tukey HSD test with Bonferroni amendment. Comparison in terms of one variety at columns.

**Table 3**
Parameters of chromosomal aberrations spectra for winter wheat mitotic cells (x ± SD, n = 1000)

| Variety | Variant | fragments (single + double) | | bridges (chromosomal + chromatid) | | fragments / bridges | other (micronucleus, lagging chromosomes) | | double and more | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | | n | % | n | % |
| Balaton | water | $4.0 \pm 0.4^{a}$ | 44.4 | $4.0 \pm 1.4^{a}$ | 44.4 | 1.0 | $1.0 \pm 0.9^{a}$ | 11.1 | $0.0 \pm 0.0^{a}$ | 0.0 |
| | EMS 0.025% | $59.0 \pm 0.6^{b}$ | 57.8 | $30.0 \pm 1.9^{b}$ | 29.4 | 2.0 | $13.0 \pm 1.8^{b}$ | 12.8 | $14.0 \pm 1.4^{b}$ | 13.7 |
| | EMS 0.05% | $98.0 \pm 1.1^{c}$ | 60.9 | $42.0 \pm 2.4^{c}$ | 26.1 | 2.3 | $21.0 \pm 1.9^{c}$ | 13.0 | $30.0 \pm 2.5^{c}$ | 18.6 |
| | EMS 0.10% | $103.0 \pm 1.9^{d}$ | 48.8 | $78.0 \pm 3.9^{d}$ | 37.0 | 1.3 | $30.0 \pm 3.0^{d}$ | 14.2 | $51.0 \pm 0.5^{d}$ | 14.2 |
| Zeleny Hai | water | $4.0 \pm 1.0^{a}$ | 50.0 | $3.0 \pm 0.6^{a}$ | 37.5 | 1.3 | $1.0 \pm 1.1^{a}$ | 12.5 | $0.0 \pm 0.0^{a}$ | 0.0 |
| | EMS 0.025% | $67.0 \pm 0.9^{b}$ | 62.0 | $31.0 \pm 3.2^{b}$ | 28.7 | 2.2 | $10.0 \pm 1.5^{b}$ | 9.3 | $16.0 \pm 2.2^{b}$ | 14.8 |
| | EMS 0.05% | $111.0 \pm 1.8^{c}$ | 62.0 | $48.0 \pm 2.6^{c}$ | 26.8 | 2.3 | $20.0 \pm 3.2^{c}$ | 11.2 | $24.0 \pm 2.9^{c}$ | 13.4 |
| | EMS 0.10% | $96.0 \pm 2.9^{d}$ | 47.8 | $69.0 \pm 3.5^{d}$ | 34.3 | 1.4 | $36.0 \pm 30^{d}$ | 17.9 | $49.0 \pm 3.3^{d}$ | 24.4 |
| Zoloto Ukrainy | water | $5.0 \pm 1.5^{a}$ | 62.5 | $3.0 \pm 1.2^{a}$ | 37.5 | 1.7 | $0.0 \pm 0.0^{a}$ | 0.0 | $0.0 \pm 0.0^{a}$ | 0.0 |
| | EMS 0.025% | $62.0 \pm 3.7^{b}$ | 62.6 | $25.0 \pm 2.5^{b}$ | 25.3 | 2.5 | $12.0 \pm 1.5^{b}$ | 12.1 | $11.0 \pm 1.1^{b}$ | 11.1 |
| | EMS 0.05% | $94.0 \pm 4.3^{c}$ | 59.5 | $43.0 \pm 3.6^{c}$ | 27.2 | 2.2 | $21.0 \pm 2.5^{c}$ | 13.3 | $31.0 \pm 3.0^{c}$ | 19.6 |
| | EMS 0.10% | $102.0 \pm 2.9^{d}$ | 48.8 | $69.0 \pm 4.1^{d}$ | 33.0 | 1.5 | $38.0 \pm 2.5^{d}$ | 18.2 | $56.0 \pm 4.1^{d}$ | 26.8 |
| Nyva Odeska | water | $4.0 \pm 1.6^{a}$ | 44.4 | $4.0 \pm 1.2^{a}$ | 44.4 | 1.0 | $1.0 \pm 1.2^{a}$ | 11.1 | $1.0 \pm 1.5^{a}$ | 11.1 |
| | EMS 0.025% | $62.0 \pm 1.9^{b}$ | 61.4 | $31.0 \pm 1.6^{b}$ | 30.7 | 2.0 | $8.0 \pm 2.0^{b}$ | 7.9 | $14.0 \pm 2.2^{b}$ | 13.9 |
| | EMS 0.05% | $85.0 \pm 4.7^{c}$ | 57.8 | $41.0 \pm 2.9^{c}$ | 27.9 | 2.1 | $21.0 \pm 3.1^{c}$ | 14.3 | $32.0 \pm 2.6^{c}$ | 21.8 |
| | EMS 0.10% | $112.0 \pm 5.5^{d}$ | 52.6 | $63.0 \pm 2.2^{d}$ | 29.6 | 1.8 | $38.0 \pm 3.4^{d}$ | 17.8 | $51.0 \pm 3.2^{d}$ | 23.9 |

*Note*: different letters significant differences at P < 0.05 by Tukey HSD test with Bonferroni amendment; comparison in terms of one variety at columns.

The Tukey HSD test showed that in terms of concentrations, there were differences in all the cases except the variants Polyanka EMS 0.025% and Polyanka EMS 0.05%. However, it is also interesting what proportion was bridged by genotypes and concentrations. Therefore, the specific share of the bridges varied in the case EMS 0.025% from 25.3% (variety Zoloto Ukrainy) to 36.6% (variety Borovytsia); 21.7% (variety Kalancha) to 30.4% (variety Borovytsia) in the case of EMS 0.05%; 28.9% (variety Kalancha) to 37.0% (variety Balaton) in the variant with EMS 0.10%. In this case, the specific weight of the induction of bridges first had decreased, and then increased again after changing the second concentration to the third one. The variety Polianka was observed with lower variability in this trait (F = 2.56; $F_{0.05}$ = 2.48; P = 0.03). The ratio of fragments and bridges is characteristic of chemical supermutagens. We observed a clear predominance of the fragments and double fragments over the bridges in all the cases under the action of chemical mutagen, accounting for significantly more than 1. The ratio increased to maximum values in the variant with EMS 0.05% and decreased under the action of EMS 0.10%, so the specificity in the effect of EMS drops with increasing concentration after some peak value.

As for the other types of aberrations (lagging chromosomes and micronuclei), the genotype factor had no significance (F = 2.13; $F_{0.05}$ = 2.48; P = 0.11), an increase in the concentration significantly increased the

frequency of these types of aberrations ($F = 211.79$; $F_{0.05} = 3.07$; $P = 3.22*10^{-9}$) The Tukey HSD test revealed that with increasing concentrations, the differences appeared in all the cases for all the varieties. As for the total number of abnormalities, the share of micronuclei and lagging chromosomes ranged 7.9% (variety Niva Odeska) to 14.3% (variety Pochayna) in the variant with EMS 0.025%, 11.2% (variety Zeleny Gai) to 23.1% (variety Kalancha) in EMS 0.05% variant, and 14.2% (variety Balaton) to 23.4% (variety Kalancha) in EMS 0.1% variant. Therefore, in general, as the concentration increased, the share of such abnormalities increased as well, but the process can hardly be considered linear. The variety Kalancha stood out with a significantly higher value of this parameter ($F = 4.24$; $F_{0.05} = 2.48$; $P = 0.007$).

**Table 4**

Parameters of chromosomal aberrations spectra for winter wheat mitotic cells ($x \pm SD$, $n = 1000$)

| Variety | Variant | Fragments (single + double) | | Bridges (chromosomal + chromatid) | | Fragments / bridges | Other (micronucleus, lagging chromosomes) | | Double and more | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | | n | % | n | % |
| Borovytsia | water | $3.0 \pm 1.0^{a}$ | 42.9 | $3.0 \pm 1.1^{a}$ | 42.9 | 1.0 | $1.0 \pm 1.6^{a}$ | 14.3 | $0.0 \pm 0.0^{a}$ | 0.0 |
| Borovytsia | EMS 0.025% | $58.0 \pm 2.6^{b}$ | 51.8 | $41.0 \pm 2.0^{b}$ | 36.6 | 1.4 | $13.0 \pm 2.0^{b}$ | 11.6 | $12.0 \pm 2.2^{b}$ | 10.7 |
| Borovytsia | EMS 0.05% | $86.0 \pm 2.9^{c}$ | 53.4 | $49.0 \pm 3.5^{c}$ | 30.4 | 1.8 | $26.0 \pm 2.3^{c}$ | 16.2 | $25.0 \pm 3.2^{c}$ | 15.5 |
| Borovytsia | EMS 0.1% | $109.0 \pm 4.1^{d}$ | 46.6 | $76.0 \pm 4.0^{d}$ | 32.5 | 1.4 | $49.0 \pm 3.6^{d}$ | 20.9 | $64.0 \pm 4.1^{d}$ | 27.4 |
| Kalancha | water | $4.0 \pm 0.7^{a}$ | 40.0 | $5.0 \pm 1.7^{a}$ | 50.0 | 0.8 | $1.0 \pm 0.6^{a}$ | 10.0 | $0.0 \pm 1.0^{a}$ | 0.0 |
| Kalancha | EMS 0.025% | $54.0 \pm 2.1^{b}$ | 55.1 | $31.0 \pm 1.6^{b}$ | 31.6 | 1.7 | $13.0 \pm 1.6^{b}$ | 13.3 | $18.0 \pm 2.0^{b}$ | 18.4 |
| Kalancha | EMS 0.05% | $79.0 \pm 3.0^{c}$ | 55.2 | $31.0 \pm 2.8^{b}$ | 21.7 | 2.6 | $33.0 \pm 2.6^{c}$ | 23.1 | $32.0 \pm 3.0^{c}$ | 22.4 |
| Kalancha | EMS 0.1% | $96.0 \pm 4.1^{d}$ | 47.8 | $58.0 \pm 3.2^{c}$ | 28.9 | 1.7 | $47.0 \pm 3.2^{d}$ | 23.4 | $48.0 \pm 3.5^{d}$ | 13.9 |
| Polianka | water | $2.0 \pm 1.2^{a}$ | 33.3 | $2.0 \pm 2.0^{a}$ | 33.3 | 1.0 | $2.0 \pm 1.3^{a}$ | 33.3 | $0.0 \pm 0.0^{a}$ | 0.0 |
| Polianka | EMS 0.025% | $64.0 \pm 3.1^{b}$ | 57.7 | $36.0 \pm 3.1^{b}$ | 32.4 | 1.8 | $11.0 \pm 2.0^{b}$ | 9.9 | $14.0 \pm 1.0^{b}$ | 12.6 |
| Polianka | EMS 0.05% | $85.0 \pm 3.4^{c}$ | 55.6 | $45.0 \pm 4.6^{b}$ | 29.4 | 1.9 | $23.0 \pm 3.0^{c}$ | 15.0 | $22.0 \pm 2.0^{c}$ | 14.4 |
| Polianka | EMS 0.1% | $107.0 \pm 3.9^{d}$ | 48.4 | $72.0 \pm 4.1^{c}$ | 32.6 | 1.5 | $42.0 \pm 4.0^{c}$ | 19.0 | $48.0 \pm 3.6^{d}$ | 21.7 |
| Pochaina | water | $3.0 \pm 1.4^{a}$ | 37.5 | $5.0 \pm 1.2^{a}$ | 62.5 | 0.6 | $0.0 \pm 0.0^{a}$ | 0.0 | $0.0 \pm 0.0^{a}$ | 0.0 |
| Pochaina | EMS 0.025% | $56.0 \pm 2.4^{b}$ | 53.3 | $34.0 \pm 2.5^{b}$ | 32.4 | 1.7 | $15.0 \pm 2.5^{b}$ | 14.3 | $11.0 \pm 1.6^{b}$ | 10.5 |
| Pochaina | EMS 0.05% | $77.0 \pm 3.5^{c}$ | 53.9 | $42.0 \pm 3.4^{c}$ | 29.4 | 1.8 | $24.0 \pm 3.3^{c}$ | 16.8 | $23.0 \pm 2.5^{c}$ | 16,2 |
| Pochaina | EMS 0.1% | $97.0 \pm 4.5^{d}$ | 44.7 | $71.0 \pm 4.4^{d}$ | 32.7 | 1.4 | $49.0 \pm 4.3^{d}$ | 22.6 | $52.0 \pm 3.5^{d}$ | 24,0 |

*Note*: different letters significant differences at $P < 0.05$ by Tukey HSD test with Bonferroni amendment; comparison in terms of one variety in the columns.

The frequency of cells with the presence of two or more aberrations was characterized by complex changes, a generally linear increase in this value after increasing the concentration. At the same time, the influence of the genotype on this process was insignificant. ($F = 1.17$; $F_{0.05} = 2.48$; $P = 0.23$); increase in concentration significantly raised the frequency of complex changes ($F = 473.22$; $F_{0.05} = 3.07$; $P = 1.32*10^{-15}$). The Tukey HSD test revealed differences in all the cases for all the varieties with increasing concentrations. The share of cells with two or more aberrations in the case of EMS 0.025% ranged 10.5% (variety Pochayna) to 18.4% (variety Kalancha). In the case of EMS 0.05%, it varied from 13.4% (variety Zeleny Gai) to 22.4% (variety Kalancha), and 13.9% (variety Kalancha) to 27.4% (variety Borovytsia) in the variant with EMS 0.1%. Again, with increasing concentration, the share of those abnormalities increased for all the varieties (except the varieties Kalancha and Balaton). The varieties Kalancha ($F = 7.11$; $F_{0.05} = 2.48$; $P = 0.001$) and Balaton ($F = 5.69$; $F_{0.05} = 2.48$; $P = 0.009$) stood out according to the dynamics of this ratio.

The discriminant analysis (Tables 5, 6 and 7, Fig. 1) confirmed the determined regularities and made it possible to identify the model variables from those studied in the cytogenetic analysis. Therefore, for the concentration factor, the number of model traits was much greater than for genotypes – in the first case, this was fertility, the total rate of chromosomal abnormalities, the frequency of fragments, lagging chromosomes and micronucleus, the presence of two or more aberrations in one dividing cell (the stage of late metaphase – early anaphase, when the evaluation of the dividing cell was carried out). This method confirmed that genotypic diversity under the action of EMS was much less important than it is usually for chemical mutagens.

**Table 5**

Factor loadings (unrotated) for winter wheat cytogenetic parameters

| Parameter | Concentration | Genotype |
|---|---|---|
| Pollen fertility | 0.896* | 0.916* |
| General rates | 0.911* | –0.342 |
| Fragments | 0.817* | –0.814* |
| Bridges | 0.317 | –0.117 |
| Other abnormalities | –0.799* | 0.255 |
| Double and more | 0.919* | 0.415 |
| Explanation variants | 4.243 | 0.998 |
| Non-explanation | 0.653 | 1.344 |

As can be seen from the resulting percentages of classification of objects in the given factor space genotype-mutagen (mutagen concentration) for changing concentrations, the success of assigning an object to the desired population ranged 87.5% to 100.0%, so in fact only a small amount of data can be lost, but in general - as a method for the EMC resolution – it was very precise.

**Table 6**

Discriminant function for winter wheat cytogenetic parameters

| Parameter | Genotype | | | Concentration | | |
|---|---|---|---|---|---|---|
| | Wilks'-Lambda | $F_{remove}$ (7.85) | P | Wilks'-Lambda | $F_{remove}$ (3.89) | P |
| Pollen fertility | 0.016 | 8.14 | 0.01 | 0.022 | 11.46 | 0.01 |
| General rates | 0.004 | 4.95 | 0.08 | 0.018 | 9.30 | 0.01 |
| Fragments | 0.021 | 8.01 | 0.01 | 0.017 | 5.33 | 0.02 |
| Bridges | 0.004 | 4.72 | 0.08 | 0.001 | 2.27 | 0.09 |
| Other | 0.008 | 4.03 | 0.09 | 0.016 | 5.07 | 0.02 |
| Double and more | 0.004 | 3.55 | 0.09 | 0.018 | 9.17 | 0.01 |

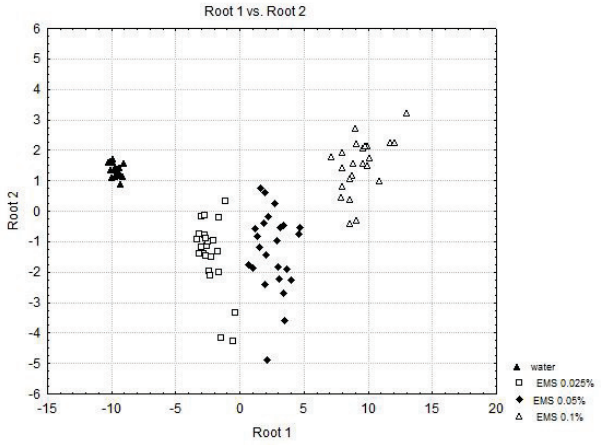


**Fig. 1.** Classification by canonical roots (discriminant analyze for concentration as factor, by Mahalanobis distances)

For genotypes (varieties), the classification frequency ranged 37.5% to 75.0%, with the average of 53.1%, which is completely insufficient. The objects belonging to the Kalancha, Polianka and (partially) Nyva Odeska genotypes can be more or less successfully classified, but even for them the error probability was quite high if the number of samples was

low. Thus, it can be considered that the genotype-mutagenic interaction is not enough for a successful division into classes in the case of the action of EMS, which may be due both to the high genetic homogeneity of the group, and the specificity of action of this mutagen, which may be significantly less site-specific.

**Table 7**
Classification ability for factor space (by canonical analysis)

| | | |
|---|---|---|
| | Water | 100 |
| Objects by concentrationss in model, % | EMS 0.025% | 87.5 |
| | EMS 0.05% | 93.8 |
| | EMS 0.10% | 100.0 |
| | Total | 96.8 |
| Objects by genotypes in model, % | Balaton | 62.5 |
| | Borovytsia | 37.5 |
| | Zeleny Hai | 37.5 |
| | Zoloto Ukrainy | 37.5 |
| | Kalancha | 75.0 |
| | Nyva Odeska | 62.5 |
| | Polianka | 75.0 |
| | Pochaina | 37.5 |
| | Total | 53.1 |

The value of the variety (genotype) was significant only for pollen fertility and this trait in its variability depends on both factors. Of the examined traits, only the rate of fragments, which has always characterized the specificity of the action of chemical mutagenic factors, had a significant representation for the genotype-mutagenic interaction. At the same time, its effect on the rate of chromosomal aberrations is clearly very strong, and this mutagen belongs to those causing a number of hereditary apparatus abnormalities in relatively low concentrations (in accordance with the above mathematical and statistical analyses).

## Discussion

According to the ontogenetic traits recorded visually and by studying the plant phenotype, germination, survival, winter tolerance, yield structures, photosynthetic activity, the main share of the cytogenetic activity parameters, with the exception of pollen sterility, did not show such an effect of the genotype and mainly depended (with the exception of some varieties) on the concentrations of the mutagen (Beiko & Nazarenko, 2022). Thus, inhibition of the mutagenic effect to a higher degree characterizes (in terms of variability of parameters) the genotype specificity than parameters of cytogenetic activity (rate and spectra of chromosomal abnormalities) (Wu et al., 2019).

The only possible conclusion is that this group of genotypes is genetically more homogeneous, which contradicts the data obtained during the study of the phenotype level (parameters of inhibition of mutagenic effect) (Wu et al., 2019). Only one suggestion remains, according to which the already noted variability is associated not with the genetic systems that are responsible for the adaptive tolerance to environment, but with those that are responsible for the creation of the adaptive potential (Abdelsalam et al., 2019).

Thus, in this case, the variability recorded in the previous stage depended on the real adaptive potential for the environmental conditions of the region. However, it manifested only in conjunction with systems that were responsible for the resistance of the variety to those environmental conditions (El-Azab et al., 2018; Von Well et al., 2018). Otherwise, it is impossible to explain the contradiction between the observed evidence of inhibition of mutagen effect, the general recommendation for the growing zones according to the yield potential realization and the effects recorded at the cytogenetic level (Chaudhary et al., 2019; Abaza et al., 2020). It could be concluded that in this case, the adaptive response mechanism simply turned out to be more complex and would be impossible to reveal without analyzing chromosomal aberrations (Bezie et al., 2020; Holeckova et al., 2021). This is the main reason why this stage of research is so important, despite the seemingly already high-level knowledge of action of individual agents (Nazarenko, 2016). In terms of interaction between mutagens and new genotypes, those studies still provide a lot of information about the course of complex processes at the level of the genetic apparatus in the first generation after mutagenic treatment (Dwinanda et al., 2020; Nazarenko, 2020). In all the cases, by the sterility reduction, the applied mutagen concentrations should be placed in the moderate range according to the generally accepted classifications (Shabani et al., 2022). However, by the induction of chromosomal abnormalities, they should clearly be classified to the extreme segment of the range of high concentrations (Nazarenko, 2016; Bhat & Wani, 2017). This is also seen from the decrease in the number of cells in the proper phases for the study. However, there were seen no critical values (which, as noted earlier, already lead to a decrease in the observed general rate of aberrations due to increased elimination of mitotic cells as mitotic index) (Han et al., 2016; Bezie et al., 2020).

This mutagen, which is typical for chemical mutagens, mainly induces fragments (i.e., the ratio of fragments to bridges in all the cases was more than one) (Badr et al., 2014; Oney-Birol & Balkan, 2019). However, after increasing the concentration of mutagen, this ratio for the second concentration increased, then decreased, which also indicates the transition of concentrations to the high range (Hase et al., 2020), since a decrease in the identifying ability of markers of cytogenetic activity is just characteristic of all the ecogenetic factors in high doses and concentrations (Li et al., 2019; Nazarenko et al., 2019). Thus, in this case, the analyzed factor was no different from the others (Amri-Tiliouine et al., 2018; Chaudhary et al., 2019).

Thehe number of complex aberrations (i.e., cells with two or more chromosomal abnormalities) significantly increases depending on concentration, whereas no such aberrations were in the control (Han et al., 2016; Nazarenko & Izhboldin, 2017) had also previously noted. The varieties were characterized by a normal level of spontaneous aberrations, slightly higher due to the lower modern plant genome stability, which has been repeatedly noted for modern cultivated plants (Caplin & Willey, 2018; Oney-Birol & Balkan, 2019). It is also typical for this mutagen that in high concentrations, the number of fragments could be lower than the number of bridge-type abnormalities with the presence of lagging chromosomes and micronucleus, which in general is not always seen for other supermutagens or is not so uniform depending on an object of action genotype (Nazarenko, 2016; Pane et al., 2018).

It should be noted that this agent is characterized by a higher level of rare types of aberrations, such as lagging chromosomes and micronucleus, especially in high doses, where they already characterize a significant part of the spectra (Nazarenko et al., 2019; Nazarenko & Izhboldin, 2017). It was also noted that the significance of this type of abnormality and its significant proportion also increase with increasing concentration, as in the case of previously studied mutagens. In general, this mutagen is characterized by a lower level of genotype-mutagenic interaction and its more complex nature than those previously studied (Nazarenko & Izhboldin, 2017). At the same time, this allows one to hope for more predictable changes in subsequent generations, already associated with obtaining valuable hereditary changes.

## Conclusion

The use of any agent causes hereditary changes, especially such a specific group of chemical compounds as supermutagens. It can never be a routine procedure and cannot be provided with a standard protocol. Nonetheless, in-depth study, the level of knowledge about the mechanisms of adaptive response at the cell level is still insufficient, and the possibilities of genetic control in the area of DNA reactivity, both in the chemical and biological senses, are excessively broad issues. The introduction of germplasm from various new and old samples (landraces), the maximum use of biodiversity and local material, and the avoidance of using exclusively super varieties, leads to the fact that new features constantly appear even when standard, well-studied substances are used according to the conventional protocols. Cytogenetic activity in this case may contradict the observed phenoeffects and, at the same time, have a closer relationship with the further rate and frequency of hereditary changes, not so much in terms of establishing a connection with individual types of changes, which is hardly possible at this level, but more with predicting the value of polymorphism for a particular genotype in combination with a specific agent's action and a significant increase in monitoring in subsequent generations to identify promising forms for various genetically and agriculturally valuable changes.

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