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Cytogenetic effects of low-damaging chemical supermutagen action on winter wheat samples

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Abstract. By using specifics of initial material for activation of the mutagenic process, the variability of the resulting material can be increased significantly. This is especially important for mutagens with low damaging effect, but with a high affinity for certain structural DNA peculiarities at the same time. We studied the cytogenetic activity of mutagens according to several parameters of chromosomal abnormalities during mutagenic depression in the first generation subject to water solution of DAB (1,4-bisdiazoacetylbutane) in 0.1%, 0.2%, 0.3% concentrations. Eight varieties of winter wheat were used as objects for maximal characterization of possible genetic variability and its influence on the cytogenetic activity. The varieties were obtained from both national and international breeding institutions. It was found that this mutagen agent induced cytogenetic activity at a significantly lower level than other substances generally accepted for the practice of genetic improvement using this method. The total number of chromosomal aberrations was 10 times lower, but significantly higher than in the case of using epimutagens. The absence of significant effects consisting in cell elimination as a result of disorders in the chromosomal apparatus indicates moderate concentrations. We determined that in the case of increasing concentrations, the model parameters can be the total rate of rearrangements, presence of fragments, presence of cells with two or more aberrations. For the variety factor, only the presence of micronuclei and lagging chromosomes was significant. This feature is not sufficient for effective classification of the group of genotypes, i.e., the variability of the group as a whole is too low. However, there are two varieties significantly differing from the other six by this parameter. This gives hope for a significant increase in mutational activity at the plant level due to optimization of the genotype-mutagenic interaction in the case of DAB. Also, despite the low damaging effect, this mutagen can be promising for induction of certain types of changes, as indicated by presence of a fairly significant number of rare changes. The initial material showed a fairly high stability, which practically excludes the occurrence of spontaneous changes in the future. It is planned to screen the obtained forms to detect changes in the phenotype, primarily in the architecture of the plant, identifying the inheritance of the identified traits in the next generations in order to identify change in the mutation. Also, we plan to analyse the obtained forms for valuable properties in terms of grain quality.

Keywords: bread wheat; genotype; chemical mutagenesis; chromosomal rebuildings; supermutagen; rate of chromosomal aberrations; mutations; cereals; 1,4-bisdiazoacetylbutane.

Introduction

The use of chromosomal aberrations to monitor mutagenic damage at the level of the chromosomal apparatus of a cell has a rather long history both in terms of studying the cytogenetic activity of individual substances and for monitoring the impact of various anthropogenic factors, primarily associated with various types of chemical and radiation pollutions (Wu et al., 2019). Methodologically, a protocol for the application of a particular mutation-inducing factor cannot be developed without a test of its cytogenetic activity, determining the overall level of variability after the action (El-Azab et al., 2018), and identifying threshold values in terms of lethal effects on the hereditary apparatus and further elimination of cells (Hase et al., 2020). Due to the site-specific effect on chemically related DNA regions, their effect is much less harmful to the viability of organisms than the effect of physical mutagens (Von Well et al., 2018), primarily gamma rays and X-rays (Nazarenko, 2016). Chromosomal aberrations also affect the fertility of an organism, the most significant and limiting effects occurring in the first generation (Hassine et al., 2022). In subsequent generations, various disorders can also occur, primarily in meiosis, but much less frequently and without such a decisive importance (Bhat & Wani, 2017). In some cases, certain substances can have low effect due to the genetic characteristics of a subject (Bezie et al., 2020).

At first, when studying the nature of a chemical mutagenic factor, significant indicators include the general rate of chromosomal rearrangements, the ratio of individual types of aberrations (Horshchar & Nazarenko, 2022b), and presence of rare types of changes. Also, the dynamics of rearrangement frequency can be used to estimate threshold values for this factor from the perspective of practical application for the genetic improvement of an initial variety or hybrid (Shabani et al., 2022). It is impossible to directly link certain types of rearrangements with changes in economically valuable traits. Nonetheless, a correct selection (which begins from tests of cytogenetic activity) of genotype can enhance changes in the hereditary apparatus by 60–80%, while maintaining viability at the same level (Dwinanda et al., 2020). Such a selection depends on the mechanisms of genetically-determined tolerance to mutagenic effects (Horshchar & Nazarenko, 2022a) and differences in the genome (which leads to changes in affinity to chemical supermutagen and different activity of individual sites, the emergence of new gene associations) (Ram et al., 2019). Given the presence of phylogenetically distinct groups of varieties and affinity when using certain basic components of the germplasm, study of the activity of even well-known mutagens on new varietal material (Lykhovyd, 2021) seems to be quite promising for experimental plant mutagenesis (Nazarenko & Izhboldin, 2017; Oney-Birol & Balkan, 2019).

The aim of our studies was demonstrating the genotype-mutagenic interaction on new winter wheat genotypes of different origin recently released for our zone (Dorrani-Nejad et al., 2022), first of all, those that are rarely used as objects for mutagenic effects, i.e., certain new

patterns are possible in the occurrence of certain types of chromosomal aberrations, their ratios, threshold values of mutagen concentrations for optimal genetic improvement of winter wheat. Also, we attempted to identify model parameters for the mutation process at the cytogenetic level.

Materials and methods

Seed material was subjected to 1,4-bisdiazoacetylbutane (DAB) in the concentrations of 0.1%, 0.2%, 0.3% in water solutions as optimal for winter wheat mutation breeding purposes (Sigma-Aldrich, Germany). The samples were prepared according to the protocol of mutagen treatment with 24 hour exposure, as recommended by FAO/IAEA Division. These concentrations are standard for the genetic improvement of winter wheat. The control comprised untreated seeds (Spencer-Lopes et al., 2018).

Eight wheat varieties – Balaton, Borovytsia, Zeleny Gai, Zoloto Ukrainy, Kalancha, Niva Odeska, Polyanka, and Pochayna – were included in the experiment in 32 variants (total). The varieties were included regarding the maximum possible characteristic of the adaptability of existing genotypes for the research area. Cytological analysis of chromosome aberrations was carried out to identify mutagenic effects (Spencer-Lopes et al., 2018; Yang et al., 2019).

Analyses of chromosomal aberrations were performed on mitoses preparations of primary root tips of winter wheat varieties during the late stage of metaphase and early anaphase using light microscopy. After DAB treatment, parts of the primary root system were grown in Petri dishes with filter papers and distilled water in a thermostat device at +20–22 °C. Then, 0.9–1.2 cm long parts of the tips (22–26 samples) was cut and fixed in Clarke's solution, consisting of 3 parts of 96% ethyl alcohol and 1 part of ocular acid, for 24 hours. The samples were kept in 70% ethyl alcohol solution at +2 °C. For each variant, about 22–26 tips of the roots were analyzed. Cytogenetic analyses were provided by the temporary pressure preparations stained with acetocarmine. The tips were treated with 45% acetic acid (Oney-Birol & Balkan, 2019). Root tip samples were made by the recommended method. The samples of root tips were analyzed on a Micromed XS-3330 (Micromed, Poltava, Ukraine) light microscope (X600 times) (Spencer-Lopes et al., 2018). Statistical analysis of the values was performed by Statistica 10.0 (TIBCO, Palo Alto, USA). Values in the tables are given as x ± SD (mean ± standard deviation). The differences between the variants were determined using the ANOVA (single-factor analysis) and were considered significant at $P < 0.05$. The normality of the data distribution was examined using the Shapiro–Wilk W-test. Differences between the samples were determined by the Tukey HSD test.

Results

The first parameters to pay attention to when analyzing the cytogenetic activity of a mutagen are the general rate of chromosome aberrations. Therefore, when analyzing the data in Table 1, we are interested in how much this parameter depended primarily on the genotype (variety) of the target and on the concentration of the mutagen. The factor analysis revealed that in general, the factor of the genotype did not significantly affect the total sample ($F = 2.13$; $F_{0.05} = 2.48$; $P = 0.08$), while increase in concentration increased the total level of rearrangements in the chromosomal apparatus of the cell ($F = 116.19$; $F_{0.05} = 3.07$; $P = 1.25*10^{-11}$). However, individual genotypes still significantly stood out in pairwise comparison. This was observed for the Borovytsia variety ($F = 5.16$; $F_{0.05} = 2.48$; $P = 0.01$) and, to a lesser extent, the Polyanka variety ($F = 2.96$; $F_{0.05} = 2.48$; $P = 0.04$), which turned out to be significantly less resistant to DAB effects than the rest of the group with more or less similar reaction. Generally, the general rate of chromosomal aberrations varied 3.99% (Zeleny Hai variety) to 6.67% (Polyanka variety) under the action of DAB 0.10%, 5.93% (variety Zeleny Gai) to 9.46% (variety Balaton) at 0.20% DAB concentration, and 9.56% (variety Kalancha) to 11.40% (variety Borovytsia) under the action of 0.30% DAB. Therefore, in general, the cytogenetic activity of this mutagen was not high; and neither have we observed significant decrease in the gen-eral rate after increasing the concentration, i.e., thresholds in the use of various concentrations were not reached.

Thus, to summarize the aforesaid, there are two varieties – Borovytsia and Polianka, which were somewhat more sensitive to the action of this mutagen. The rest of the genotypes exhibited more or less the same reaction in terms of the frequency of chromosomal rearrangements, and in general, rather low, indicating first of all, a rather high degree of affinity of this mutagen to certain key features of the genome in domestic varieties, and secondly, to the low DNA-damaging ability of this substance. We should also note higher monitoring sensitivity of even the total cytogenetic activity in comparison with the parameters of depression at the plant level in general.

Table 1
General rates of chromosomal rearrangements
for cells under mitotic division (x ± SD, n = 1000)

Cultivar	Treatment	Number of mitosis	Chromosomal rearrangements	
			number	%
Balaton	water	1002	10	1.00 ± 0.12^{a}
	DAB 0.1	1008	51	5.06 ± 0.17^{b}
	DAB 0.2	1004	95	9.46 ± 0.30^{c}
	DAB 0.3	1007	104	10.33 ± 0.15^{d}
Zeleny Gai	water	1008	9	0.89 ± 0.32^{a}
	DAB 0.1	1002	56	3.99 ± 0.21^{b}
	DAB 0.2	1011	93	5.93 ± 0.10^{c}
	DAB 0.3	1009	99	9.81 ± 0.10^{d}
Zoloto Ukrainy	water	1001	8	0.80 ± 0.21^{a}
	DAB 0.1	1005	57	5.67 ± 0.11^{b}
	DAB 0.2	1009	88	8.72 ± 0.10^{c}
	DAB 0.3	1002	99	9.88 ± 0.10^{d}
Niva Odeska	water	1009	8	0.79 ± 0.23^{a}
	DAB 0.1	1000	53	5.30 ± 0.15^{b}
	DAB 0.2	1004	67	6.67 ± 0.12^{c}
	DAB 0.3	1009	104	10.31 ± 0.06^{d}
Borovytsia	water	1001	7	0.70 ± 0.20^{a}
	DAB 0.1	1010	56	5.54 ± 0.16^{b}
	DAB 0.2	1003	77	7.68 ± 0.21^{c}
	DAB 0.3	1000	114	11.40 ± 0.10^{d}
Kalancha	water	1000	10	1.00 ± 0.15^{a}
	DAB 0.1	1009	50	4.96 ± 0.15^{b}
	DAB 0.2	1004	69	6.87 ± 0.17^{c}
	DAB 0.3	1004	96	9.56 ± 0.10^{d}
Polyanka	water	1007	6	0.60 ± 0.26^{a}
	DAB 0.1	1005	67	6.67 ± 0.36^{b}
	DAB 0.2	1008	76	7.54 ± 0.41^{b}
	DAB 0.3	1000	109	10.90 ± 0.35^{c}
Pochayna	water	1005	8	0.80 ± 0.06^{a}
	DAB 0.1	1001	49	4.90 ± 0.06^{b}
	DAB 0.2	1007	69	6.85 ± 0.17^{c}
	DAB 0.3	1001	105	10.49 ± 0.13^{d}

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

Pairwise comparison confirmed the overall results of the factor analysis. Therefore, despite the low damaging ability of the mutagen, in all cases, except for the Polyanka variety, during the transition between DAB concentrations of 0.2 and 0.3 ($F = 2.03$; $F_{0.05} = 2.08$; $P = 0.07$), the general rate of chromosome aberrations increased with each increase in the mutagen concentration. Also, in all cases, significant differences from the control were seen for the lowest concentration of the mutagen. The general characteristics of the sample show that even the highest concentration did not approach the threshold concentrations of this substance in terms of the elimination of the chromosome apparatus, which is absent in significant quantities. In all cases, it was not difficult to collect the required number of samples.

With regard to the spectrum of rearrangements of the chromosomal apparatus of the cell, we studied such aberrations as fragments (single and double, which are generally more characteristic of the action of chemical supermutagens), bridges (also single (chromatids) and double (chromosomes), which are more characteristic in the spectrum of physical mutagens, first of all gamma rays and X-ray exposure), as well as other, rarer aberrations such as micronucleus, lagging chromosomes. Separately, we took into account cells with multiple chromosomal aberrations (two or more cases in one cell) (Table 2 and 3), which are a fairly powerful integrative indicator of the damaging effect of a mutagen.

As in the case of the general rate of rearrangements in the chromosomal apparatus, no significant difference in the genotype factor was found for the total frequency of the fragments and double fragments. ($F = 1.49$; $F_{0.05} = 2.48$; $P = 0.10$), but only increases with increasing

concentration of the chemical mutagen (F = 83.11; $F_{0.05}$ = 3.07; P = $1.22*10^{-4}$). However, pairwise comparison showed that although the first concentration had a significant effect compared with the control, the difference was not always significant when switching between individual concentrations. Generally, number of the fragments varied 17 (variety Zeleny Hai) to 46 (variety Polianka) under the action of 0.1% DAB, 28 (variety Zeleny Gai) to 54 (variety Balaton) at 0.2% DAB concentration, and 45 (variety Kalancha) to 56 (variety Nyva Odeska) under the action of 0.3% DAB.

Therefore, in the first group of genotypes, which were more sensitive at the level of depression effects to DAB action, there was no difference for the Balaton variety upon transition from DAB 0.2 to DAB 0.3 (F = 1.97; $F_{0.05}$ = 2.08; P = 0.06; Table 2). For the second group of genotypes, there was also no difference in the Polianka variety during the transition from DAB 0.1 to DAB 0.2 (F = 2.04; $F_{0.05}$ = 2.08; P = 0.06; Table 3). At the same time, the dynamics of change in terms of this aberration in the total number of rearrangements was quite different for all the varieties, however, in general, in all cases of mutagen exposure, their number prevailed over bridges, as evidenced by the integrative index of the ratio of fragments to bridges, which always exceeded one, i.e. indicated the predominance of fragments over bridges for all the varieties and all the concentrations.

Table 2

Parameters of range of chromosomal aberrations for mitotic cells of winter wheat: first group (x ± SD, n = 1000)

Cultivar	Treatment	Fragments		Bridges		Ratio	Other types of aberrations		Cells with double or more aberrations	
		n	%	n	%		n	%	n	%
Balaton	water	4.0 ± 0.4^{a}	44.4	4.0 ± 1.4^{a}	44.4	1.0	1.0 ± 0.9^{a}	11.1	0.0 ± 0.0^{a}	0.0
	DAB 0.1	30.0 ± 0.4^{b}	58.8	14.0 ± 1.0^{b}	27.5	2.1	7.0 ± 0.6^{b}	13.7	7.0 ± 1.0^{b}	13.7
	DAB 0.2	54.0 ± 0.7^{c}	56.8	31.0 ± 1.3^{c}	32.6	1.7	10.0 ± 0.7^{c}	10.5	15.0 ± 1.1^{c}	15.8
	DAB 0.3	52.0 ± 0.7^{c}	50.0	38.0 ± 1.4^{d}	36.5	1.3	14.0 ± 1.0^{d}	13.5	25.0 ± 1.4^{d}	24.0
Zeleny 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
	DAB 0.1	17.0 ± 0.5^{b}	42.5	16.0 ± 1.1^{b}	40.0	1.1	7.0 ± 0.7^{b}	17.5	7.0 ± 0.6^{b}	17.5
	DAB 0.2	28.0 ± 0.8^{c}	46.6	22.0 ± 1.3^{c}	36.6	1.3	10.0 ± 1.1^{c}	16.6	11.0 ± 1.0^{c}	18.3
	DAB 0.3	49.0 ± 1.2^{d}	49.4	34.0 ± 1.7^{d}	34.3	1.4	16.0 ± 1.4^{d}	16.1	23.0 ± 1.3^{d}	23.2
Zoloto Ukrainy	water	5.0 ± 1.5^{a}	62.5	3.0 ± 1.2^{a}	37.5	1.7	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
	DAB 0.1	33.0 ± 1.7^{b}	57.8	17.0 ± 1.0^{b}	29.8	1.9	7.0 ± 0.5^{b}	12.2	7.0 ± 0.8^{b}	12.2
	DAB 0.2	47.0 ± 1.9^{c}	53.4	31.0 ± 1.6^{c}	35.2	1.5	10.0 ± 1.1^{c}	11.3	16.0 ± 1.0^{c}	18.1
	DAB 0.3	50.0 ± 1.9^{d}	50.5	32.0 ± 2.1^{c}	32.3	1.5	17.0 ± 1.5^{d}	17.1	27.0 ± 2.1^{d}	27.2
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
	DAB 0.1	32.0 ± 1.0^{b}	60.3	16.0 ± 1.0^{b}	30.1	2.0	5.0 ± 1.0^{b}	9.4	7.0 ± 1.2^{b}	13.2
	DAB 0.2	38.0 ± 1.7^{c}	56.7	18.0 ± 1.9^{b}	26.8	2.1	11.0 ± 1.1^{c}	16.4	16.0 ± 1.6^{c}	23.8
	DAB 0.3	56.0 ± 1.9^{d}	53.8	31.0 ± 2.2^{c}	29.8	1.8	17.0 ± 1.4^{d}	16.3	25.0 ± 2.2^{d}	24.0

Note: see Table 1.

In the case of bridges, which were taken into account as chromosome and chromatid together, no significant differences were seen in the first group during pairwise comparison of the Zoloto Ukrainy variety during the transition from DAB 0.2 to DAB 0.3 (F = 1.99; $F_{0.05}$ = 2.08; P = 0.06) and for variety Nyva Odeska during the transition from DAB 0.1 to DAB 0.2 (F = 2.01; $F_{0.05}$ = 2.08; P = 0.06; Table 2). In all other genotypes, the number of this type of rearrangements differed significantly. At the same time, as the concentration increased more and more, most genotypes shifted towards induction in favour of bridges, i.e., in general, site-specificity as a property weakened quite significantly after increasing concentration. Although not always. For the second group, there were also no differences in the Kalancha variety during the transition from DAB 0.1 to DAB 0.2 (F = 1.24; $F_{0.05}$ = 2.08; P = 0.13) and for variety Polianka during the transition from DAB 0.1 to DAB 0.2 (F = 1.71; $F_{0.05}$ = 2.08; P = 0.08; Table 3). Thus, number of the bridges varied 14 (variety Balaton) to 19 (variety Borovytsia) under the action of DAB 0.1%, 15 (variety Kalancha) to 31 (varieties Balaton and Zoloto Ukrainy) at 0.2% DAB concentration, and 27 (variety Borovytsia) to 38 (variety Balaton) under the action of 0.3% DAB.

Table 3

Parameters of range of the chromosomal rearrangements for cells under mitotic division: second group (x ± SD, n = 1000)

Cultivar	Treatment	Fragments		Bridges		Ratio	Other types of aberrations		Cells with double or more aberrations	
		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
	DAB 0.1	30.0 ± 1.6^{b}	53.5	19.0 ± 1.0^{b}	33.9	1.5	7.0 ± 1.0^{b}	12.5	7.0 ± 1.0^{b}	12.5
	DAB 0.2	42.0 ± 1.9^{c}	54.5	23.0 ± 1.5^{c}	29.8	1.8	12.0 ± 1.3^{c}	15.5	11.0 ± 1.2^{c}	14.2
	DAB 0.3	53.0 ± 2.1^{d}	46.4	27.0 ± 2.0^{d}	32.4	1.4	24.0 ± 1.6^{d}	21.0	31.0 ± 2.1^{d}	27.1
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
	DAB 0.1	27.0 ± 0.6^{b}	54.0	16.0 ± 0.6^{b}	32.0	1.6	7.0 ± 0.6^{b}	14.0	8.0 ± 1.0^{b}	16.0
	DAB 0.2	38.0 ± 1.0^{c}	55.0	15.0 ± 1.0^{b}	21.7	2.5	16.0 ± 1.0^{c}	23.1	16.0 ± 1.5^{c}	23.1
	DAB 0.3	45.0 ± 1.1^{d}	46.8	28.0 ± 1.6^{c}	29.1	1.6	23.0 ± 1.2^{d}	23.9	25.0 ± 2.1^{d}	26.0
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
	DAB 0.1	46.0 ± 1.1^{b}	68.6	16.0 ± 1.1^{b}	23.8	2.8	5.0 ± 1.0^{b}	7.4	7.0 ± 1.0^{b}	10.4
	DAB 0.2	43.0 ± 1.4^{b}	56.5	22.0 ± 1.6^{b}	28.9	1.9	11.0 ± 1.2^{c}	14.4	10.0 ± 1.2^{c}	13.1
	DAB 0.3	53.0 ± 1.9^{c}	48.6	35.0 ± 1.9^{c}	32.1	1.5	21.0 ± 1.5^{d}	19.2	23.0 ± 1.6^{d}	21.1
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
	DAB 0.1	27.0 ± 0.4^{b}	55.1	16.0 ± 0.5^{b}	32.6	1.6	6.0 ± 0.5^{b}	12.2	5.0 ± 0.6^{b}	10.2
	DAB 0.2	38.0 ± 1.0^{c}	55.1	20.0 ± 1.0^{c}	28.9	1.9	11.0 ± 1.0^{c}	15.9	11.0 ± 1.0^{c}	15.9
	DAB 0.3	47.0 ± 1.5^{d}	44.7	35.0 ± 1.4^{d}	33.3	1.3	23.0 ± 1.3^{d}	21.9	25.0 ± 1.5^{d}	23.8

Note: see Table 1.

As with other types of chromosomal rearrangements, such as lagging chromosomes and micronuclei, the genotype factor turned out to be significant for them (F = 2.54; $F_{0.05}$ = 2.48; P = 0.05), however, the increase in this type of aberrations was also significant with increase in the concentration (F = 84.17; $F_{0.05}$ = 3.07; P = $1.12*10^{-4}$). When comparing options in pairs, all the variants had statistically significant differences, without exception. There were also significant differences from control in all the cases. Generally, the number of other abnormalities varied 14 (Balaton variety) to 19 (Borovytsia variety) under the action of DAB 0.1%, 15 (variety Kalancha) to 31 (varieties Balaton and

Zoloto Ukrainy) at 0.2% DAB concentration, 27 (variety Borovytsia) to 38 (variety Balaton) under the action of 0.3% DAB.

The number of the cells with two or more aberrations is usually a highly reliable and significant indicator reflecting increase in concentration (dose) of a mutagen. At the same time, the influence of the genotype on this process was insignificant ($F = 1.32$; $F_{0.05} = 2.48$; $P = 0.19$). Increase in concentration significantly raised the frequency of complex changes ($F = 117.11$; $F_{0.05} = 3.07$; $P = 2.17*10^{-7}$). In the case of DAB 0.1, the number of the cells with two or more aberrations ranged 5 (Pochaina variety) to 8 (Kalancha variety), 10 (Polianka variety) to 16 (Kalancha and Nyva Odeska varieties) at 0.2 DAB, 23 (Zeleny Hai and Polianka varieties) to 31 (variety Borovytsia) when subject to 0.3 DAB. Pairwise comparison of the variants revealed statistically significant differences for all of them without exception. There were also significant differences from control in all the cases.

Factor analysis showed (Table 4) that all the studied parameters, except for number of the bridges, were significant for increasing the concentration of DAB, while the genotype only affected changes in number of the aberrations by the type of micronucleus and lagging chromosomes. The process of changing the concentration and changes in the cytogenetic activity associated with it is much more suitable for modeling the mutation process and, in general, the group of genotypes selected for the study was quite homogeneous. In general, the method is quite sensitive, taking into account the low damaging ability of the mutagen.

Table 4
Factor loadings (unrotated) for winter wheat cytogenetic parameters

Indicator	Mutagen concentration	Variety
General rates	0.845*	–0.441
Fragments	0.788*	–0.314*
Bridges	0.412	–0.117
Other abnormalities	–0.611*	0.655*
Double and more	0.779*	0.422
Explanation variants	3.111	0.789
Non-explanation	0.796	1.456

Note: * indicate significant differences at $P < 0.05$.

To determine model qualities and characteristics of the influence of cytogenetic activity, depending on factors of the genotype of the initial object of influence and concentration of the mutagen, a discriminant analysis was carried out (Tables 5 and 6, Fig. 1). As demonstrated, in the case of the genotype, the discriminant analysis revealed significance for the genotype of only one parameter in the model of double and more chromosomal rearrangements in one cell, which is quite consistent with the data of factor analysis. In the case of concentration, the picture is characteristic of the action of chemical supermutagens (presence of the bridges was not included in the model parameters) and some difference from factor analysis, namely absence of other chromosomal rearrangements, such as micronucleus and lagging chromosomes, in the model parameter. Thus, if the resolution of features in the case of increase in concentration is sufficient to build a model (Fig. 1), then there is only one parameter axis for genotypes in general. However, this does not mean that modeling and classifying cases for individual varieties is impossible.

Table 5
Discriminant Function for winter wheat cytogenetic parameters

Parameter	Genotype			Concentration		
	Wilks'-Lambda	F_{remove} (5.85)	P	Wilks'-Lambda	F_{remove} (3.88)	P
General rates	0.003	4.90	0.11	0.040	22.82	0.01
Fragments	0.002	4.51	0.12	0.032	12.22	0.01
Bridges	0.004	4.62	0.12	0.024	2.36	0.07
Other	0.022	7.03	0.04	0.006	1.01	0.12
Double and more	0.002	4.11	0.12	0.027	5.91	0.01

Classification analysis according to canonical roots (Table 6) showed that in the case of mutagen concentrations, they were significantly displayed for all the studied parameters. Only in the case of DAB 0.2 was the classification power slightly less than the absolute one (97.5% of the classification cases). This suggests that despite the weaker damaging effect, the mutagen is effective in inducing cytogenetic damage. As for the varieties, on the contrary, the classification power of the genotype was extremely low. It was worth taking it into account only in the case of Balaton and Polianka varieties (62.5% of objects in the factor space were assigned to these varieties from the total sample of varieties).

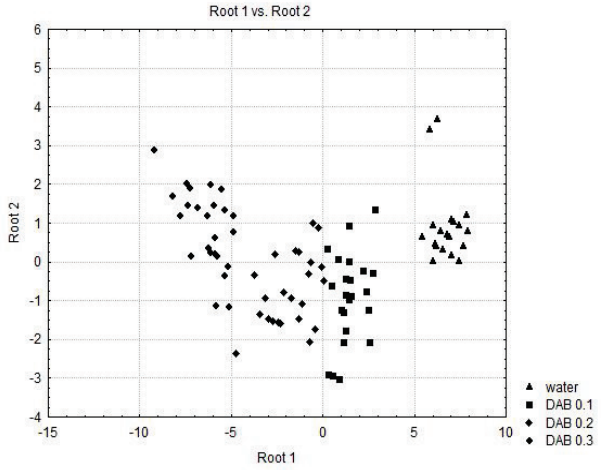

Fig. 1. Classification in canonical fuction space (as resault for concentration)

Table 6
Classification ability for factor space (by canonical analysis)

Objects by concentrations in model, %	water	100.0
	DAB 0.1	100.0
	DAB 0.2	95.9
	DAB 0.3	100.0
	Total	98.8
Objects by genotypes in model, %	Balaton	62.5
	Borovytsia	37.5
	Zeleny Gai	37.5
	Zoloto Ukrainy	37.5
	Kalancha	37.5
	Niva Odeska	37.5
	Polyanka	62.5
	Pochayna	37.5
	Total	43.3

Thus, according to the model parameters for genotypes, only the presence of rare types of aberrations differed (micronucleus, lagging chromosomes). There were no other significant differences. Apparently, it was this part of the spectrum that caused changes in the general rate of cytogenetic changes, which influenced the differences between the two varieties from the others in terms of the nature of variability at the cellular level. At the same time, changes in concentration were much more significant. However, not for the same parameter: in this situation, it did not significantly respond to changes in the mutagen activity. It can be concluded that the site-specific ability of the mutagen manifests in this way, and not through the induction of the fragments and bridges, which are of a more general nature. At the same time, in general, one should not expect particularly high parameters of variability at the level of the organism as a whole; also, the mutagen in its applied concentrations did not reach significant lethal values for the material used.

Discussion

The results have once again indicated that the cytogenetic parameters of activity are worth studying even on relatively well-studied mutagenic factors in terms of their interaction (Horshchar & Nazarenko, 2022b). Moreover, even relatively low-damaging substances sometimes show quite significant effects in terms of both the induction of the general rate of rearrangements (Nazarenko, 2016) and their ratios in the spectrum, which depends primarily on the peculiarities of DNA architecture of specific varieties (Wu et al., 2019; Beiko & Nazarenko, 2022a). In our case, this was shown by significant differences between the two varieties, reaction of which to DAB action was quite different from the rest of the objects of influence (Bezie et al., 2020). At the same time, even despite the relatively low damaging ability of this

supermutagen, it was enough to cause significant differences between the individual variants regarding the action at the level of the chromosomal apparatus of the cell (Bhat & Wani, 2017). At the same time, these studies have shown that significant effects in terms of both the general induction of cytogenetic disorders (Nazarenko & Izhboldin, 2017) and ratio of their various types are more different for individual genotypes (Horshchar & Nazarenko, 2022a), rather than their characteristics in the de-velopment of mutagenic depression at the level of the organism as a whole (Pane et al., 2018; Zaidi et al., 2019).

However, it is worth reiterating a number of general points. Although this chemical mutagen is less specific in induction of the fragments and double fragments characteristic of the chemical mutagenic activity, they still occupy a significant, prevailing, share in the total cytogenetic activity (Oney-Birol & Balkan, 2019). The ratio between them and the bridges is much higher than one (Pane et al., 2018). At the same time, the value of such an indicator as the induction of other types of rearrangements sharply decreases. Micronucleus and lagging chromosomes are comparatively rarer under the influence of DAB and do not belong to the modeling ones (El-Azab et al., 2018). We should also note a great decrease in the significance of such a parameter as the presence of cells with two or more aberrations (Hussain et al., 2021), which is associated with a lower activity of the mutagen and its low damaging ability (Mamenko & Yakymchuk, 2019). However, the latter parameter is still significant (Hassine et al., 2022), in contrast to the rare types of chromosomal rearrangements (Oney-Birol & Balkan, 2019; Beiko & Nazarenko, 2022b).

It should be noted that this mutagen exerted a rather high degree of site-specificity and was more dependent on the target of action than the ones studied earlier (Horshchar & Nazarenko, 2022a), at least at the cellular level. At the same time, it should be noted that this specificity, in general, manifests primarily in a slightly higher activity in a number of varieties and it is still unknown how much this will be linked to specific mutations at the organism level (Dwinanda et al., 2020). In general, as practice shows, this can be significant for the induction of some rather specific changes, primarily associated with traits of the plant architecture and changes in physiological processes in its ontogenesis (Gharib et al., 2021), but usually, these changes are of interest only as general activity, but have no practical value (Abaza et al., 2020).

Conclusion

The induction of plant variability using various genetically active substances constantly faces the problem of genome peculiarities of new varieties and hybrids of agricultural plants, particularly different mechanisms of resistance-sensitivity to a type of action and different susceptibility at DNA level and different polymorphic ability, which is provided simultaneously by differences in the structure and site-specific nature of the action of some agents. Therefore, despite the standardization and study of the application protocols, the question of the limits of effectiveness remains open, which can be significantly expanded by understanding the peculiarities of the genotype-mutagenic interaction in each specific case. This is also caused by peculiarities of the germplasm, which strongly depends on the specific regional program of the genetic improvement of a particular crop. Our research shows that it is too early to consider these issues already solved and there are constantly additional points that significantly affect the effectiveness of experimental mutagenesis programs. Of course, research on cytogenetic activity in themselves will be further developed in our studies in determining variability, primarily in terms of valuable traits, already at the level of plants as a whole in subsequent generations. In the future, we are planning to analyze both visual changes and their biometric analysis, identifying microchanges, and recording biochemical and physiological changes.

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