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Cytogenetic activity of a mutagenic factor with high damaging capacity in winter wheat

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Abstract. The analysis of cytological abnormalities is an important method for identifying the potential of a chemical as a mutagen for future heritable genetic changes, the level of genotype-mutagen interaction and site-specific activity for the nature and/or different concentrations of the mutagen. The research aims to determine the limits of the variability of genotypes of different origins, especially those with wide ecological and genetic variability, and to show the interaction between the variety and the mutagen. Winter wheat grains of several varieties (Balaton, Borovytsia, Zelenyi Hai, Zoloto Ukrainy, Kalancha, Niva Odeska, Polianka, Pochayna) were treated with dimethyl sulphate at concentrations of 0.0125%, 0.025%, 0.05%, and exposure was 24 hours. The cytogenetic activity was studied by the frequency and spectrum of chromosomal rearrangements in the corresponding phases of cell division, depending on the variety and concentration of the mutagen as the main factors affecting these parameters, as well as the main features of the spectrum, such as the overall rate of chromosomal rearrangements, the number of fragments and double fragments, bridges, micronuclei, and lagging chromosomes. The studied concentrations of the supermutagen were found to have a significant effect on all analysed parameters and can be classified as optimal and high concentration levels in terms of the effect on cytological activity and mitotic problems for the factor, despite previous studies. The variety factor has a much greater impact on the nature and frequency of certain types of aberrations than an increase in the mutagen concentration, it was characterised by a much greater site-specific effect than other chemical agents, and various variants in mutagenic effects were identified according to the subject's genotype. It was generalised that the features that reproduced the effect of the mutagen, according to the discriminant

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analysis, were the total frequency, the frequency of fragments and double fragments, and bridges. In practical use in the genetic improvement of plants and for obtaining valuable traits, the optimal concentrations are 0.0125%, and 0.025%, which is planned to be further confirmed on a wider range of genotypes and by mutation studies for the next (second or third) generations

Keywords: chromosomal aberrations; mutagen; winter wheat; dimethyl sulphate; cytological analysis

INTRODUCTION

The study of the chemical factors' activity identifies the success of the respective substance in inducing both general variability in plant material and specificity in the possibility of detecting certain types of changes in treated plants in subsequent generations. As long-term studies have shown, the level of activity depends on the future overall frequency of changes and is significantly adjusted by the subject of the action, while the specificity of the action directly depends on the reproduction of the specifics of the factor's effect by individual indicators.

Following previous studies (Küçük & Liman, 2018; Cann *et al.*, 2022), the use of mutagenic factors for genetic improvement of cultivated plants should be preceded by careful monitoring of their mutational activity and damage capacity. The study was carried out both at the level of the plant as a whole (mutagenic depression in terms of plant growth and development in the first generation) and at the level of the organism, to determine cytogenetic activity at the cellular level (analysis of chromosomal aberrations, their frequencies depending on the genotype and dose (concentration) of the mutagen, characteristics of the spectrum of cytogenetic variability and analysis of the ratios of certain types of rearrangements).

Monitoring of cytogenetic activity allows not only to establish the potential activity of a chemical as a mutagen, as indicated in previous studies (Ariraman *et al.*, 2018; Abaza *et al.*, 2020; Yali & Mitiku, 2022) but also to conclude the nature of the aberrations formed and their correlation with the possible genotype-mutagenic interaction and the suitability of individual factors for acting on certain genotypes, which has already been established many times. This is of particular importance for chemical mutagenesis, given the significant specific feature of chemicals (mechanism of action through structural correspondence to certain DNA sites) established by previous studies (Von Well *et al.*, 2018; Chaudhary *et al.*, 2019).

Several researchers (Yang *et al.*, 2019; Hussain *et al.*, 2021) have shown that varietal material is rather poorly studied in this area, and even for classical factors, there is still variability that can reach quite significant limits. S. Nurmansyah *et al.* (2018) and E. El-Azab *et al.* (2018) proved that only by using the DNA structure of the original forms, firstly, it is possible to increase mutational variability or reduce possible depressive effects when using forms that are very sensitive to this factor (especially on the verge of semi- and sublethality of certain indicators), and secondly, to predict the level of variability of the resulting material. H. Ram *et al.* (2019) found that an important feature of identifying a mutagenic effect at the cellular level is the ability to determine the lethality of a particular factor and its practically useful concentrations in terms of its genetic activity. When certain thresholds are reached, the use of higher concentrations is inappropriate and leads, instead of increasing, to a decrease in the induction of new forms and their variability, which is associated with a too-high toxic effect and an increase in the magnitude of changes in DNA (Hong *et al.*, 2022).

M. Spencer-Lopes *et al.* (2018) showed that sensitivity to mutagens is genetically determined, and while two main genetically determined resistance mechanisms are known for physical factors, in particular gamma rays, summarised in the relevant protocols, a significantly larger number of them are predicted for chemical agents. It should be noted that one of these mechanisms was discovered precisely when studying the cytogenetic activity of local forms of cereal crops, so cases where individual genotypes in studies (Yang *et al.*, 2019; Hase *et al.*, 2020) demonstrate a rather specific, uncharacteristic genotype response to a particular factor may indicate a new or modified mechanism of resistance and/or sensitivity to mutagenic effects. This is especially true given the predominantly recessive control of these types of resistance.

The research aims to identify the peculiarities of genotype mutagenic action given the ecological and genetic variability of subjects in terms of cytogenetic activity parameters under the influence of a factor with high damaging ability.

MATERIALS AND METHODS

Cytological analysis studies were conducted in the laboratory at the Department of Breeding and Seed Production of Dnipro State Agrarian and Economic University in 2021-2022. The experiment was conducted on the seeds of eight winter wheat varieties, which were selected in such a way as to maximise the biodiversity of the crop varieties used for agriculture (Balaton, Borovytsia, Zelenyi Hai, Zoloto Ukrainy, Kalancha, Nyva Odeska, Polianka, Pochaina). These varieties in the first generation were classified into two groups in the field studies under the action of the mutagen according to the effects of depression, so to simplify the research

design, we further studied not only the behaviour of each genotype separately but also linked the data obtained to the groups.

Samples of winter wheat grain were treated with an aqueous solution of DMS (dimethyl sulphate, hereinafter referred to as DMS) at concentrations of 0.0125, 0.025 and 0.05%. The control was soaked in distilled water. The number of treated grains should have ensured the maintenance of at least 25-30 samples with a developed primary root system in the future (at least 100 grains). The selected concentrations were experimentally proposed for protocols for the mutational improvement of winter wheat. The exposure period was 24 hours.

The cytological analysis of chromosomal abnormalities under the action of the mutagen was performed on temporary preparations of the primary root system meristem at the corresponding stages of mitosis in late metaphase and early anaphase. All types of aberrations were studied using Micromed XS-3330 light microscopy with a 600x magnification on pressed root tip preparations (2 mm with root length of 1.0-1.2 cm). In each variant, up to 1000 corresponding cells were used if available. At a high level of elimination, at least 500 were used.

After exposure to the appropriate concentration of DMS, the samples were cultured in Petri dishes on filter paper with distilled water in a thermostat at 20-22°C. The tips of the roots were then cut off and fixed in Clark's fixative, consisting of 3 parts 96% medical alcohol and 1-part ice-cold acetic acid, for at least 24 hours. The samples were stored in a solution of 70% ethyl alcohol at 2°C in a refrigerator. For each variant, up to 30 samples were prepared (but not necessarily all were used). The resulting pressed root tip preparations were stained with acetocarmine. For this method of cytogenetic analysis, such abnormalities as fragments and double fragments, chromatid and chromosomal bridges, micronuclei, and lagging chromosomes were examined. Cells with multiple abnormalities were counted separately.

The values in the tables are presented as means with standard deviation x±SD. The difference between the variants was determined by factor analysis (ANOVA module) and was considered significant at $P<0.05$. The normality of the distribution was investigated by the Shapiro-Wilks test, pairwise comparison was performed by the Tukey HSD test, and discriminant analysis was performed by the multiple statistics modules of Statistica 10.

RESULTS AND DISCUSSION

The study of the cytogenetic activity of a mutagenic factor includes such steps as calculating the total frequency of chromosomal aberrations, indicators of the spectrum of existing changes, and studying the nature and concentration of mutagens on the variability of these indicators. The total frequency of chromosomal aberrations generally depended on the change in concentration according to the factor analysis ($F=46.17$; $F_{0.05}=3.07$; $P=3.19*10^{-6}$) and, especially, on the genotype of the source material ($F=112.47$; $F_{0.05}=2.48$; $P=8.26*10^{-9}$), which significantly differed from the previously studied genetic activity of other, less harmful mutagens (Table 1). The total frequency, in general, varied in the control from 0.6 % in Polianka variety to 1.00% in Balaton variety, from 13.47 (Nyva Odeska variety) to 17.78% in Balaton variety under the influence of DMS concentration 0. 0125%, under the action of DMS 0.025% from 14.18% in Kalancha to 20.26% in Balaton, from 12.99% in Nyva Odeska to 23.91% in Balaton with increasing concentration to DMS 0.05%. In contrast to the previously studied mutagens, the specificity of the dynamics of frequency changes in different genotypes was noted. Thus, the frequency gradually increases in Balaton and Polyanka, the frequency remains at the same level and then increases in Zelenyi Hai, Zoloto Ukrainy, Borovytsia, and Kalancha (i.e. this is the most typical reaction), the frequency first increases and then decreases in Nyva Odeska, increases and then remains at the same level in Pochaina.

Table 1. *The total frequency of chromosomal rearrangements in mitotic cells in the corresponding phases (x±SD, n=500-1000)*

Variety	Concentration	Mitoses, pcs.	Chromosomal rearrangements	
			pcs.	%
Balaton	water	1002	9	0.90±0.11[a]
Balaton	DMS 0.0125%	1007	179	17.78±0.51[b]
Balaton	DMS 0.025%	923	187	20.26±0.48[c]
Balaton	DMS 0.05%	640	153	23.91±0.37[d]
Zelenyi Hai	water	1008	8	0.79±0.12[a]
Zelenyi Hai	DMS 0.0125%	1001	175	17.48±0.39[b]
Zelenyi Hai	DMS 0.025%	910	161	17.69±0.50[b]
Zelenyi Hai	DMS 0.05%	610	123	20.16±0.69[c]
Zoloto Ukrainy	water	1001	8	0.80±0.21[a]

Table 1, Continued

Variety	Concentration	Mitoses, pcs.	Chromosomal rearrangements	
			pcs.	%
Zoloto Ukrainy	DMS 0.0125%	1008	174	17.26±0.39[b]
Zoloto Ukrainy	DMS 0.025%	890	150	16.85±0.37[b]
Zoloto Ukrainy	DMS 0.05%	610	117	19.18±0.43[c]
Nyva Odeska	water	1009	9	0.89±0.19[a]
Nyva Odeska	DMS 0.0125%	1010	136	13.47±0.40[b]
Nyva Odeska	DMS 0.025%	889	130	14.62±0.28[c]
Nyva Odeska	DMS 0.05%	670	87	12.99±0.47[b]
Borovytsia	water	1001	7	0.70±0.20[a]
Borovytsia	DMS 0.0125%	1009	157	15.56±0.32[b]
Borovytsia	DMS 0.025%	930	148	15.91±0.33[b]
Borovytsia	DMS 0.05%	578	106	18.34±0.41[c]
Kalancha	water	1000	10	1.00±0.15[a]
Kalancha	DMS 0.0125%	1003	139	13.86±0.34[b]
Kalancha	DMS 0.025%	924	131	14.18±0.39[b]
Kalancha	DMS 0.05%	619	97	15.67±0.41[c]
Polianka	water	1007	6	0.60±0.26[a]
Polianka	DMS 0.0125%	1005	148	14.73±0.36[b]
Polianka	DMS 0.025%	917	145	15.81±0.39[c]
Polianka	DMS 0.05%	734	129	17.57±0.51[d]
Pochaina	water	1005	8	0.80±0.06[a]
Pochaina	DMS 0.0125%	1002	139	13.87±0.35[b]
Pochaina	DMS 0.025%	901	152	16.87±0.33[c]
Pochaina	DMS 0.05%	712	118	16.57±0.37[c]

Note: *indicates a significant difference at P<0.05 for the Tukey test with Bonferroni correction. Comparison within the same variety*

Source: *developed by the authors*

Tukey's test for pairwise comparison showed that Balaton (F=11.09; $F_{0.05}$=2.48; P=0.001), Nyva Odeska (F=8.34; $F_{0.05}$=2.48; P=0.005), Pochaina (F=4.53; $F_{0.05}$=2.48; P=0.02) were significantly different from other varieties. Thus, regarding the difference in response between genotypes, the following was found in the pairwise comparison: varieties Zelenyi Hai, Zoloto Ukrainy, Borovytsia, Kalancha, and Polianka form a more or less homogeneous group, while varieties Balaton, Nyva Odeska, Pochaina differ from them and each other.

Considering such an indicator as the number of cells studied, it can be established that only a concentration of 0.0125% of DMS allowed us to study the appropriate number of cells. Already at a concentration of 0.025%, there was a sufficiently high elimination of the genetic apparatus of cells to fail to collect such a number (the difference between genotypes is insignificant), and a concentration of 0.025% of DMS led to high cell death in the corresponding phases, especially for the varieties Balaton, Zelenyi Hai, Zoloto Ukrainy, Borovytsia, Kalancha. The varieties Nyva Odeska, Polianka, and Pochaina were more resistant (F=8.90; $F_{0.05}$=4.10; P=0.004), but they have a more characteristic plateau in the effect of the mutagen at high concentrations (except for Polyanka). That is, the overall decrease in frequency at high concentrations cannot be explained by cell death alone; the mechanism is more complex and includes a ban on the rearrangement of individual sites after reaching a certain limit in aberrations. The peak of genetic activity should be between the second and third concentrations.

Individual indicators of the chromosomal aberration spectrum are presented following the level of depression at the level of the organism as a whole in Tables 2 (higher depression) and 3 (more stable). The analysis was based on the following parameters: the presence of bridges, fragments, the ratio between fragments and bridges (since the first type of aberration is mainly induced by chemical mutagens, the second – by physical ones), the presence of less frequent types of aberrations such as micronuclei and lagging chromosomes, and the presence of cells with two or more aberrations were calculated separately.

Table 2. *Parameters of the spectrum of adjustments. First group (x±SD, n=500-1000)*

Variety	Concentration	Fragments (single + double)		Bridges (chromosomal + chromatid)		fragments/ bridges	Other (micronuclei, lagging chromosomes)		Double or more	
		n	%	n	%		n	%	n	%
Balaton	water	4.0±0.4[a]	44.4	4.0±1.7[a]	44.4	1.0	2.0±0.9[a]	22.2	0.0±0.0[a]	0.0
Balaton	DMS 0.0125%	96.0±1.6[b]	53.6	61.0±2.1[b]	34.1	1.6	22.0±1.7[b]	12.3	32.0±1.7[b]	17.9
Balaton	DMS 0.025%	101.0±1.9[b]	54.2	64.0±2.2[b]	34.2	1.6	22.0±1.5[b]	11.8	30.0±2.7[b]	16.0
Balaton	DMS 0.05%	75.0±2.4[c]	49.0	62.0±3.4[b]	40.5	1.2	16.0±1.9[c]	10.5	22.0±1.5[c]	14.4
Zelenyi Hai	water	4.0±1.0[a]	50.0	3.0±0.6[a]	37.5	1.3	1.0±1.1[a]	12.5	0.0±0.0[a]	0.0
Zelenyi Hai	DMS 0.0125%	109.0±1.2[b]	62.3	47.0±3.1[b]	26.9	2.3	19.0±2.5[b]	10.9	26.0±2.0[b]	14.9
Zelenyi Hai	DMS 0.025%	97.0±1.3[c]	60.2	46.0±2.7[b]	28.6	2.1	18.0±2.2[b]	11.2	23.0±2.5[b]	14.3
Zelenyi Hai	DMS 0.05%	75.0±2.2[d]	61.0	34.0±2.5[c]	27.6	2.2	14.0±2.1[b]	11.4	16.0±2.3[c]	13.0
Zoloto Ukrainy	water	5.0±1.5[a]	62.5	3.0±1.2[a]	37.5	1.7	0.0±0.0[a]	0.00	0.0±0.0[a]	0.0
Zoloto Ukrainy	DMS 0.0125%	93.0±3.0[b]	53.5	59.0±2.4[b]	33.9	1.6	22.0±1.8[b]	12.6	29.0±1.4[b]	16.7
Zoloto Ukrainy	DMS 0.025%	78.0±3.3[c]	52.0	52.0±3.1[b]	34.7	1.5	20.0±2.0[b]	13.3	29.0±2.3[b]	19.3
Zoloto Ukrainy	DMS 0.05%	61.0±3.1[d]	52.1	42.0±2.9[c]	35.9	1.5	14.0±2.1[c]	12.0	17.0±3.0[c]	14.6
Nyva Odeska	water	4.0±1.6[a]	44.4	4.0±1.2[a]	44.4	1.0	1.0±1.2[a]	11.1	1.0±1.5[a]	11.1
Nyva Odeska	DMS 0.0125%	79.0±2.3[b]	58.1	35.0±1.8[b]	25.7	2.3	22.0±2.0[b]	16.2	32.0±2.0[b]	23.5
Nyva Odeska	DMS 0.025%	74.0±3.2[b]	56.9	33.0±2.1[b]	25.4	2.2	23.0±2.1[b]	17.7	29.0±2.0[b]	22.3
Nyva Odeska	DMS 0.05%	55.0±4.1[c]	60.9	20.0±2.1[c]	23.0	2.7	14.0±2.2[c]	16.1	19.0±2.2[c]	21.8

Note: *indicates a significant difference at P<0.05 for the Tukey test with Bonferroni correction. Comparison within the same variety*

Source: *developed by the authors*

Analysing the data for individual types of rearrangements, it can be seen that according to the factor analysis, the frequency of fragments is highly dependent on the concentration (F=34.33; $F_{0.05}$=3.07; P=1.16*10^{-5}) and, to a lesser extent, on the genotype of the source material (F=29.90; $F_{0.05}$=2.48; P=4.17*10^{-4}). In general, the number of fragments varied at 0.0125% DMS from 79 in Nyva Odeska in the first group (Table 2) and 73 in Pochaina and Kalancha (second group, Table 3) to 109 in Zelenyi Hai and 85 in Polianka. Under the influence of 0.025% DMS, from 74 in Nyva Odeska in the first group, 70 in Kalancha to 97 in Zelenyi Hai (first group), 87 in Polianka (second group). Under the influence of DMS, 0.05% from 55 in Nyva Odeska to 75 in Balaton and Zelenyi Hai (first group), from 54 in Kalancha to 76 in Polianka (second group).

In the first group, the absolute amount is higher, and the relative weight in the spectrum is lower in the second group, except for the variety Polyanka, i.e. the effect of the mutagen is less pronounced. At the same time, in Balaton, Nyva Odeska, Borovytsia, Polianka, Pochaina (F=3.11; $F_{0.05}$=4.10; P=0.07) there is no statistically significant difference between the effect of the first and second concentration, while in Zelenyi Hai, Zoloto Ukrainy and Kalancha (F=11.25; $F_{0.05}$=4.44; P=0.004) the difference at each concentration is significant. The number of fragments between the control and the first concentration, the second and the third concentration always changes significantly within the grade. It should be noted that, in general, the proportion of this type of chromosomal aberration varies between 50 and 60% of the total number in the spectrum. The following groups can be distinguished by variability: in the Balaton variety (F=4.45; $F_{0.05}$=2.48; P=0.01), the proportion is generally decreasing, in all other varieties it is at the same level, without significant variations.

As for the frequency of bridges, there is a characteristic dependence on the concentration (F=5.09, $F_{0.05}$=3.07, P=0.02), and a relatively stronger dependence on the genotype of the source material (F=9.34, $F_{0.05}$=2.48, P=0.007). In general, the number of bridges varied at 0.0125% DMS from 35 in Nyva Odeska in the first group (Table 2) and 34 in Kalancha (second group, Table 3) to 61 in Balaton and 47 in Borovytsia. Under the influence of 0.025% DMS, from 33 in Nyva Odeska in the first group, 30 in Borovytsia to 64 in Balaton (first group), and 44 in Borovytsia (second group). Under the influence of DMS 0.05% from 20 in Nyva Odeska to 62 in Balaton (first group), from 23 in Kalancha to 37 in Pochaina (second group).

Table 3. *Parameters of the restructuring spectrum. The second group (x±SD, n=500-1000)*

Variety	Concentration	Fragments (single + double)		Bridges (chromosomal + chromatid)		fragments/ bridges	Other (micronuclei, lagging chromosomes)		Double or more	
		n	%	n	%		n	%	n	%
Borovytsia	water	3.0±1.0[a]	42.9	3.0±1.1[a]	42.9	1.0	1.0±1.6[a]	14.3	0.0±0.0[a]	0.0
Borovytsia	DMS 0.0125%	82.0±3.1[b]	52. 2	47.0±2.0[b]	30.0	1.7	28.0±1.9[b]	17.8	26.0±2.0[b]	16.6
Borovytsia	DMS 0.025%	78.0±1.9[b]	52.7	44.0±2.5[b]	30.0	1.8	26.0±2.4[b]	17.6	25.0±2.2[b]	16.9
Borovytsia	DMS 0.05%	57.0±3.0[c]	53.7	30.0±3.0[c]	28.3	1.9	19.0±2.0[c]	17.9	20.0±3.1[b]	18.9
Kalancha	water	4.0±0.7[a]	40.0	5.0±1.7[a]	50.0	0.8	1.0±0.6[a]	10.0	0.0±1.0[a]	0.0
Kalancha	DMS 0.0125%	73.0±2.2[b]	52.5	34.0±1.8[b]	24.5	2.2	32.0±1.4[b]	23.0	29.0±1.5[b]	20.9
Kalancha	DMS 0.025%	70.0±2.0[b]	53.4	32.0±2.2[b]	24.4	2.2	29.0±2.1[b]	22.0	28.0±2.0[b]	21.4
Kalancha	DMS 0.05%	54.0±1.1[c]	55.7	23.0±2.2[c]	23.7	2.4	20.0±2.2[c]	20.7	19.0±2.5[c]	19.6
Polianka	water	2.0±1.2[a]	33.3	2.0±2.0[a]	33.3	1.0	2.0±1.3[a]	33.3	0.0±0.0[a]	0.0
Polianka	DMS 0.0125%	85.0±2.1[b]	57.4	43.0±2.1[b]	29.0	2.0	20.0±1.4[b]	13.5	23.0±1.4[b]	15.4
Polianka	DMS 0.025%	87.0±2.4[b]	60.0	41.0±2.6[b]	28.3	2.1	17.0±2.0[b]	11.7	22.0±2.1[b]	15.1
Polianka	DMS 0.05%	76.0±2.9[c]	58.9	31.0±3.1[c]	24.0	2.5	22.0±3.0[b]	17.1	31.0±2.6[c]	24.0
Pochaina	water	3.0±1.4[a]	37.5	5.0±1.2[a]	62.5	0.6	0.0±0.0[a]	0.0	0.0±0.0[a]	0.0
Pochaina	DMS 0.0125%	73.0±2.1[b]	52.5	40.0±2.6[b]	28.8	1.8	26.0±2.4[b]	18.7	25.0±1.4[b]	18.0
Pochaina	DMS 0.025%	78.0±2.5[b]	51.3	42.0±3.3[b]	27.6	1.9	32.0±3.0[c]	21.1	28.0±1.5[bc]	18.4
Pochaina	DMS 0.05%	60.0±3.1[c]	50.9	37.0±3.4[b]	31.3	1.6	21.0±3.3[b]	17.8	24.0±1.9[b]	20.3

Note: *indicates a significant difference at P<0.05 for the Tukey test with Bonferroni correction. Comparison within the same variety*

Source: *developed by the authors*

The absolute amount is again higher in the first group, and the relative weight in the spectrum is approximately equal in both groups and depends more on the individual variety, but it is impossible to establish patterns. The variability in concentrations is much lower. Thus, in the Balaton and Pochaina varieties (F=2.46, $F_{0.05}$=4.40, P=0.09) there is no statistically significant difference between the effects of individual concentrations, while in other varieties there is no difference between the first and second concentration (F=1.34, $F_{0.05}$=2.55, P=0.08), only between the control and the first and third and all other concentrations. In general, the proportion of this type of chromosomal aberration varies between 20-40% of the total number in the spectrum. The following groups can be distinguished by their variability: in the Balaton variety, the proportion is generally increasing, in the Polianka variety it is decreasing, and in all other varieties it is at the same level, without significant variations. As for the integrative index of the ratio of bridges to fragments, in all cases under the action of the mutagen it exceeded 1, which is typical for the action of chemical mutagens. This is least pronounced in the Balaton variety, with a significant predominance of fragments in the varieties Zelenyi Hai, Nyva Odeska, Kalancha, and Polianka.

As for the presence of liquid aberrations, their number depends on the concentration (F=3.91; $F_{0.05}$=3.07; P=0.04) but does not depend on the genotype of the source material (F=2.40; $F_{0.05}$=2.48; P=0.06). In general, the number of micronuclei and lagging chromosomes varied at 0.0125% DMS from 19 in Zelenyi Hai in the first group (Table 2) and 20 in Polianka (second group, Table 3) to 22 in Balaton, Zoloto Ukrainy, Nyva Odeska and 32 in Kalancha. Under the effect of 0.025% DMS, from 18 in Zelenyi Hai in the first group, 17 in Polianka to 23 in Nyva Odeska (first group), and 32 in Pochayna (second group). Under the influence of 0.05% of the DMS, from 14 in three varieties to 16 in Balaton (first group), there is virtually no variation, from 19 in Borovytsia to 22 in Polyanka (second group). In general, the number is much lower than other types of aberrations.

In this case, the absolute amount is higher in the second group, and the relative weight in the spectrum is relatively higher in the second group (except for Polianka and considering that Nyva Odeska is approximately at the same level as other varieties in the second group) and is more dependent on the individual variety. The variability in concentrations is even lower than that of bridges. Thus, in the varieties Zelenyi Hai and Polianka (F=2.99; $F_{0.05}$=4.40; P=0.08) there is no statistically significant difference between the effect of individual concentrations, while in other varieties there is no difference between the first and second concentration (F=2.55; $F_{0.05}$=3.01; P=0.07), only between the control and the first and third concentrations, and all other varieties with a significant decrease in the

number under the highest concentration (except for Pochaina, which stood out with a higher number under the second concentration). In general, the proportion of this type of chromosomal aberration varies between 10 and 25% of the total number in the spectrum. The following groups can be distinguished by their variability: in the first group and varieties Borovytsia and Kalancha, it is approximately at the same level, in the variety Polianka it decreases at the second concentration and increases again to the same level at the third concentration, in the variety Pochaina it increases to increases to the second concentration and then decreases to the same level.

As for the presence of cells with complex aberrations (two or more rearrangements), their number depends on the concentration (F=5.99, $F_{0.05}$=3.07, P=0.03), but again does not depend on the genotype of the source material (F=2.12, $F_{0.05}$=2.48, P=0.07) according to the factor analysis. When counting the number of cells with complex chromosomal rearrangements, the effect of 0.0125% DMS varied from 26 in Zelenyi Hai in the first group (Table 2) and 23 in Polyanka (second group, Table 3) to 32 in Balaton, Nyva Odeska and 29 in Kalancha. Under the influence of DMS 0.025% from 23 in Zelenyi Hai in the first group, 22 in Polyanka to 30 in Balaton (first group), and 28 in Polianka, Pochaina (second group). Under the influence of 0.05% DMS, from 16 in Zelenyi Hai to 22 in Balaton (first group), from 19 in Kalancha to 31 in Polianka (second group). In general, the number of such cells is quite high in comparison with other mutagens, but the lethal effect of high concentrations is affected.

In this case, in the second group, the absolute number is higher at higher concentrations, which is associated with increased cell elimination in the first group, the relative weight in the spectrum is relatively higher in the second group (considering the lower one in the variety Polyanka from the second group and the higher one in the variety Nyva Odeska), this parameter shows a high genotype-mutagenic interaction in the form of a variety reaction as a subject of mutagenic action. The variability in concentrations is quite high but lower than in the previous parameters. Thus, only in the Pochaina variety, the effect of the second concentration is partially different, while in other varieties there is no difference between the first and second concentration (F=2.01; $F_{0.05}$=3.40; P=0.09), there is a statistically significant difference only between the control and the first and second concentration, between the first and second concentrations and the third, highest concentration. In general, the proportion of this type of chromosomal aberration varies between 10-25% of the total number in the spectrum. Such varieties as Polianka (the number of cells with multiple aberrations increases at the third concentration) (F=9.46; $F_{0.05}$=2.48; P=0.001) and Pochaina (the indicator does not reflect any increase in concentration) (F=4.00; $F_{0.05}$=2.48; P=0.03) stood out for their variability in pairwise comparison, which indicates the absence of threshold concentrations of significance for viability. All other varieties are characterised by approximately the same value at the first and second concentrations and a sharp decline at the third.

The analysis of the factor loadings of individual signs of cytogenetic variability (Table 4) showed that for the genotype, such parameters as the total frequency, fragments, bridges, rare types of aberrations, and the presence of cells with multiple rearrangements were significantly variable, although some were on the verge of being unreliable. That is, concentration as a factor was more significant, since in the case of genotype, two indicators of the presence of other types of rearrangements and complex rearrangements were not significant, indicating the fact of the reliability of deviations in the reaction of only two varieties of Polianka and Pochaina (in one case, somewhat Zelenyi Hai).

Table 4. *Factor load by cytogenetic parameters*

Parameter	Concentration	Genotype
General frequency	0.817*	-0.934*
Fragments	0.816*	0.844*
Bridges	0.790*	-0.749*
Other types of aberrations	-0.678*	0.436
Complex	0.717*	0.501
Overall explained	2.983	2.114
Unexplained	0.990	1.095

Note: ** – important parameters*

Source: *developed by the authors*

The discriminant analysis (Table 5, Fig. 1) confirmed the data obtained, somewhat specifying the traits that reproduced the effect of the mutagen. The total frequency, number of fragments, and bridges depended on the genotype. It should be noted that for chemical mutagens, especially weaker ones, the number of bridges is not always included in the model. All parameters changed when the concentration of DMS changed. In the classification

space of the canonical functions, the control was clearly distinguished with a large distance. The grouping also showed a statistically significant difference in the case of higher concentrations. Concentrations of 0.0125 and 0.025% of DMS did not differ in the factor space and formed one continuous mixed group.

Table 5. *Discriminant function values for cytogenetic activity parameters*

Parameter	Genotype			Concentration		
	Wilks' - Lambda	F_{remove} (7.85)	p-level	Wilks' - Lambda	F_{remove} (3.89)	p-level
General frequency	0.022	10.87	0.01	0.017	8.07	0.01
Fragments	0.020	11.34	0.01	0.018	9.76	0.01
Bridges	0.015	8.77	0.03	0.015	4.56	0.03
Other	0.004	3.67	0.07	0.014	4.66	0.04
Two or more	0.003	3.16	0.08	0.014	4.19	0.05

Source: *developed by the authors*

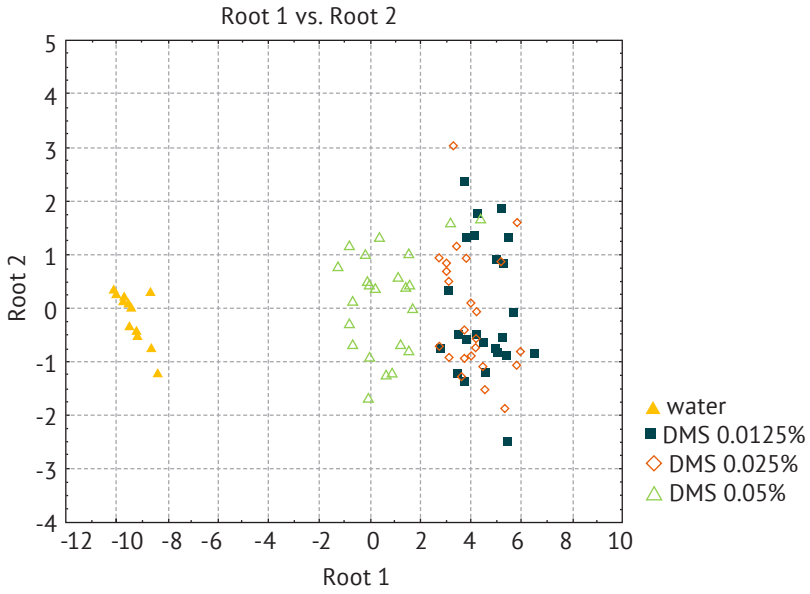

Figure 1. *Classification by concentrations in the factor space of canonical functions*

The strength of the classification of individual parameters was the same for both genotypes and concentrations, which is significantly different from previous studies, where concentrations had a significantly (50%) higher strength when divided into groups (Table 6). First of all, this is due to the low potency for the first two concentrations, while for the control and 0.05% DMS, it is quite high. Among the genotypes, we can hope for a reliable classification for Balaton, Nyva Odeska, Polianka, and Pochaina, especially for the first and third. While the other genotypes form a separate group, with no significant difference between them. That is, the studied varieties can be divided into three separate groups according to their efficiency: Balaton and Polianka, with high genotype-mutagenic interaction (although it can be different in consequences, always with high identification ability), Nyva Odeska and Pochaina – mediocre genotype-mutagenic interaction (can be different) and the other 4 varieties with low genotype-mutagenic interaction.

Table 6. *Classification ability of objects belonging to separate concentrations and genotypes*

	water	100
Objects in the model, concentration, %	DMS 0.0125%	32.0
	DMS 0.025%	32.0
	DMS 0.05%	83.5
	Total	62.0

Table 6, Continued

	water	**100**
Objects in the model, genotype, %	Balaton	92.5
	Borovytsia	37.5
	Zelenyi Hai	52.5
	Zoloto Ukrainy	37.5
	Kalancha	37.5
	Nyva Odeska	75.0
	Polianka	92.5
	Pochaina	75.0
	Total	62.5

Source: *developed by the authors*

According to such an integrative sign of mutagen concentration lethality as the number of cells with multiple aberrations (with the addition of the total frequency), for most varieties, the critical concentration can be between the DMS 0.025% and DMS 0.05% with a plateau in the range of DMS 0.0125% and DMS 0.025% or to the right of the higher one. Based on the nature of the expression of individual parameters, it can be concluded that the first hypothesis is more justified. For the Polianka and Balaton varieties, the critical concentration lies further away from the 0.05% DMS, while the Pochaina variety is characterised by a long plateau under the influence of all concentrations.

This study is part of a series of studies analysing the effects of a genotype-mutagenic set of chemical factors on a corresponding set of genotypes. Previous analyses of the data obtained showed (Nazarenko, 2017; Beiko & Nazarenko, 2022; Horshchar & Nazarenko, 2022) a decrease in site specificity with increasing mutagen activity, but when using DMS, the trend reversed, and site specificity increased significantly. This is evident from the pairwise comparison of genotypes but was not reflected in the number of traits with a significant effect of genotype, which may indicate the contrast of only individual varieties.

The plateau found in the studies, depending on the increase in the dose of the mutagen and the variety, has already been noted by researchers (Oney-Birol & Balkan, 2019), with a general decrease in mitotic activity and a gradual increase in the frequency of rearrangements in other material. This effect is typical in the range of high values of the agents of action. In addition, it demonstrated the ability to reduce the genotoxicity index, which in total allowed the researchers to conclude that the mutagenic effect is highly dependent on the characteristics of a particular variety as its subject.

Protocols of cytogenetic studies of mutagenic factors show (Spencer-Lopes *et al.*, 2018) that such values, although they belong to high and ultra-high doses and concentrations, have quite wide limits of variability in the case of continuous mutagens, but are 1.5-2 times lower for chemical supermutagens. The difference in recommendations is the use of such doses in the case of physical factors, while for chemical factors these are pre-critical concentrations (Bezie *et al.*, 2020), given the maximum values of usefulness in future generations (in the case of the studied material, DMS 0.0125 and 0.025%, except for the varieties Polyanka and Pochayna, for which the use of DMS 0.05% is more effective for obtaining complex changes)

Previous studies (El-Azab *et al.,* 2018; Pane *et al.,* 2018) have often found a lower significance of the subject of mutagenic influence, mainly at the level of the organism as a whole, The experiment confirms the significance of this conclusion and proves the preservation of the identified trends in a partial form at the cellular level, taking into account some changes that are related to the structural features of the genome of certain varieties and that may not affect the level of fixation of depressive effects in terms of phenotypic indicators in the first generation. This may indicate the possible presence of separate genetically determined mechanisms of resistance to the mutagenic factor and their increasing importance with the increase in the strength of the mutagen, even though previous studies (Nader *et al.,* 2022) also associated a decrease in the site specificity of some chemicals, especially new classes of nanoparticles, which, despite the overall negative effect on mitotic activity and induction of a significant number of rearrangements, may not have shown any dependence on the subject of action.

Despite the peculiarities of the action of each mutagen in the group as a whole, the greater significance of such classical indicators (Nurmansyah *et al.*, 2018) as the total frequency, number and ratio of fragments and bridges does not decrease. They are the main indicators of mutagenic activity at the cytogenetic level, as established by cycles of previous studies (Bhat & Wani, 2017), especially in terms of identifying the properties of a chemical agent, while other indicators are more complementary (Nurmansyah *et al*, 2018), even when applying agents with high correspondence to DNA structures and using insertional mutagenesis (Ram *et al.*, 2019), which has a higher level of dependence on the subject

of action. Of great importance for establishing the significance of individual concentrations in terms of peak activity is the rate of complex aberrations, which has been observed in both less harmful agents (Nazarenko & Izhboldin, 2017; Bhat & Wani, 2017) and aspects of continuous action (Pramanik *et al.*, 2018), although in the latter case, it is proposed to give priority to the use of sublethal and lethal doses. In the first case, the recommendation to use more moderate options remains, which can be based on both mutagenic depression and monitoring of cytogenetic activity.

CONCLUSIONS

According to the pairwise comparison of cytogenetic activity for individual varieties, the role of genotype in determining the reaction of the subject of mutagenic action to the increase in the damaging properties of the mutagen (considering both its nature and the increase in the concentration of the factor) was found to be increasing. As the concentration of the agent of genotype-mutagenic interaction increases, the difference between individual varieties in the variability of cytogenetic parameters of the spectrum increases, which is reproduced by the power of classification analysis of objects by a set of indicators and by the significance of three parameters (total frequency, number of bridges and fragments) according to factor and discriminant analysis. The sensitivity of the varieties manifested at the organism level in terms of mutagenic depression is partially preserved, although it may differ for individual varieties (Niva Odeska, Polyanka). A rather significant variety of reactions to the action of the studied mutagenic factor was shown. The data obtained on predicting the effectiveness of individual concentrations (DMS 0.0125 and 0.025%) in terms of inducing certain types of mutations require both the expansion of the source material to confirm the obtained patterns and further research at the level of consideration of visual and biochemically identified micro- and macro- changes that are heritable during field trials in the next generations, which is included in the plans (involvement of additional varieties of Western European ecotypes, research of the second or third generation).

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CONFLICT OF INTEREST

None.

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Цитогенетична активність мутагенного чинника з високою ушкоджувальною здатністю у пшениці озимої

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Анотація. Аналіз цитологічних аномалій є важливим методом ідентифікації можливостей хімічної речовини як мутагену для майбутніх спадкових генетичних змін, рівня генотип-мутагенної взаємодії та сайт-специфічної активності для природи та/або різних концентрацій мутагену. Метою експерименту було визначити межі мінливості генотипів різного походження, насамперед із широкою еколого-генетичною мінливістю, показати взаємодію між сортом та мутагеном. Зерно пшениці озимої кількох сортів (Балатон, Боровиця, Зелений Гай, Золото України, Каланча, Нива Одеська, Полянка, Почайна) обробляли диметилсульфатом у концентраціях 0,0125 %, 0,025 %, 0,05 %, експозиція становила 24 години. Було досліджено цитогенетичну активність досліджували за частотою та спектром хромосомних перебудов у відповідних фазах клітинного поділу в залежності від сорту та концентрації мутагену як основних факторів, що впливають на ці показники, також основні ознаки спектру, такі як загальна швидкість хромосомних перебудов, кількість фрагментів і подвійних фрагментів, містків, мікроядер і відстаючих хромосом. Було встановлено, що досліджувані концентрації супермутагену суттєво вплинули на всі аналізовані параметри, їх можна віднести до оптимальних і високих рівнів концентрацій за впливом на цитологічну активність і проблемами у мітозі, для чинника, незважаючи на попередні дослідження, фактор сорт значно більше впливає на природу та частоту деяких типів аберацій, ніж збільшення концентрації мутагену, він характеризувався значно більшою сайт-специфічною дією, ніж інші хімічні агенти, різні варіанти по змінам мутагенної дії були ідентифіковані відповідно до генотипу суб'єкту. Було узагальнено, що ознаками, які відтворювали вплив мутагену, згідно дискримінантного аналізу, є загальна частота, частота фрагментів і подвійних фрагментів, містки. При практичному використані в генетичному поліпшені рослин та для отримання цінних ознак оптимальним є використання концентрацій 0,0125 %, 0,025 %, що планується додатково підтвердити на більш широкому спектрі генотипів та дослідженнями мутацій для наступних (другого-третього) поколінь

Ключові слова: хромосомні аберації; мутаген; пшениця озима; диметилсульфат; цитологічний аналіз