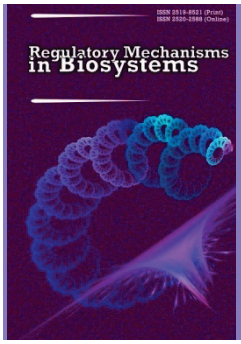

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Genotype-mutagenic interaction in the cytogenetic variability of winter wheat for a new ecogenetic factor

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The study of cytogenetic parameters of the activity of new mutagenic factors aims to reliably establish the possibilities of these factors in terms of variability depending on the subject of mutagenic action, the optimal use of certain factor sizes, the combination of the selected protocol with optimization of the yield of mutant forms in the future. Seeds of eight varieties of winter wheat (Balaton, Borovytsia, Zeleny Gai, Zoloto Ukrainy, Kalancha, Niva Odeska, Polyanka, Pochayna) were treated by SA (sodium azide) at concentrations of 0.010%, 0.025%, 0.05%, 0.10%. They were soaked in water solution for 24 hours. Cytogenetic activity was evaluated by pollen sterility, evaluation of general rates and indicators of spectra of chromosomal abnormalities at medium phases of cell mitosis according to wheat variety and chemical agent concentrations. As a result of the study, the key importance of the genotype-mutagenic interaction was demonstrated within the limits of variability of the main indicators of the frequency and spectrum of chromosomal aberrations. It has been established that in the future it will be more optimal to use two varieties whose genotype-mutagenic specificity indicators are significantly higher and one should expect a more significant yield of promising mutant forms from them in the future, especially in combination with SA concentrations in the range of 0.025% and 0.05%. It is demonstrated that the main parameters that reflect genetically determined possibilities in susceptibility to the ecogenetic factor are pollen fertility, the overall frequency of chromosome aberrations, and the number of induced fragments. The use of other parameters displays the trend only partially or does not display it at all, as is the case with the use of rarer types of chromosomal rearrangements. The least promising forms have also been identified for use as starting material in treatments with this substance. It is demonstrated that, in general, this agent is characterized by the same patterns in the induction of cytogenetic activity as for other chemical supermutagens, with some variations depending on the starting material. In the future, it is planned to link the obtained data with the frequency and quality of the resulting hereditary changes, primarily complex biochemical and physiological ones, in order to improve the quality of plant products and various types of plant tolerance to adverse environmental conditions.

Keywords: cereals; chromosomal abnormalities; sodium azide; pollen sterility; cytogenetic analyze; site-specific activity; mutagenesis.

Introduction

One of the main provisions of the practical use of mutagenesis in plant improvement is the search for new agents and protocols for their use to increase the efficiency in obtaining new economically valuable forms and using modifications of treatment protocols with this factor. The wide variability of the already existing procedures provides significant opportunities in the study of various compositions of the initial material (Bondarenko & Nazarenko, 2020), the components of the processing method, the state of the subject of influence and the modification of the forms of activity of the factor. However, with all the diversity, the key in determining the effectiveness of a mutation agent is still, first of all, a cytogenetic test of the activity of this factor at the cellular level (Hintzsche et al., 2017; Ergün et al., 2023).

The study of the frequency of chromosomal damage allows us to draw conclusions about the general genetic and epigenetic activity of this factor, while the analysis of the spectrum of subsequent changes makes it possible to link the nature of future mutations and the features of the induction of rare types of changes (Nazarenko & Izhboldin, 2017; Bilgın et al., 2022). Although there is no test system that would directly link changes at the body level with DNA damage, some patterns can still be noted (Dwinanda et al., 2020). Chemical agents, which include sodium azide, which is gaining popularity as a mutagen, are characterized by increased site specificity, which in itself is reflected in a higher frequency of certain types of changes (Horshchar & Nazarenko, 2022a). This has been repeatedly demonstrated at the level of the plant organism in the form of relatively higher frequencies of certain rare mutations, the occurrence of certain types of dwarfism and sterility (Jalal et al., 2021; Bilgın et al., 2022).

Another point associated with testing a new mutagen at the cellular level is the elimination of cells at high concentrations or doses of a certain factor (Bhat & Wani, 2017). As a rule, the number of changes in the chromosomal apparatus at certain values of the dose or concentration reaches a maximum with a subsequent decrease. Such a maximum is in fact a practical limit on the amount of agent used (Horshchar & Nazarenko, 2022b). Quite often there is some kind of plateau in the effects of a number of doses or concentrations and in general, it is much more logical to stop immediately after reaching it. At the same time, the maximum damage to the nuclear apparatus is by no means always associated with a critical decrease in the mutant population in terms of survival factor (LD_{50}) or a decrease in morphometric parameters (RD_{50}) (Handa et al., 2018). In some situations, it is possible to use doses and concentrations of the activity plateau and even go beyond it, if the task is to induce some rare changes in order to establish the mechanism of genetic control of this trait (Hong et al., 2022). This is quite widely used in modern genetic study, primarily with the use of chemical supermutagens (Hase et al., 2020).

Quite often, appropriate test systems are also used for the purpose of environmental monitoring of pollution in a given area (Gupta et al., 2019). The data obtained may indicate not only the magnitude of the pollution itself, but also its nature, causes, and features of the consequences (Navid,

2021). The methods developed on the basis of testing chromosomal changes are appropriately standardized and internationally recognized (Ahumada-Flores et al., 2020).

Of particular importance in this case are the subjects of mutagenic effects, primarily for chemicals, since the features of the genotype also lead to features in damage to the corresponding DNA sections, a change in the location of the main mutable sites, and the transition of sites from mutability to stability (Von Well et al., 2023b). Optimal compositions of the genotype and concentration of a certain mutagen contribute to obtaining exactly the required type of changes (Chernysky & Gumentyk, 2020) with a high frequency, eliminating the associated negative changes, and maximizing the potential for improving individual traits and properties (Aly et al., 2019).

At the same time, various fluctuations in the behaviour of a particular subject during treatment with a mutagen, including in terms of the induction of the overall frequency and individual types of chromosomal rearrangements, are the main ones for determining such genetically possible features of the DNA structure (Nazarenko et al., 2019). Also important are individual features in response to the impact of the corresponding factor – increased susceptibility or, less often, resistance to this type of activity (Bhat & Wani, 2017). This aspect, however, may also depend on the presence of a certain mechanism of tolerance to the genetic activity of a particular substance (Von Well et al., 2023b).

For chemical mutagenesis, such studies on the genotype-mutagenic interaction are more important than for other types of agents of variability due to an order of magnitude greater dependence, which is found in differences in the effects of even similar mutagenic factors (Nazarenko et al., 2019). At the same time, the use of identification of changes at the chromosomal level gives a much more reliable and faster result than a study according to the scheme of classical experimental wheat mutagenesis, which requires obtaining at least the second and third generations. It should be noted that in this regard, sodium azide is rather poorly studied and the genotypes of domestic winter wheat varieties have not been studied at all (especially in comparison). Meanwhile, according to the mechanism of action, it differs quite seriously from the previously studied chemical genetically active substances (Beiko, & Nazarenko, 2022).

The purpose of the study was to demonstrate the potential of sodium azide as a mutagen in inducing the overall frequency and spectrum of chromosome aberrations, the specifics of its interaction with different genotypes, the features of the formation and manifestation of genotype-mutagenic interaction, the possibility of its use in the future, the prognostic moment when testing at the cellular level in terms of establishing the ability of the factor to induce mutations at the plant level, the effect on the fertility of the material in the first generation as a significant limitation for practical use in terms of obtaining the necessary plant sample.

Materials and methods

Study on establishing the viability of pollen and frequencies, the spectrum of chromosomal rearrangements was carried out by the laboratory of the Department of Breeding and Seed Production of Dnipro State Agrarian Economic University. Pollen samples were taken from the first-generation mutant population at the experimental fields station of the Science-Education Center.

A sample of a thousand grains for each concentration was treated with sodium azide (SA) (Sigma-Aldrich, Germany) at concentrations of 0.010%, 0.025%, 0.05%, and 0.10%; grains soaked in water in the same amount served as a control. The concentrations used were based on available data from those previously used for cereals in experimental mutagenesis. The exposure of the grains was 24 hours in accordance with standard processing protocols for these cultivars (Spencer-Lopes et al., 2018).

In total, 40 different variants were involved in the experiment based on 8 winter wheat varieties Balaton, Borovytsia, Zeleny Gai, Zoloto Ukrainy, Kalancha, Niva Odeska, Polyanka, Pochayna. The genotypes of varieties were selected in such a way as to affect all the main breeding centers of Ukraine and all the features of the main programs for the creation of commercial varieties of winter wheat, and one variety of the Western European ecotype, typical for European breeding, was added as a comparison. At the same time, we were also guided by different degrees of manifestation of the adaptability of the material involved for the conditions of the arid and sharply continental northern part of the Steppe of Ukraine. The agricultural technology of growing the population of the first generation of mutant varieties corresponded to the generally accepted for this zone. To analyze the viability of pollen, an average of 25 samples (well developed and without ear morphoses) were taken at the flowering stage of winter wheat in such a way that yellow anthers appeared in the middle part of the ear, which gives the maximum amount of material for study. The obtained pollen grains were stained in acetocarmine, the degree of fertility was judged by the intensity of staining when observing preparations by light microscopy.

Cytogenetic analysis of sodium azide activity was also performed by light microscopy (observation of temporary pressure preparations) using a Micromed XS-3330 device (Micromed, Poltava, Ukraine) with a magnification of 600 times. The microscope was equipped with a 5M camera for better observation and recording of the obtained results. Mitotic cells of preparations of the primary root system of winter wheat seedlings were observed and counted in stages ranging from late metaphase to anaphase with the fixation of all types of chromosomal abnormalities (fragments, double fragments, chromatid and chromosomal bridges, micronuclei, lagging chromosomes), cells with complex (two or more) rearrangements were separately counted. To prepare preparations, swollen seeds were germinated at a temperature of 20–22 °C until primary roots no longer than 1.1 cm long were obtained, followed by cutting off their tips and fixing the material obtained for 24 hours in Clarke's fixative, consisting of 1 part of glacial acetic acid and 3 parts of medical alcohol (96%). The samples thus obtained were stored in a refrigerator in a 70% ethanol solution. In total, at least 25 temporary pressure preparations were prepared with the observation of a sample of up to 1000 cells in the corresponding phases for each concentration and control (at high concentrations, the sample decreased). Preparations for observations were stained with acetocarmine. In the case of excessive rigidity of the resulting root tips, soaking in a 45% solution of glacial acetic acid was used.

Statistical analysis was performed using the Statistica 10.0 software (Tibco, Palo Alto, USA). The ANOVA module (at a significance level of $P < 0.05$) was used to establish the influence of factors and determine the genotype-mutagenic interaction with graphical visualization of the result obtained; pairwise comparison was performed using the Tukey HSD test. In the descriptive part of the statistics, the arithmetic mean and standard deviation ($x \pm SD$) were calculated; the normality of the obtained sample was confirmed by the Shapiro–Wilk test (W-test). Discriminant analysis (standard modules of the program) was used to determine the model character of the obtained features and their significance.

Results

An analysis of the fertility of the obtained samples of the mutant population of winter wheat (Table 1) demonstrated that, in general, a fairly gradual increase in the amount of sterile pollen is characteristic of increasing the concentration, while this feature is observed to divide varieties into two groups. Varieties Balaton, Zeleny Gai, Zoloto Ukrainy, Niva Odeska belong to the first group, which is characterized by a greater decrease in viability to the level of 60–63% at SA 0.1%. At the same time, the trend began to manifest itself already at the first concentration, and the difference changed moderately with each increase in the amount of the factor (from the initial 2–3% to the subsequent 5–6% difference between the varieties of the first and second groups on average). The second group includes varieties with a higher level of fertility Borovytsia, Kalancha, Polyanka, Pochayna. In any case, the differences between the two groups in terms of sterility are statistically significant ($F = 4.02$; $F_{0.05} = 2.17$; $P = 0.03$), while pairwise comparison demonstrates the same trend – a significant difference between any variety of the first group from the second and insignificant within the group.

Pollen fertility is a rather important indicator for determining the possibility of obtaining the required amount of material for study in the first generation, and, from this point of view, even the use of an extreme dose did not lead to significant problems – the amount of viable pollen received is more than enough for effective fertilization. In the control as a whole, in all years of the study, fertility was significant and no significant violations

were noted. A general analysis of the genotype and mutagen concentration factors demonstrated that the effect of the genotype factor was not significant ($F = 1.78$; $F_{0.05} = 2.35$; $P = 0.07$), while an increase in concentration significantly affected the increase in sterility ($F = 274.01$; $F_{0.05} = 2.71$; $P = 4.17*10^{-15}$), however, the interaction of the genotype and mutagen was very significant ($F = 20.98$; $F_{0.05} = 1.87$; $P = 3.22*10^{-5}$), with the varieties of the second group standing out especially. Apparently, this influence determined the differentiation of varieties. Thus, for this trait, we should expect to find the desired varieties under the action of sodium azide, first of all, in the second group, while the varieties Borovytsia and Polyanka stand out somewhat. Also, in the case of the first group, varieties Zeleny Gai and Zoloto Ukrainy can be theoretically promising, but less than the varieties of the second group. The varieties Balaton and Niva Odeska are of the least interest. At the same time, promising varieties of the first and second groups have the highest genotypically determined variance, although the overall variability of this component is insufficient. In all cases, at all concentrations and controls, the differences are statistically significant for each genotype separately.

Table 1

Influence of SA action on pollen sterility trait of first-generation spikes ($x \pm SD$, $n = 25$)

Variety	Control	SA 0.010%	SA 0.025%	SA 0.050%	SA 0.100%
Balaton	99.26 ± 1.00^{a}	84.17 ± 0.97^{b}	75.17 ± 1.16^{c}	69.57 ± 1.57^{d}	63.14 ± 1.67^{e}
Zeleny Gai	99.55 ± 0.95^{a}	82.02 ± 1.09^{b}	76.40 ± 1.27^{c}	69.79 ± 1.58^{d}	63.29 ± 1.58^{e}
Zoloto Ukrainy	99.22 ± 0.95^{a}	83.15 ± 1.15^{b}	75.15 ± 1.20^{c}	69.66 ± 1.48^{d}	62.31 ± 1.68^{e}
Niva Odeska	98.94 ± 0.94^{a}	84.02 ± 1.12^{b}	74.14 ± 1.23^{c}	70.10 ± 1.51^{d}	60.19 ± 1.71^{e}
Borovytsia	99.29 ± 1.11^{a}	86.17 ± 1.02^{b}	79.95 ± 1.34^{c}	73.17 ± 1.54^{d}	66.92 ± 1.39^{e}
Kalancha	98.98 ± 1.10^{a}	86.28 ± 1.02^{b}	79.82 ± 1.18^{c}	73.53 ± 1.49^{d}	66.94 ± 1.33^{e}
Polyanka	99.17 ± 1.13^{a}	88.07 ± 1.03^{b}	81.13 ± 1.29^{c}	75.12 ± 1.44^{d}	68.73 ± 1.44^{e}
Pochayna	99.32 ± 1.01^{a}	88.11 ± 1.11^{b}	81.41 ± 1.26^{c}	74.17 ± 1.49^{d}	69.22 ± 1.49^{e}

Note: indicate significant differences at $P < 0.05$ by Tukey HSD test with Bonferroni amendment. Comparison in terms of one variety at rows.

In the case of the overall frequency of chromosome aberrations (Table 2), the situation with the effect of the mutagen changes somewhat, although, as we will see below, the classification into two groups, which coincide with the same case with pollen sterility, remains. In this case, not only the genotype-mutagenic interaction, but also the influence of the genotype acts as a section on the specifics of the action. So, in factor analysis, we find that the effect of the genotype factor was already significant ($F = 9.08$; $F_{0.05} = 2.35$; $P = 0.007$), however, an increase in concentration significantly affected the increase in the total number of cells with aberrations ($F = 298.01$; $F_{0.05} = 2.71$; $P = 2.22*10^{-16}$), the interaction of the genotype and mutagen was again very significant ($F = 29.99$; $F_{0.05} = 1.87$; $P = 2.92*10^{-6}$). It is also worth noting that the main differences between the two groups were in the effect of the extreme dose. So, in the first group, a decrease in the overall frequency was observed, while in the second, the frequency increased linearly until the last concentration. The only exception was Zoloto Ukrainy, which had no statistically significant differences between the effects of the third and fourth concentrations ($F = 2.12$; $F_{0.05} = 2.48$; $P = 0.06$). Again, the effect of the genotype factor was most pronounced in the varieties Borovytsia and Polyanka, the genotype-mutagenic interaction was more pronounced in the variety Borovytsia, the difference between the two groups was also significant – such an interaction was more significant for the second group, and the contrast between the groups increased even more, and within the groups – significantly decreased. So, the first group, in principle, is much more homogeneous.

The overall frequency of rearrangements was insignificant in the control, no more than 1.00%. At the same time, even the first SA concentration of 0.01% sharply and statistically significantly increased the frequency from 8.91% (variety Kalancha) to 12.19% (variety Polyanka), which is generally very significant and indicates a high damaging ability of this mutagen. Under the action of a second SA concentration of 0.025%, the number of rearrangements varied from 12.14% (variety Pochayna) to 16.73% (variety Zeleny Gai), the difference with the control and the previous concentration being statistically significant in all cases. As for the third SA concentration of 0.05%, the frequency of aberrations again increased significantly in all variants, changing from 18.97% (variety Pochayna) to 25.74% (variety Zeleny Gai). In the case of switching to the action of the last SA concentration of 0.1%, the frequency significantly decreased in varieties Balaton, Zeleny Gai, Niva Odeska, remained at the same level in variety Zoloto Ukrainy and increased in varieties Borovytsia, Kalancha, Polyanka, and Pochayna, changing from 20.07% (variety Niva Odeska) to 26.81% (variety Borovytsia). Thus, the boundaries of variability in the first group are still somewhat more significant for this indicator, as in the case of the first trait.

It may be said that the selection of concentrations from the point of view of the analysis of cytogenetic variability is extremely successful – all of them are more or less contrasting for a given material (obviously, one should not expect any other fluctuations when using other genotypes, even those that are very ecologically and geographically far from the varieties used).

Table 2

General rates of chromosomal abnormalities in proper division mitotic cells of root tips ($x \pm SD$, $n = 800–1000$)

Variety	Variant	Mitosis, number	Chromosomal aberrations	
			number	%
Balaton	water	1002	10	1.00 ± 0.12^{a}
Balaton	SA 0.010%	1001	92	9.19 ± 0.31^{b}
Balaton	SA 0.025%	1009	161	15.96 ± 0.24^{c}
Balaton	SA 0.050%	912	215	23.57 ± 0.39^{d}
Balaton	SA 0.100%	801	163	20.35 ± 0.30^{e}
Zeleny Gai	water	1008	9	0.89 ± 0.32^{a}
Zeleny Gai	SA 0.010%	1007	97	9.63 ± 0.27^{b}
Zeleny Gai	SA 0.025%	1004	168	16.73 ± 0.36^{c}
Zeleny Gai	SA 0.050%	847	218	25.74 ± 0.41^{d}
Zeleny Gai	SA 0.100%	798	176	22.06 ± 0.32^{e}
Zoloto Ukrainy	water	1001	8	0.80 ± 0.21^{a}
Zoloto Ukrainy	SA 0.010%	1002	100	9.98 ± 0.27^{b}
Zoloto Ukrainy	SA 0.025%	1004	159	15.84 ± 0.35^{c}
Zoloto Ukrainy	SA 0.050%	889	201	22.61 ± 0.43^{d}
Zoloto Ukrainy	SA 0.100%	812	173	21.31 ± 0.39^{d}
Niva Odeska	water	1009	8	0.79 ± 0.23^{a}
Niva Odeska	SA 0.010%	1004	92	9.16 ± 0.26^{b}
Niva Odeska	SA 0.025%	1010	126	12.48 ± 0.31^{c}
Niva Odeska	SA 0.050%	920	198	21.52 ± 0.42^{d}
Niva Odeska	SA 0.100%	817	164	20.07 ± 0.35^{e}
Borovytsia	water	1001	7	0.70 ± 0.20^{a}
Borovytsia	SA 0.010%	1007	104	10.33 ± 0.21^{b}
Borovytsia	SA 0.025%	1003	151	15.05 ± 0.32^{c}
Borovytsia	SA 0.050%	941	207	22.00 ± 0.40^{d}
Borovytsia	SA 0.100%	854	229	26.81 ± 0.52^{e}
Kalancha	water	1000	10	1.00 ± 0.15^{a}
Kalancha	SA 0.010%	1010	90	8.91 ± 0.20^{b}
Kalancha	SA 0.025%	1004	139	13.84 ± 0.28^{c}
Kalancha	SA 0.050%	980	188	19.18 ± 0.35^{d}
Kalancha	SA 0.100%	893	199	22.28 ± 0.42^{e}
Polyanka	water	1007	6	0.60 ± 0.26^{a}
Polyanka	SA 0.010%	1001	122	12.19 ± 0.26^{b}
Polyanka	SA 0.025%	1004	146	14.54 ± 0.33^{b}
Polyanka	SA 0.050%	968	198	20.45 ± 0.41^{c}
Polyanka	SA 0.100%	890	209	23.48 ± 0.52^{e}
Pochayna	water	1005	8	0.80 ± 0.06^{a}
Pochayna	SA 0.010%	1010	93	9.21 ± 0.19^{b}
Pochayna	SA 0.025%	1005	122	12.14 ± 0.22^{c}
Pochayna	SA 0.050%	954	181	18.97 ± 0.31^{d}
Pochayna	SA 0.100%	901	203	22.53 ± 0.42^{e}

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

The study of the spectrum of changes made it possible to identify such types of rearrangements as fragments and double fragments, bridges of various types, micronuclei and lagging chromosomes (other types of aberrations), which were separately taken into account as a reliable parameter for enhancing the cell mutation process in the corresponding division phases with complex changes (two or more rearrangements) (Table 3 and 4). The ratio of fragments to bridges (as the main types of aberrations characterizing the nature of the mutagenic factor) was also calculated.

The frequency of fragments and double fragments in the first group in the control is characterized by an insignificant amount, however, they are present without fail. Already the first SA concentration of 0.01% leads to a sharp and significant increase in their amount in all varieties, with the amount in the first group from 47.0 (variety Balaton) to 61.0 (variety Zeleny Gai), for the second group from 48.0 (variety Kalancha) to 84.0 (variety Polyanka), which is quite significant in general and indicates the predominant induction of this type of aberration. Under the action of the second SA concentration of 0.025%, the number of fragments varies in the first group from 75.0 (variety Niva Odeska) to 104.0 (variety Zeleny Gai), for the second group from 71.0 (variety Kalancha) to 92.0 (variety Polyanka), the difference from the control and the previous concentration was statistically significant in all cases. As for the third SA concentration of 0.05%, in the first group the number of fragments varies from 101.0 (variety Balaton) to 116.0 (variety Zeleny Gai), for the second group from 88.0 (varieties Kalancha, Pochayna) to 103.0 (variety Polyanka), while again all differences from the previous concentration in all varieties are observed upwards. Differences in cultivars and groups begin at the transition to the last SA concentration of 0.1%, where the number of fragments in the first group varies from 75.0 (variety Niva Odeska) to 91.0 (variety Zeleny Gai), in the second group from 94.0 (varieties Kalancha, Pochayna) to 110.0 (variety Borovytsia). As can be seen, in the case of the first group, the absolute number of fragments drops rather sharply, in the second group it increases, but not so significantly and with variations in varieties, so in the first group, in contrast to the total frequency, there is no difference in the number of fragments at the third and fourth concentration already in two varieties Zoloto Ukrainy ($F = 2.09$; $F_{0.05} = 2.48$; $P = 0.07$) and Niva Odeska ($F = 1.99$; $F_{0.05} = 2.48$; $P = 0.07$), in other varieties it significantly decreases. For the second group, there are no significant differences between Kalancha ($F = 2.26$; $F_{0.05} = 2.48$; $P = 0.06$) and Polyanka ($F = 2.32$; $F_{0.05} = 2.48$; $P = 0.06$), the number of fragments remains at the same level, while the number of fragments increases in the other two.

If we analyze a part of the total number of aberrations, then all varieties of the first group are characterized by a slight decrease in the part, except for the variety Balaton, which again has a rather sharp increase at the maximum concentration. For the second group, the same trend is observed, except for the behaviour similar to the previous one in the variety Borovytsia. For the indicator of fragment induction, the effect of the genotype factor was significant ($F = 18.19$; $F_{0.05} = 2.35$; $P = 0.001$), however, an increase in concentration significantly affected the change in the total number of fragments ($F = 321.01$; $F_{0.05} = 2.71$; $P = 1.97*10^{-17}$), the interaction of the genotype and mutagen was also very significant here ($F = 25.54$; $F_{0.05} = 1.87$; $P = 2.32*10^{-5}$). At the same time, varieties of the second group stood out again, however, three of them were already at the same level, this was much weaker in the Kalancha variety.

Table 3

Spectra of chromosomal abnormalities in proper division mitotic cells of root tips, first group ($x \pm SD$, $n = 800–1000$)

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 ± 0.4^{a}	51.1	4.0 ± 1.4^{a}	44.4	1.0	1.0 ± 0.9^{a}	11.1	0.0 ± 0.0^{a}	0.0
Balaton	SA 0.010%	47.0 ± 0.7^{b}	53.4	32.0 ± 1.7^{b}	34.8	1.5	13.0 ± 1.3^{b}	14.1	13.0 ± 1.4^{b}	14.1
Balaton	SA 0.025%	86.0 ± 1.3^{c}	47.0	56.0 ± 2.3^{c}	34.8	1.5	19.0 ± 1.8^{c}	11.8	34.0 ± 1.5^{c}	21.1
Balaton	SA 0.050%	101.0 ± 1.8^{d}	46.6	85.0 ± 3.2^{d}	39.5	1.2	29.0 ± 2.7^{d}	13.5	52.0 ± 2.9^{d}	24.2
Balaton	SA 0.100%	76.0 ± 1.2^{e}	51.1	65.0 ± 2.7^{e}	39.9	1.2	22.0 ± 2.0^{e}	13.5	45.0 ± 2.3^{d}	27.6
Zeleny Gai	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
Zeleny Gai	SA 0.010%	61.0 ± 1.1^{b}	62.9	26.0 ± 2.2^{b}	26.8	2.4	10.0 ± 1.5^{b}	10.3	13.0 ± 2.1^{b}	13.4
Zeleny Gai	SA 0.025%	104.0 ± 1.9^{c}	61.9	47.0 ± 2.9^{c}	28.0	2.2	17.0 ± 3.0^{c}	10.1	21.0 ± 2.3^{c}	12.5
Zeleny Gai	SA 0.050%	116.0 ± 2.8^{d}	53.2	68.0 ± 3.4^{d}	31.2	1.7	34.0 ± 3.1^{d}	15.6	44.0 ± 2.9^{d}	20.2
Zeleny Gai	SA 0.100%	91.0 ± 2.7^{e}	51.7	58.0 ± 3.3^{e}	33.0	1,6	26.0 ± 3.1^{e}	15.3	38.0 ± 2.6^{d}	21.6
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
Zoloto Ukrainy	SA 0.010%	59.0 ± 2.7^{b}	59.0	31.0 ± 2.1^{b}	31.0	1.9	10.0 ± 1.7^{b}	10.0	10.0 ± 1.0^{b}	10.0
Zoloto Ukrainy	SA 0.025%	89.0 ± 2.3^{c}	55.9	53.0 ± 2.6^{c}	33.3	1.7	17.0 ± 2.1^{c}	10.7	24.0 ± 2.5^{c}	15.1
Zoloto Ukrainy	SA 0.050%	106.0 ± 2.5^{d}	52.7	64.0 ± 2.1^{d}	31.8	1.7	31.0 ± 2.2^{d}	15.4	55.0 ± 3.1^{d}	27.4
Zoloto Ukrainy	SA 0.100%	88.0 ± 2.2^{c}	50.9	56.0 ± 2.5^{cd}	32.4	1.6	29.0 ± 2.0^{d}	16.8	50.0 ± 3.1^{d}	28.9
Niva 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
Niva Odeska	SA 0.010%	55.0 ± 1.5^{b}	59.8	29.0 ± 1.4^{b}	31.5	1.9	8.0 ± 1.0^{b}	8.7	13.0 ± 2.2^{b}	14.1
Niva Odeska	SA 0.025%	75.0 ± 2.7^{c}	59.5	33.0 ± 2.2^{b}	26.2	2.3	18.0 ± 2.1^{c}	14.3	31.0 ± 2.4^{c}	24.6
Niva Odeska	SA 0.050%	105.0 ± 2.5^{d}	53.0	58.0 ± 2.3^{c}	29.3	1.8	35.0 ± 2.4^{d}	17.7	49.0 ± 2.2^{d}	24.8
Niva Odeska	SA 0.100%	75.0 ± 2.4^{e}	45.7	61.0 ± 2.5^{c}	37.2	1.2	28.0 ± 2.0^{e}	17.1	48.0 ± 2.3^{d}	29.3

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

As for chromatid and chromosomal bridges, all genotypes in the control are characterized by a small amount, however, they are again present without fail. Again, the SA concentration of 0.01% leads to a sharp and significant increase in their amount in all varieties, with the amount in the first group varying from 26.0 (variety Zeleny Gai) to 32.0 (variety Balaton), in the second group from 28.0 (variety Polyanka) to 39.0 (variety Borovytsia). This is a fairly significant amount, but less than the fragments by an order of magnitude, it is also noticeable that again mainly the same genotypes and limits of variations are involved. The features of the groups, although they intersect, are still different. Under the action of a second SA concentration of 0.025%, the number of bridges varied in the first group from 33.0 (variety Niva Odeska) to 56.0 (variety Balaton), in the second group from 34.0 (variety Pochayna) to 46.0 (variety Borovytsia), the difference from the control and the previous concentration was statistically significant in all cases, except for the variety Niva Odeska ($F = 2.11$; $F_{0.05} = 2.48$; $P = 0.06$) for the first group, in the second group, on the contrary, the difference was significant only for one variety Polyanka ($F = 3.87$; $F_{0.05} = 2.48$; $P = 0.03$) The groups reacted approximately equally to this concentration in terms of the boundaries of the trait's variability, but it is already noticeable that it is less typical for this mutagenic effect. As for the third SA concentration of 0.05%, the number of bridges in the first group varied from 58.0 (variety Niva Odeska) to 85.0 (variety Balaton), for the second group from 53.0 (varieties Kalancha) to 69.0 (variety Borovytsia), the differences between the groups become smaller, the variety Balaton is increasingly singled out ($F = 4.14$; $F_{0.05} = 2.48$; $P = 0.02$), while again, all differences from the previous concentration in all varieties are observed upwards for the first group in comparison with the previous concentration, for the second group it is similar. Differences in cultivars and groups begin again upon passing to the last SA concentration of 0.1%, where the number of bridges in the first group is from 56.0 (variety Zoloto Ukrainy) to 65.0 (variety Balaton), in the second group from 58.0 (varieties Kalancha) to 71.0 (variety Borovytsia). As you can see, in the case of the first group, the absolute number of bridges also drops quite sharply, in the second group it remains approximately at the same level with some

variation in genotypes. In this case, in the first group for the Balaton variety, the number of bridges differs from the previous concentration, but at the level of the second, for the Zeleny Gai variety it differs, but significantly decreases, for the Zoloto Ukrainy variety it is intermediate between the second and third concentrations, for the Niva Odeska variety it slightly increases, at the level of the previous one. As for the second group, only in the variety Pochayna ($F = 5.56$; $F_{0.05} = 2.48$; $P = 0.01$) are the differences significant in relation to the previous concentration, in the other three the trait remains at the same level. Thus, in the case of bridges, the situation is much more complicated and contradictory; in some cases, there are no differences at all.

As for the percentage of the total number of aberrations, all varieties of the first group are characterized by a slight decrease in the part, followed by an increase at the third or fourth concentration. This is generally a fairly typical reaction for a chemical supermutagen where site specificity drops at high concentrations. For the second group, the trend is even more pronounced. For the indicator of bridge induction, the effect of the genotype factor was not significant ($F = 1.99$; $F_{0.05} = 2.35$; $P = 0.06$), however, an increase in concentration significantly affected the change in the total number of this type of rearrangements ($F = 204.13$; $F_{0.05} = 2.71$; $P = 3.08*10^{-12}$), the interaction of the genotype and mutagen was still significant ($F = 13.72$; $F_{0.05} = 1.87$; $P = 5.16*10^{-3}$). At the same time, all varieties were approximately at the same level, even the differences at the group level can hardly be called significant, except for the variety Borovytsia.

As for the ratio between the number of fragments and bridges, it was approximately the same at all concentrations, in favour of fragments, which is typical for chemical mutagenesis. According to the dynamics for all varieties, except for one, it first slightly increased (an increase in the proportion of fragments), then decreased again, which also indicates site-specific variability, however, in the Balaton variety, it changed slightly from concentration to concentration, which may indicate a lower susceptibility of this genotype to sodium azide.

With regard to more rare types of chromosomal aberrations, they are not always found in the control (absent for two varieties) and their role increases significantly at high concentrations. For all varieties, the SA concentration of 0.01% leads to a sharp and significant increase in their number with the number in the first group ranging from 8.0 (variety Niva Odeska) to 13.0 (variety Balaton), for the second group from 10.0 (variety Polyanka) to 13.0 (variety Kalancha).This is a fairly significant amount, but less than bridges by an order of magnitude, it is also noticeable that mainly the same genotypes and the limits of variability of groups are again involved. Clearly, there is clearly no effect of the genotype. Under the action of the second SA concentration of 0.025%, the number of rare rearrangements varied in the first group from 17.0 (varieties Zeleny Gai, Zoloto Ukraine) to 19.0 (variety Balaton), for the second group from 16.0 (variety Polyanka) to 33.0 (variety Kalancha), the difference from the control and the previous concentration was statistically significant in all cases, the limits of variation were significantly higher in the second group, but again differers little overall. As for the third SA concentration of 0.05%, in the first group rare aberrations varied from 29.0 (variety Balaton) to 35.0 (variety Niva Odeska), for the second group from 33.0 (varieties Polyanka) to 47.0 (variety Kalancha).The differences between the groups are insignificant, again all differences from the previous concentration in all varieties are observed upwards in comparison with the previous concentration. Differences in cultivars begin again upon passing to the last SA concentration of 0.1%, where the number of other types of rearrangements in the first group ranges from 22.0 (variety Balaton) to 29.0 (variety Zoloto Ukrainy), in the second group from 34.0 (varieties Polyanka) to 48.0 (variety Borovytsia). At the same time, the differences between the groups again become significant ($F = 3.43$; $F_{0.05} = 2.17$; $P = 0.04$), and there is also no statistically significant difference in the induction of this type of aberration between the third and first concentrations in all varieties of the first group and in the varieties Borovytsia and Pochayna of the second group.

As for the percentage of the total number of aberrations, all varieties of the first group are characterized by a gradual increase in the proportion of rare aberrations, followed by stabilization or some decrease at the third or fourth concentration. This is also a typical reaction for a chemical supermutagen, which indicates the achievement of critical concentrations. For the indicator of induction of rare types of aberrations, the effect of the genotype factor was not significant ($F = 1.51$; $F_{0.05} = 2.35$; $P = 0.08$), however, an increase in concentration significantly affected the change in the total number of rearrangements of this type ($F = 345.13$; $F_{0.05} = 7.62$; $P = 3.08*10^{-17}$), the interaction of the genotype and mutagen was not significant for the first time ($F = 1.19$; $F_{0.05} = 1.87$; $P = 0.08$). At the same time, all varieties were approximately at the same level, even differences at the group level were not significant. Only the variety Niva Odeska stood out somewhat, for which this type of change is more characteristic individually.

Table 4

Spectra of chromosomal abnormalities in proper division mitotic cells of root tips, second group ($x \pm SD$, $n = 800–1000$)

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 ± 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	SA 0.010%	54.0 ± 2.4^{b}	51.9	39.0 ± 2.1^{b}	37.5	1.4	11.0 ± 2.1^{b}	10.6	11.0 ± 1.5^{b}	10.6
Borovytsia	SA 0.025%	81.0 ± 2.5^{c}	53.6	46.0 ± 3.1^{b}	30.5	1.8	24.0 ± 2.2^{c}	15.9	23.0 ± 2.2^{c}	15.2
Borovytsia	SA 0.050%	93.0 ± 3.2^{d}	44.9	69.0 ± 3.0^{c}	33.3	1.4	45.0 ± 2.6^{d}	21.7	60.0 ± 2.1^{d}	29.0
Borovytsia	SA 0.100%	110.0 ± 3.1^{e}	48.0	71.0 ± 3.0^{c}	31.0	1.6	48.0 ± 2.7^{d}	21.0	65.0 ± 2.5^{d}	28.4
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	SA 0.010%	48.0 ± 2.2^{b}	49.0	29.0 ± 1.5^{b}	29.6	1.7	13.0 ± 1.5^{b}	13.3	14.0 ± 2.1^{b}	14.3
Kalancha	SA 0.025%	71.0 ± 2.7^{c}	49.7	35.0 ± 2.6^{b}	24.5	2.0	33.0 ± 2.4^{c}	23.1	28.0 ± 2.0^{c}	19.6
Kalancha	SA 0.050%	88.0 ± 3.0^{d}	43.8	53.0 ± 3.1^{c}	26.4	1.7	47.0 ± 3.1^{d}	23.4	45.0 ± 3.0^{d}	22.4
Kalancha	SA 0.100%	94.0 ± 3.2^{d}	46.8	58.0 ± 3.0^{c}	28.9	1.6	47.0 ± 3.1^{d}	23.4	54.0 ± 3.1^{e}	26.9
Polyanka	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
Polyanka	SA 0.010%	84.0 ± 3.2^{b}	68.9	28.0 ± 2.1^{b}	23.0	3.0	10.0 ± 2.0^{b}	8.2	13.0 ± 0.9^{b}	10.7
Polyanka	SA 0.025%	92.0 ± 3.0^{c}	63.0	38.0 ± 2.6^{c}	26.0	2.4	16.0 ± 2.1^{c}	11.0	20.0 ± 1.7^{c}	13.7
Polyanka	SA 0.050%	103.0 ± 3.5^{d}	52.0	62.0 ± 2.6^{d}	31.3	1.7	33.0 ± 2.6^{d}	16.7	50.0 ± 2.6^{d}	25.3
Polyanka	SA 0.100%	107.0 ± 4.0^{d}	51.2	68.0 ± 3.1^{d}	32.5	1.6	34.0 ± 2.7^{d}	16.3	57.0 ± 3.1^{e}	27.3
Pochayna	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
Pochayna	SA 0.010%	50.0 ± 1.6^{b}	53.8	31.0 ± 2.0^{b}	33.3	1.6	12.0 ± 1.5^{b}	12.9	10.0 ± 1.5^{b}	10.8
Pochayna	SA 0.025%	68.0 ± 2.1^{c}	55.7	34.0 ± 2.4^{b}	27.9	2.0	20.0 ± 2.0^{c}	16.4	23.0 ± 2.0^{c}	18.9
Pochayna	SA 0.050%	88.0 ± 2.5^{d}	48.6	54.0 ± 2.8^{c}	29.8	1.6	39.0 ± 2.3^{d}	21.5	57.0 ± 2.5^{d}	31.5
Pochayna	SA 0.100%	99.0 ± 3.1^{e}	48.8	62.0 ± 3.0^{d}	30.5	1.6	42.0 ± 2.6^{d}	20.7	65.0 ± 3.1^{d}	32.0

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

The presence of cells with complex chromosomal aberrations is uncharacteristic for the control and there is only one case in the variety Niva Odeska. Already with the first SA concentration of 0.01%, the picture changes radically both in the first group from 10.0 (variety Zoloto Ukraine) to 13.0 (the other three varieties), and for the second group from 10.0 (variety Pochayna) to 14.0 (variety Kalancha).This is a rather signifi-

cant amount, the differences from the control are always significant, but approximately the same as with the frequency of rarer types of aberrations. At the same time, it is noticeable that there are absolutely no differences between groups, as well as significant variation in genotypes. Under the action of the second SA concentration of 0.025%, the number of cells with complex aberrations varied in the first group from 21.0 (variety Zeleny Gai) to 34.0 (variety Balaton), in the second group from 20.0 (variety Polyanka) to 28.0 (variety Polyanka).The difference from the control and the previous concentration was statistically significant in all cases, there were no significant differences between the groups. As for the third SA concentration of 0.05%, in the first group the number of such cells ranged from 44.0 (variety Zeleny Gai) to 55.0 (variety Zoloto Ukraine), for the second group from 45.0 (varieties Kalancha) to 60.0 (variety Borovytsia). There is no difference between the groups, as well as differences in varieties, all values are statistically significantly different from the effect of the previous concentration. Some variability occurs under the action of the last SA concentration of 0.1%, where in the first group the number of cells with complex aberrations varies from 38.0 (variety Zeleny Gai) to 50.0 (variety Zoloto Ukraine), in the second group from 54.0 (varieties Kalancha) to 65.0 (varieties Borovytsia, Pochayna). At the same time, there is no statistically significant difference from the third concentration for most varieties; in fact, in the range of the third – fourth concentration, a mutagen action plateau is observed, which indicates the achievement of critical values, except for the varieties of the second group Kalancha and Polyanka, in which this indicator has statistically significantly increased.

As for the percentage of the total number of aberrations, all varieties of the first group are characterized by an increase in the specific gravity of the number of such cells, then, when the third concentration is reached, it reaches a maximum and declines to the last one. For the second group, the trend is different, although the indicator is much weaker, but it also increases from the third concentration to the fourth. For the indicator of the number of cells with complex aberrations, the effect of the genotype factor was not significant ($F = 1.55$; $F_{0.05} = 2.35$; $P = 0.09$), however, an increase in concentration significantly affected the change in the total number of this type of cells ($F = 392.17$; $F_{0.05} = 4.67$; $P = 3.08*10^{-19}$), the interaction of the genotype and mutagen was significant ($F = 19.31$; $F_{0.05} = 1.87$; $P = = 1.17*10^{-5}$). At the same time, all varieties were approximately at the same level, even the differences at the level of groups can hardly be called significant, only the variety Borovytsia and Pochayna stood out somewhat.

The performed discriminant analysis (Table 5, Fig. 1), in turn, confirmed the model character of all traits for an increase in mutagen concentration, except for the presence of bridges, while the effect of the variety was noted only for pollen sterility, total frequency, and number of fragments. However, the interaction of factors was also significant in the case of the presence of bridges and cells with two or more types of changes.

Table 5
Trait in model after discriminant analyses

Parameter	Genotype			Concentration		
	Wilks' - Lambda	F_{remove} (7.85)	P	Wilks' - Lambda	F_{remove} (3.89)	P
Pollen fertility	0.018	9.92	0.02	0.026	18.17	0.01
General rates	0.022	12.44	0.01	0.024	16,92	0.01
Fragments	0.020	9.14	0.02	0.021	14.51	0.01
Bridges	0.003	3.12	0.09	0.014	5.33	0.03
Other	0.002	3.01	0.09	0.018	9.12	0.01
Double and more	0.002	2.97	0.09	0.019	10.38	0.01

It can also be answered from Figure 1 that, in general, SA concentrations of 0.010%, 0.025% were clearly contrasting in action. The difference between them and the last two concentrations of 0.05% and 0.10% was also significant. However, the third and fourth are clearly served by a mixed group, thus the question arises as to the appropriateness of using these both concentrations. However, this seems to be justified for a certain part of the genotypes, given the previous factor analysis, approximately a quarter of the total number.

The classification of the totality of objects according to the influence of both different concentrations of the mutagen, and depending on belonging to a particular variety, demonstrated, on the whole, extremely satisfactory results. In the case of concentrations, in fact, the error even at the third or fourth is insignificant and the given number of objects (no more than one eighth) can be neglected. The influence of the first three studied parameters is demonstrated. In the case of the cultivar (genotype), even in the most unfavourable variant of the Balaton cultivar, more than half of the objects turn out to be in the required population, although, in general, the use of sodium azide for mutagenesis in its case is generally unpromising. On average, at least 75% of the objects were clearly classified, which confirms the significant resolution of the parameters chosen for the study.

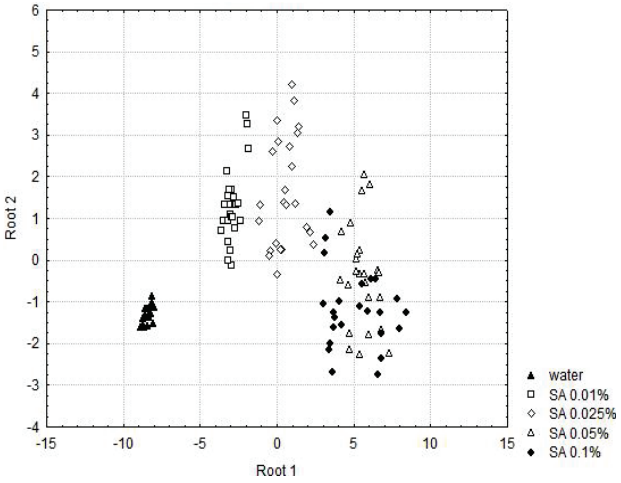

Fig. 1. Classification by canonical functions
(discriminant analysis for SA as factor, by Mahalanobis distances)

Table 6
Classification ability for factor interaction (by canonical function)

Objects by concentrations in model, %	Water	100
	SA 0.010%	93.8
	SA 0.025%	93.8
	SA 0.050%	87.5
	SA 0.100%	87.5
	Total	92.5
Objects by genotypes in model, %	Balaton	52.5
	Borovytsia	87.5
	Zeleny Gai	75.0
	Zoloto Ukrainy	75.0
	Kalancha	82.5
	Niva Odeska	62.5
	Polyanka	87.5
	Pochayna	82.5
	Total	75.6

It may be said that the resulting model, in terms of the features included in it and taking into account the component in the overall variability of individual factors, demonstrated a full opportunity to predict the subsequent effectiveness of the use of sodium azide as a genetically active substance in terms of obtaining a high level of variability. This is especially true for varieties Borovytsia and Polyanka, a little less for other varieties of the second group. Varieties of the first group, especially Balaton and Niva Odeska, are less suitable for this type of impact, however, in general, a significant yield of mutant forms is well within the confidence interval. Moreover, this substance clearly demonstrates very high levels of potential genetic variability.

Discussion

Identification of the key components of cytogenetic variability makes it possible to significantly simplify the future selection of the required doses and concentrations of the mutagenic factor (Amri-Tiliouine et al., 2018). At the same time, they are guided primarily by such factors as the achievement by certain values of the corresponding milestones with the maximization of activity or a plateau in activity for some indicators (Shabani et al., 2022). For chemical supermutagens, such variants were repeatedly considered signs of the induction of fragments and double fragments in the spectrum of chromosomal aberrations, the general frequency of rearrangements (Beyaz et al., 2016).

Some studies have repeatedly noted the possibly key role of this type of changes as micronuclei, which form the basis of a qualitative test for ecogenetic activity and monitoring of environmental pollution. However, these studies demonstrate that what is suitable for low doses and concentrations of genetically active factors is not always suitable for primary screening in studies on inducible biodiversity (Beyaz et al., 2016). Quite often, this requires the use of factor values that would be far beyond the limits of what is possible in the environment, even at high degrees of pollution (Balkan et al., 2019). Proceeding from this, it should be noted that although rarer types of chromosomal aberrations can serve as an indicator of an increase in concentrations to a certain extent (until the mitotic activity of cells as a whole is critically suppressed), they still do not reflect the specifics of the genotype in cytogenetic studies, and even more so, its interaction with a specific chemical agent (Ahumada-Flores et al., 2020; Shabani et al., 2022).

In turn, the use of the number of cells with complex (two or more aberrations), as well as the use of a less characteristic bridge type rearrangement to establish the features of the genotype-mutagenic interaction in individual varieties and groups of varieties as a whole, is quite promising (Ram et al., 2019; Navid, 2021). However, it is not entirely clear whether it is worth using them if there are more reliable indicators that also reflect the influence of the genotype of each variety separately with the desired level of confidence (Aly et al., 2019).

Indicators that reliably identify the significance of the genotype-mutagenic interaction and fix the characteristics of the reaction of varieties are pollen fertility, the total frequency of rearrangements, the number of fragments and double fragments (Rozman, 2015). At the same time, the increased variability in certain genotypes according to these traits may be the basis for isolating this variety (Beiko & Nazarenko, 2022) as a promising form for obtaining valuable and diverse changes using sodium azide (Shabani et al., 2022; Von Well et al., 2023a).

We can also agree with previous studies of this mutagen in other cereal crops, primarily in terms of depressive effects in the first generation and induction of mutations, that, in general, the concentrations proposed for practical use are sufficiently contrasting to obtain a wide variety in the largest possible group of genotypes (Zhao et al., 2019; Ahumada-Flores et al., 2020). Other effects are possible, but primarily in groups that are of more limited interest – with the presence of significant translocations from wild relatives, perhaps some specific local forms and landraces (Pekol et al., 2016). Perhaps, given the similar effects under the action of high concentrations of SA mutagen 0.05% and 0.10%, it would be worthwhile to make a choice in favour of one of these concentrations, especially the lower one (Spencer-Lopeset et al., 2018). However, as can be seen from the response of some variants, this may narrow the possibilities for the emergence of promising forms in the future for some less variable initial subjects (Nazarenko, 2020). Thus, the position on expansion of the used gradations of the mutagenic factor remains unchanged, since even in this study there are at least two of these forms out of eight, in fact, a quarter of the sample, which is very significant (Ahumada-Flores et al., 2020; Von Well et al., 2022).

The study of cytogenetic activity has demonstrated its importance not only as a test system for the mutagenic factor itself and a way to determine the limits of its activity and the optimal use of concentrations or doses, but also as an opportunity to assess the suitability of this factor for a specific genotype, a specific subject of exposure, which may be an extremely promising component of the system in the future with an optimized yield of valuable modified material (El-Mouhamady & Ibrahim, 2020). Thus, through many years of study, both from the side of selecting individual concentrations, types of substances, genotypes, it was possible to increase the efficiency of the mutation process by an order of magnitude (Spencer-Lopeset et al., 2018). Also, such an approach is promising from the point of view of obtaining complex biochemical mutations, i.e. in the fulfillment of tasks both in obtaining food products that are more complete in terms of microelements and biologically active substances, and in creating forms with physiologically determined characteristics of growth and development, which serve as the basis for high tolerance to adverse environmental factors (Nazarenko et al., 2022), possibly through qualitatively new mechanisms, as was the case with forms with an increased nitrogen reutilization period in cereals.

Conclusion

To optimize the yield of promising valuable forms, it would be worthwhile to use, first of all, two varieties Borovytsia and Polyanka with this ecogenetic factor, while the use of the concentrations involved will obviously be generally effective. Although, if we link the data on mutational variability, we should expect the use of the third-fourth concentration range to be more successful from the point of view of high biodiversity. From the point of view of optimizing the process of obtaining economically valuable forms, the interval of the second or third concentration looks attractive, where, moreover, the level of genotype-mutagenic interaction is ascending. At higher concentrations, it reaches a kind of flat plateau, and in this variant, complex changes with additional negative features and large, abrupt mutations are more likely to appear, which are of interest only from the point of view of genetic control mechanisms or as a starting material for selection. In the future, it is planned to check the resulting forecast in terms of the release of the desired forms in the second or third generation, including in terms of complex valuable biochemical and physiological changes.

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