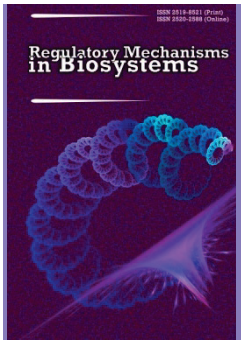

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Occurrence of cytogenetic effects under the action of epimutagen in winter wheat

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Research on studying the features of 0.01%, 0.05%, 0.10% and 0.50% epimutagen Triton-X-305 impact at the cellular level, which means the way of identifying the viability of pollen in plants of the first generation, and cytological analysis of chromosomal aberrations in the cells of the primary meristem of germinal roots during germination of bread winter wheat seeds have been conducted. Research in this direction allows us to significantly improve the monitoring of same type of substances in the environment and to forecast the nature of their action at the DNA-level. Genotypes Podolyanka and Spivanka created by Ukrainian selection and varieties of French selection Altigo, Courtot, Lyrik, Flamenko have been studied. These genotypes were selected in order to characterize possible genotype-mutagenic interactions for a compound of complex hereditary pattern for a range of concentrations with maximum contrast taking into account the possible high site-specific effect. The main purpose of this research was to identify the specificity of impact of the agent Triton X-305 at the cellular level and identify parameters fully showing the effect of this substance on the subsequent induction of biodiversity and the enhancement of hereditary variability at the cellular level. Such indicators as pollen sterility effected by various concentrations, the total frequency of chromosomal rearrangements, the spectrum of chromosomal rearrangements, including fragments and double fragments, single and double bridges, micronuclei and lagging chromosomes have been investigated. The ratio of fragments to bridges as an indicator of the active factor nature, the number of cells with two or more rearrangements as an indicator of genetic toxicity of this substance has been established. As a result, a significantly weaker effect of the epimutagen on the overall frequency of chromosomal rearrangement has been shown unlike other factors of a mutagenic nature, at the same time changing the ratio of the obtained aberrations in favour of micronuclei and lagging chromosomes. In addition, other parameters are determined as more meaningful for identifying a specific agent of impact, and the differences in concentrations are less contrasting. There is also a less significantly decreasing fertility, however, this parameter is highly dependent on the source material. In the future, we intend to assess the variability, primarily of a hereditary nature, by the way of a visual analysis of the obtained material in subsequent generations, as well as through biochemical analysis, yield qualities analysis and morphometry of the obtained material.

Keywords: cereals; chromosomal aberrations; epimutagenesis; pollen fertility; bridges; fragments; cytogenetic; Triton X-305.

Introduction

Research on not only the consequences at the plant level (Nazarenko & Beiko, 2022) as a whole in assessing the variability in the activity of individual factors in hereditary variability is being used in contemporary ecological genetics and genotoxicology (Nader et al., 2022). As a rule, the changes at the chromosomal apparatus level of the cell are more objective indicators of the beginning of variability (OlaOlorun et al., 2021). The cytogenetic researches may be identified as an integral part of the experiments connected with studying the first generation of plants affected in an appropriate way (Verma & Khah, 2016; Spencer-Lopes et al., 2018).

The analysis of subsequent changes reveals the consequences of chromosomal rearrangements that are as diverse as the specific causes affecting them. There may be both the consequences of the effect of carcinogenic substances, and spontaneous ontogenesis mutations (Semenov et al., 2020). These are the main causes of all genetic and evolutionary changes as long as gene mutations (although this interaction nature is just on the first level of being studied). In general, chromosomal abnormalities have become the most accurate tool for identifying both individual chromosomes and genes, cell nucleus and its components (Shu et al., 2013; Umavathi et al., 2020).

Chromosomal aberrations have been identified for quite a long time as the main biomarker of the impact of natural manifestation of various agents on a living organism at the cellular level. Numerous structural aberrations affect the growth and development of plants. The level of spontaneous chromosomal aberrations for any living creature generally reaches 0.6%, but this is highly dependent on the structure of the genome. Chromosomal analysis of spontaneous aberrations shows that in almost 50% of cases, the abortion of embryos is due to them (Semenov et al., 2020). Many hereditary changes are directly associated with chromosome regions characterized by a high probability of such changes (Nikolova et al., 2015). Modern studies show a high level of association between the frequency of spontaneous chromosome aberrations in a population and the level of mutability (Yali & Mitiku, 2022). These observations highlight the importance of understanding the mechanisms involved in the occurrence of chromosomal aberrations (Nazarenko & Izhboldin, 2017; Nader et al., 2022).

Changes in the structure and number of chromosomes can be caused by both external and internal factors. Chromosomal changes leading to mutations were first described in the genus *Oenothera*. Subsequent studies of some plant species showed that these changes are a complex set of translocations. But even earlier, studies of other objects have shown that other types of changes (in particular, paracentric inversions) are quite often more likely causes of hereditary changes than much rarer translocations (Palmieri et al., 2016), although this type of change is more promising for cultivated plants. Already at the early stages of research, it became clear that chromosomal aberrations play an essential role in the evolution of living organisms (Oney-Birol & Balkan, 2019; Nazarenko, 2020). The study of plant chromosomes in pachytene (Küçük & Liman, 2018) made it possible to establish that such types of rearrangements as dele-

tions, duplications, inversions and translocations have a complex and complex character (Spencer-Lopes et al., 2018).

Two phenomena are directly related to the induction of chromosomal aberrations, the so-called adaptive response and genome instability. An adaptive response was first demonstrated using mutations in bacteria, and later the same phenomenon was identified in other objects. It is especially characteristic of physical mutagens (Holme et al., 2019; Hong et al., 2022). Although there are hypotheses about the mechanisms of this phenomenon, there is no definitive justification for this effect (Khursheed et al., 2015; Palmieri et al., 2016). As for the instability of the genome, there is a phenomenon that is directly related to the nature of the genotype of a particular individual, obviously with the presence of highly variable loci. The mechanism of the phenomenon is not entirely clear, since it is not explained by any of the basic principles of radiobiology and genotoxicology, such as dependence on the dose (concentration), the nature of the mutagen, or the interaction of the mutagen and genotype (Nazarenko, 2018, 2020; Yali & Mitiku, 2022).

Plants as an object of this type of research, in contrast to other model objects, make it possible to study the types and frequencies of chromosome rearrangements directly during the first mitotic division after treatment (Khursheed et al., 2019). It is believed that the main factors influencing the dependence of the reaction on the action of the mutagen are the difference in the genotype of the original form, the size of the chromosomes, the activity of the repair systems, and the duration of the mitotic cycle (Shu et al., 2013). Also, the genetic activity in cereals literally changes several times depending on the presence of appropriate genetic resistance systems, of which only a few basic ones are known at the present time (Chernysky & Gumentyk, 2020; Gumentyk et al., 2020).

The aim of the research was to establish the consequences for the action of an epigenetic substance at the plant cell level for comparison with conventional physical mutagens and chemical supermutagens, to establish the variability of individual parameters, the possibilities of modeling and predicting the process, and the suitability of classical methods in the study of epimutagens.

Materials and methods

Field experiments were sown on the field of the Department of Breeding and Seed Production of the Dnipro State Agrarian Economic University. Pollen for analysis was selected from well, properly developed main ears of plants of the first generation of varieties that received a mutagenic effect in the flowering phase. 25 samples were taken and analyzed.

Seeds of common wheat of six varieties (1000 seeds in each treatment variant and in control) were soaked in an aqueous solution of epimutagen Triton-X-305 (hereinafter TX-305, Merck KGaA, Darmstadt, Germany) at concentrations of 0.01%, 0.05%, 0.1%, and 0.5%. The exposure of each of the options was 36 hours, which corresponds to the generally accepted method for the use of chemical mutagens. More contrasting concentrations of this agent were determined from the data of previous studies of substances of a similar nature in order to identify the boundaries of the transition in terms of survival and variability of the material and to determine the curve of action of concentrations in the future for the practical use of the epimutagen. The control was soaked in distilled water.

A total of 30 variants of varieties Podolyanka, Spivanka (Ukrainian selection), Altigo (Lemagrain, France), Courtot, Lyrik, Flamenko (INRA, France) were processed. The genotypes were selected in such a way as to maximally characterize the possible genotype-mutagenic interaction, taking into account the high site-specificity of chemical agents (Shu et al., 2013; Bondarenko & Nazarenko, 2020). Laboratory studies were carried out to determine the degree of fertility of pollen grains and cytological analysis of chromosome aberrations. Pollen sterility was determined by acetocarmin fertilization and its intensity in a light microscope. All samples are looked through at least 25 preparations.

Cytological studies were carried out on mitoses of the primary roots of wheat during the first hour of the passage of late metaphase and early anaphase for all types. After treatment with mutagens, they were germinated in Petri dishes on a filter paper soaked with distilled water in a thermostat at a temperature of +25 °C. Then the central core with a depth of 0.8–1.0 cm was fixed in Clark's fixative solution, which consists of 3 parts of 96% alcohol and 1 part of ocular acid, for 24 hours. Fixation material was taken in 70% alcohol at a temperature of +2 °C in the refrigerator. For the skin version, 25–30 roots were fixed. Cytological analyses were performed on temporary pressure preparations prepared with acetocarmin. Like the roots, they choked badly; the tissues were macerated with 45% acetic acid. Preparations were prepared according to the standard method. Roots were placed in 70% alcohol in the refrigerator. For an additional method, it is possible to fix single pairs of fragments, dicentric chromosomes, micronuclei and mixed chromosomes. Preparations magnified by 600 times were examined under a Micromed XS-3330 (Micromed, Poltava, Ukraine) light microscope with a 5 megapixel camera. The sample consisted of at least 1000 cells in the corresponding stages of mitosis for each variant (Spencer-Lopes et al., 2018; Oney-Birol & Balkan, 2019).

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

Results

The first stage of research on the effect of epimutagen TX-305 at the cellular level included the selection of mature anthers (medium spikelets from the main spike with normal development were used) followed by microscopy and determination of the ratio of fertile and sterile pollen grains (Table 1). A general analysis of the data showed that the decrease in fertility with increasing concentration is a gradual linear process for all varieties ($r = 0.87$), however, occurring with different steps and for three varieties Courtot, Lyrik, Flamenko, characterized by a sharp drop in viability when moving from a concentration of 0.1% to a concentration of 0.5%. In general, for the experiment, the data varied depending on the genotype and epimutagen concentration with statistical significance (genotype $P = 0.0013$; $F = 4.08$; $F_{0.05} = 2.71$; concentration $P = 0.0034$; $F = 11.06$; $F_{0.05} = 2.86$.

Table 1
Pollen fertility under epimutagen action (x ± SD, n = 10)

Variant	Control	TX-305, 0.01%	TX-305, 0.05%	TX-305, 0.1%	TX-305, 0.5%
Spivanka	99.17 ± 1.04^{a}	98.34 ± 0.78^{a}	92.34 ± 1.22^{b}	84.77 ± 1.17^{c}	78.46 ± 1.18^{d}
Altigo	99.14 ± 0.67^{a}	99.01 ± 0.89^{a}	90.03 ± 0.67^{b}	87.67 ± 0.58^{c}	79.66 ± 1.99^{d}
Podolyanka	99.11 ± 0.97^{a}	98.83 ± 0.66^{a}	91.92 ± 0.80^{b}	89.13 ± 2.98^{b}	80.21 ± 2.15^{c}
Courtot	98.17 ± 0.64^{a}	90.05 ± 1.11^{b}	74.22 ± 2.85^{c}	52.10 ± 4.11^{d}	0.00 ± 0.00^{e}
Lyrik	99.34 ± 1.01^{a}	88.17 ± 1.02^{b}	64.12 ± 3.99^{c}	39.17 ± 5.64^{d}	0.00 ± 0.00^{e}
Flamenko	96.14 ± 1.12^{a}	96.01 ± 0.72^{a}	82.44 ± 2.96^{c}	66.99 ± 3.49^{d}	25.01 ± 3.61^{e}

Note: indicate significant differences at $P < 0.05$ by factor analyses; comparison in terms of one variety; results werfore confirmed Tukey HSD test.

In terms of their effect, in most varieties (except Courtot and Lyrik) no significant increase in sterility occurred under the action of TX-305 0.01% (for the first group, $P = 0.19$; $F = 2.17$; $F_{0.05} = 2.94$; for Courtot and Lyrik $P = 0.00021$; $F = 5.64$; $F_{0.05} = 3.01$). In general, indicators of growth and development were similar to those obtained in previous studies. Under the action of TX-305 0.05%, the decrease in fertility was statistically significant for all varieties ($P = 0.012$; $F = 7.20$; $F_{0.05} = 4.66$), for Courtot varieties and especially Lyrik, it was already extremely significant, in fact, by a quarter to a third of the original. The third concentration significantly reduced fertility for all varieties ($P = 0.02$; $F = 4.68$; $F_{0.05} = 3.17$), except

for Podolyanka (P = 0.17; F = 2.09; $F_{0.05}$ = 3.24). For the varieties Courtot and Lyrik, this concentration was already semi-lethal, in the variety Flamenko, an extremely sharp drop in fertility began, almost by 16%. Under the action of a TX-305 concentration of 0.5%, a significant decrease in pollen fertility occurred in all cases (P = 0.00012; F = 7.99; $F_{0.05}$ = 2.88) for the Courtot and Lyrik varieties, this concentration was already completely lethal; for the Flamenko variety, there was an even sharper drop in fertility, in fact, the indicator decreased three times.

It is possible to establish the following dynamics by the varieties – for the varieties Spivanka, Altigo and Podolyanka, a gradual, not always significant with increasing concentration, increase in sterility, an overall decrease is significant, but not more than about 20% of the control, the varieties Courtot and Lyrik are characterized by significant changes in this parameter even when using the minimum concentration, followed by an increasingly contrasting and sharp drop when using subsequent concentrations. Variety Flamenko initially demonstrates great similarity with the first group of varieties, however, upon reaching a concentration of TX-305 of 0.1%, the increase in sterility becomes more and more sharp and, in fact, this genotype occupies an intermediate position in the reaction between the first and second groups.

Thus, the vulnerability of the indicator of the ratio of fertile and sterile grains already at the first concentration reveals more vulnerable genotypes. Although this is not an absolute variety that did not show a statistically significant decrease in fertility, (Flamenko) may later also show higher sterility at subsequent concentrations. However, most of the varieties showed higher fertility and the decrease in total can hardly be called critical (only about 20%). Apparently, this agent is extremely dependent on the characteristics of the genotype on which it acts. Previously, this had not been noted for various chemicals used to induce hereditary variability.

The variability of the material in terms of the overall frequency of chromosome rearrangements showed (Table 2) that an increase in concentrations generally leads to a significant increase in the frequency of chromosome aberrations (P = $2.1*10^{-12}$; F = 78.29; $F_{0.05}$ = 2.86). However, in relation to differences between varieties, the Tukey test showed the unreliability of the result obtained and it cannot be said that, despite a slightly higher frequency for the second group of cultivars Courtot, Lyrik, and Flamenko, they somehow significantly differ from the cultivars of the first group, less susceptible to the action of this epimutagen, Spivanka, Altigo, and Podolyanka. Thus, for this indicator, we are forced to reject the hypothesis of significant differences in the effect on the chromosomal apparatus of the cell.

In the control, the rate of chromosomal rearrangements is approximately the same as for other modern varieties in the case of spontaneous changes in previous studies. In the case of the concentration factor, the general rate of chromosome aberrations increases more or less linearly with an increase in the epimutagen concentration (r = 0.93). Thus, for all cases, at the first concentration of TX-305 of 0.01%, in all cases, the frequency of chromosome aberrations significantly differed from the control (P = 0.00023; F = 18.16; $F_{0.05}$ = 3.11); it increased to the greatest extent for the varieties Courtot and Lyrik. Under the action of a TX-305 concentration of 0.05%, the rate of rearrangements again significantly increases for all variants (P = 0.00017; F = 9.07; $F_{0.05}$ = 2.23) and the same pattern is again observed, except for the Altigo variety, in which the frequency of aberrations differs from the control under these conditions (P = 0.0006; F = 14.28; $F_{0.05}$ = 2.64), but does not differ from the previous concentration (P = 0.16; F = 2.01; $F_{0.05}$ = 3.11). The impact of the third concentration of TX-305 0.1% leads to significant differences from all variants for all varieties (P = 0.00082; F = 7.34; $F_{0.05}$ = 2.89). The activity of the extreme concentration statistically significantly exceeds the previous variants for all varieties, except for the cases with Altigo and Podolyanka, where the rate of chromosome aberrations does not differ from the effect of the previous concentration.

Thus, when analyzing the overall frequency of chromosome aberrations, Altigo and, to a lesser extent, Podolyanka, in which the concentrations were not always contrasting, are periodically distinguished by activity, but, as mentioned above, this was not enough for significant differences. The overall frequency of chromosome aberrations varies from 0.60% (Flamenko) – 1.00% (Altigo) in control to 4.95% (Altigo) – 8.75% for TX-305 concentration of 0.50% (Lyrik). In general, the Altigo variety is less variable; the Courtot and Lyrik varieties demonstrate large boundaries in the variation of this indicator. This indicator is less contrasting than in the case of pollen fertility. Apparently, the peculiarities of the action of this substance (epimutagen) show its low damaging ability.

Table 2
General rates of chromosomal aberrations (x ± SD)

Parameter	Mitosis, number	Chromosomal aberrations	
		number	%
Spivanka, control	1011	10	0.99 ± 0.15^{a}
Spivanka, TX-305 0.01%	1010	16	1.58 ± 0.23^{b}
Spivanka, TX-305 0.05%	1009	40	3.96 ± 0.11^{c}
Spivanka, TX-305 0.1%	1007	51	5.06 ± 0.20^{d}
Spivanka, TX-305 0.5%	1014	61	6.02 ± 0.32^{e}
Podolyanka, control	1005	8	0.80 ± 0.25^{a}
Podolyanka, TX-305 0.01%	1009	19	1.88 ± 0.21^{b}
Podolyanka, TX-305 0.05%	1012	28	2.77 ± 0.21^{c}
Podolyanka, TX-305 0.1%	1017	49	4.82 ± 0.23^{d}
Podolyanka, TX-305 0.5%	1004	54	5.38 ± 0.26^{d}
Lyrik, control	1006	9	0.89 ± 0.17^{a}
Lyrik, TX-305 0.01%	1004	32	3.19 ± 0.45^{b}
Lyrik, TX-305 0.05%	1003	56	5.58 ± 0.35^{c}
Lyrik, TX-305 0.1%	1011	68	6.73 ± 0.41^{d}
Lyrik, TX-305 0.5%	1017	89	8.75 ± 0.89^{e}
Altigo, control	1001	10	1.00 ± 0.32^{a}
Altigo, TX-305 0.01%	1004	21	2.09 ± 0.10^{b}
Altigo, TX-305 0.05%	1015	28	2.76 ± 0.47^{b}
Altigo, TX-305 0.1%	1017	44	4.33 ± 0.30^{c}
Altigo, TX-305 0.5%	1011	50	4.95 ± 0.35^{c}
Courtot, control	1001	7	0.70 ± 0.15^{a}
Courtot, TX-305 0.01%	1002	36	3.59 ± 0.15^{b}
Courtot, TX-305 0.05%	1007	45	4.47 ± 0.40^{c}
Courtot, TX-305 0.1%	1011	61	6.03 ± 0.30^{d}
Courtot, TX-305 0.5%	1015	80	7.88 ± 0.60^{e}
Flamenko, control	1008	6	0.60 ± 0.26^{a}
Flamenko, TX-305 0.01%	1014	25	2.47 ± 0.21^{b}
Flamenko, TX-305 0.05%	1011	34	3.36 ± 0.30^{c}
Flamenko, TX-305 0.1%	1003	58	5.78 ± 0.25^{d}
Flamenko, TX-305 0.5%	1005	72	7.16 ± 0.40^{e}

Note: comparison in terms of one variety; results were confirmed by the Tukey HSD test (P < 0.05); SD was calculated for 25–35 roots.

According to the spectrum of chromosomal aberrations, researchers have studied such types of rearrangements as fragments (single and double), bridges (chromatids and chromosomes), micronuclei, lagging chromosomes, cells in the necessary stages of mitosis with two or more aberrations simultaneously (Tables 3 and 4). Each rearrangement was taken into account as a separate case; the frequency was calculated in relation to the total number of rearrangements in this variant.

An analysis of the frequencies of the fragments showed that their frequency significantly depended on the increase in concentration (P = $2.2*10^{-19}$; F = 44.47; $F_{0.05}$ = 2.86), while factor analysis showed the dependence of the parameter (P = 0.04; F = 2.84; $F_{0.05}$ = 2.71), however, pairwise comparison using Tukey's test showed no differences between varieties. Thus, varietal differences remained unconfirmed for this indicator. In the case of analysis by concentrations, the differences were statistically repeated for all variants. The number of fragments increases linearly with an increase in the concentration of TX-305 (r = 0.82). In the control, the number of fragments is insignificant and corresponds closely with the level of spontaneous rearrangements, generally at the same level as the number of bridges. Under the action of the first concentration of TX-305 0.01%, we find a statistically significant increase in the number of fragments for all varieties without exception, the highest increase in the varieties Courtot and Lyrik, the lowest in the varieties Spivanka and Podolyanka, which generally corresponds to the same trend for the overall frequency of chromosomal rearrangements (P = $1.4*10^{-14}$; F = 17.45; $F_{0.05}$ = 3.14). At the subsequent concentration, there was a statistically significant increase in the number of fragments compared to the previous variant in the varieties Spivanka, Altigo and Lyrik (P = $2.1*10^{-19}$; F = 21.08; $F_{0.05}$ = 3.43), while in other varieties the increase was not significant (P = 0.06; F = 2.18; $F_{0.05}$ = 2.99), although it differed statistically significantly from the control.

Table 3
Parameters of chromosomal aberrations spectra (x ± SD, n ≈ 1000)

Variant	Fragments (single + double)		Bridges (chromosomal + chromatid)		Fragments / bridges	Other (micronucleus, lagging chromosomes)		Double and more	
	n	%	n	%		n	%	n	%
Spivanka	4.0 ± 0.6^{a}	40.0	5.0 ± 1.5^{a}	50.0	0.8	1.0 ± 0.6^{a}	10.00	0.0 ± 0.0^{a}	0.0
Spivanka, TX-305 0.01%	8.0 ± 0.5^{b}	50.0	6.0 ± 1.0^{a}	37.5	1.3	2.0 ± 1.1^{a}	12.50	1.0 ± 0.6^{b}	6.3
Spivanka, TX-305 0.05%	24.0 ± 1.2^{c}	60.0	10.0 ± 0.6^{b}	25.0	2.4	6.0 ± 1.0^{b}	15.00	2.0 ± 1.2^{b}	5.0
Spivanka, TX-305 0.1%	29.0 ± 1.5^{d}	56.9	11.0 ± 1.1^{b}	21.6	2.6	11.0 ± 1.5^{c}	21.57	4.0 ± 0.5^{c}	7.8
Spivanka, TX-305 0.5%	28.0 ± 2.1^{d}	45.9	14.0 ± 0.6^{c}	22.9	2.0	19.0 ± 1.1^{d}	31.15	3.0 ± 1.2^{bc}	4.9
Altigo	5.0 ± 0.58^{a}	50.0	4.0 ± 1.2^{a}	40.0	1.3	1.0 ± 0.5^{a}	10.00	0.0 ± 0.0^{a}	0.0
Altigo, TX-305 0.01%	14.0 ± 1.15^{b}	66.7	6.0 ± 0.6^{a}	28.6	2.3	1.0 ± 0.2^{a}	4.76	1.0 ± 1.2^{a}	4.8
Altigo, TX-305 0.05%	11.0 ± 0.58^{c}	39.3	9.0 ± 0.5^{b}	32.1	1.2	8.0 ± 1.2^{b}	28.57	3.0 ± 0.3^{b}	10.7
Altigo, TX-305 0.1%	22.0 ± 1.53^{d}	50.0	14.0 ± 1.2^{c}	31.8	1.6	8.0 ± 1.1^{b}	18.18	4.0 ± 0.6^{c}	9.1
Altigo, TX-305 0.5%	21.0 ± 1.00^{d}	42.0	16.0 ± 1.5^{c}	32.0	1.3	13.0 ± 1.5^{c}	26.00	7.0 ± 1.1^{d}	14.0
Podolyanka	4.0 ± 1.15^{a}	50.0	3.0 ± 0.6^{a}	37.5	1.3	1.0 ± 0.5^{a}	12.50	0.0 ± 0.0^{a}	0.0
Podolyanka, TX-305 0.01%	11.0 ± 1.53^{b}	57.8	7.0 ± 1.12^{b}	36.8	1.6	1.0 ± 0.5^{a}	5.26	0.0 ± 0.0^{a}	0.0
Podolyanka, TX-305 0.05%	13.0 ± 0.58^{b}	46.4	10.0 ± 1.2^{c}	35.7	1.3	5.0 ± 1.2^{b}	17.86	2.0 ± 0.5^{b}	7.1
Podolyanka, TX-305 0.1%	24.0 ± 1.73^{c}	48.9	19.0 ± 0.6^{d}	38.8	1.3	6.0 ± 1.2^{b}	12.24	4.0 ± 1.2^{c}	8.2
Podolyanka, TX-305 0.5%	23.0 ± 1.53^{c}	42.6	21.0 ± 1.2^{d}	38.9	1.1	10.0 ± 2.1^{c}	18.52	5.0 ± 0.6^{c}	9.3

Note: see Table 2.

The TX-305 concentration of 0.1% leads to a significant increase in the number of fragments compared to the previous one ($P < 0.00014$; $F = 8.19$; $F_{0.05} = 2.82$), except for the Lyrik variety, where the number of fragments significantly differs from the control and the first concentration, but the difference from the previous concentration of TX-305 0.05% was not statistically significant ($P = 0.09$; $F = 1.96$; $F_{0.05} = 2.13$). For a concentration of TX-305 of 0.5%, the strongest in action, there is a statistically significant increase compared to the previous one for the varieties Courtot and Lyrik ($P = 1.1*10^{-9}$; $F = 13.16$; $F_{0.05} = 3.01$), the most vulnerable to this factor, while while in other varieties, the fourth concentration in action does not differ from the previous one ($P = 0.09$; $F = 2.47$; $F_{0.05} = 3.16$), but significantly exceeds the control and the first two concentrations. The largest number of fragments is produced in the varieties Courtot and Lyrik, the smallest in the variety Podolyanka. However, in general, as noted earlier, the initial material did not have a significant effect.

For bridges in the control, there is an insignificant level of variability, as well as for fragments. The increase in the number of such rearrangements was significantly affected by both the increase in concentration ($P = 1,7*10^{-9}$; $F = 28.91$; $F_{0.05} = 2.86$) and the genotype of the target ($P = 0.001$; $F = 5.96$; $F_{0.05} = 2.71$), which was confirmed by the Tukey test. In the case of bridges, the Tukey test showed differences between the varieties Spivanka and Lyrik, Altigo and Lyrik, and the differences in concentration were significant for all pairs except between the control and TX-305 0.01%, TX-305 0.1% and TX-305 0.5%.

Table 4
Parameters of chromosomal aberrations spectra (x ± SD, n ≈ 1000)

Variant	Fragments (single + double)		Bridges (chromosomal + chromatid)		Fragments / bridges	Other (micronucleus, lagging chromosomes)		Double and more	
	n	%	n	%		n	%	n	%
Courtot	3.0 ± 1.0^{a}	42.9	3.0 ± 1.0^{a}	42.9	1.0	1.0 ± 0.6^{a}	14.3	0.0 ± 0.0^{a}	0.0
Courtot, TX-305 0.01%	19.0 ± 0.6^{b}	52.8	11.0 ± 1.0^{b}	30.6	1.7	6.0 ± 1.0^{b}	16.7	2.0 ± 1.2^{a}	5.6
Courtot, TX-305 0.05%	21.0 ± 1.6^{b}	46.7	16.0 ± 1.5^{c}	35.6	1.3	8.0 ± 1.3^{b}	17.8	3.0 ± 1.2^{ab}	6.7
Courtot, TX-305 0.1%	26.0 ± 2.1^{c}	42.6	21.0 ± 1.0^{d}	34.4	1.2	14.0 ± 0.6^{c}	23.0	9.0 ± 1.1^{c}	14.8
Courtot, TX-305 0.5%	32.0 ± 1.7^{d}	40.0	30.0 ± 1.7^{e}	37.5	1.1	18.0 ± 0.6^{d}	22.5	11.0 ± 1.0^{c}	13.8
Lyrik	3.0 ± 1.1^{a}	33.3	4.0 ± 0.6^{a}	44.4	0.8	1.0 ± 1.5^{a}	11.1	0.0 ± 0.0^{a}	0.0
Lyrik, TX-305 0.01%	18.0 ± 1.0^{b}	56.3	10.0 ± 0.8^{b}	31.3	1.8	4.0 ± 0.6^{b}	12.5	3.0 ± 1.0^{b}	9.4
Lyrik, TX-305 0.05%	24.0 ± 1.1^{c}	42.9	20.0 ± 1.2^{c}	35.7	1.2	12.0 ± 1.2^{c}	21.4	8.0 ± 1.5^{c}	14.3
Lyrik, TX-305 0.1%	26.0 ± 1.2^{c}	38.2	25.0 ± 2.1^{d}	36.8	1.0	17.0 ± 1.3^{d}	25.0	13.0 ± 1.5^{d}	19.1
Lyrik, TX-305 0.5%	34.0 ± 2.1^{d}	38.2	36.0 ± 2.1^{e}	40.5	0.9	19.0 ± 2.0^{d}	21.4	18.0 ± 1.0^{e}	20.2
Flamenko	2.0 ± 1.1^{a}	33.3	4.0 ± 0.6^{a}	66.7	0.5	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
Flamenko, TX-305 0.01%	11.0 ± 0.9^{b}	44.0	9.0 ± 1.1^{b}	36.0	1.2	5.0 ± 1.0^{b}	20.0	2.0 ± 0.6^{b}	8.0
Flamenko, TX-305 0.05%	12.0 ± 1.9^{b}	35.3	11.0 ± 1.2^{b}	32.4	1.1	11.0 ± 1.0^{c}	32.4	6.0 ± 1.0^{c}	17.7
Flamenko, TX-305 0.1%	26.0 ± 2.0^{c}	44.8	18.0 ± 1.5^{c}	31.0	1.4	14.0 ± 0.5^{d}	24.1	11.0 ± 0.6^{d}	19.0
Flamenko, TX-305 0.5%	30.0 ± 2.5^{c}	41.7	21.0 ± 1.4^{c}	29.2	1.4	21.0 ± 1.3^{e}	29.2	17.0 ± 0.5^{e}	23,6

Note: see Table 2.

Under the action of an increase in concentration, an increase in the number of bridges was generally observed ($r = 0.74$), while under the action of the first concentration of TX-305 0.01%, the number of bridges in the varieties Podolyanka, Courtot, Lyrik, and Flamenko significantly increases ($F = 7.06$; $F_{0.05} = 2.99$; $P = 0.00091$), the rest were at the control level ($P = 0.07$; $F = 2.17$; $F_{0.05} = 2.93$). For the action of the next concentration of TX-305 0.05%, the number of bridges increased in all variants ($P = 0.0019$; $F = 4.45$; $F_{0.05} = 2.67$), except for Flamenko ($P = 0.07$; $F = 2.17$; $F_{0.05} = 2.82$), in which the number of bridges significantly exceeded the control, but not the first concentration. Under the action of TX-305 0.1%, this type of rearrangement did not show significant growth wuth concentration. In the rest, the number of bridges increased significantly ($P = 1.1*10^{-11}$; $F = 16.87$; $F_{0.05} = 3.43$). As for the extreme concentration of TX-305 0.5%, in Altigo, Podolyanka, and Flamenko the level of variability did not change significantly from the previous concentration ($P = 0.09$; $F = 1.99$; $F_{0.05} = 2.43$), in the rest it increased significantly ($P = 2.2*10^{-11}$; $F = 11.83$; $F_{0.05} = 3.16$). As can be seen, the lowest contrast is just observed when passing from the control to TX-305 0.01% and between TX-305 0.1% and TX-305 0.5%. In general, the indicator is much more dependent on the genotype of the source material than the number of fragments. The ratio of fragments and bridges is usually in favour of fragments, that is, more than 1, which is generally normal for the action of a chemical agent, except for the Lyrik variant, TX-305 0.5%, while in general this ratio first increases as the concentration increases, then begins a gradual decrease, that is, the same trend is observed as in earlier studies, when at high (at the level of semi-lethal) concentrations, the action of chemicals became less selective in terms of the frequencies of types of chromosomal rearrangements.

As for aberrations of the micronucleus type and lagging chromosomes, there was a significantly higher number of them in relation to other

types of chromosomal aberrations than for the action of previously studied factors characterizes the action of TX-305. That is, the value of this indicator is clearly increasing significantly. At the same time, factor analysis showed that the variability of this indicator was significantly affected by the epimutagen concentration factor ($P = 2.3*10^{-19}$; $F = 52.01$; $F_{0.05} = 2.86$) and the genotype factor of the target ($P = 0.00062$; $F = 6.01$; $F_{0.05} = 2.71$). The Tukey test confirmed that in all cases, in pairwise comparison, the difference was significant, except for Spivanka and Podolyanka varieties for genotypes and control with a TX-305 concentration of 0.01%. The number of rearrangements of this type increases with increasing concentrations ($r = 0.84$).

In the control, we find the almost complete absence of this type of aberration, which is the norm - they are extremely rare with spontaneous changes. Under the action of the first concentration of TX-305 0.01%, the entire first group of varieties, for which the action of this substance is less toxic, does not have statistically significant differences from the control ($P = 0.10$; $F = 2.01$; $F_{0.05} = 2.56$), while in the second, more vulnerable is also in all cases ($P = 1.7*10^{-10}$; $F = 14.29$; $F_{0.05} = 2.01$). For the second concentration of TX-305 0.05%, all varieties have statistically significant differences from the control and the first concentration ($P = 1.8*10^{-16}$; $F = 17.19$; $F_{0.05} = 3.48$), except for the Courtot variety ($P = 0.07$; $F = 2.09$; $F_{0.05} = 2.43$), in which the differences between the second and first concentrations are not significant.

The third concentration of TX-305 0.1% is again characterized by a statistically significant increase in this type of rearrangement for all genotypes ($P = 1.6*10^{-10}$; $F = 8.39$; $F_{0.05} = 2.99$) except for Altigo and Podolyanka ($P = 0.07$; $F = 2.47$; $F_{0.05} = 2.88$), in which this indicator did not change significantly, but differed from the control and the first concentration upwards. Under the action of TX-305 0.5%, the number of micronuclei and lagging chromosomes increases in all variants ($P = 1.9*10^{-12}$; $F = 11.16$; $F_{0.05} = 2.99$), except for cv. Lyrik ($P = 0.11$; $F = 1.93$; $F_{0.05} = 2.44$), where it remained approximately at the same level. Thus, for this indicator, the first and third concentrations are less contrasting. At the same time, this indicator is clearly more significant than the indicator of the presence of bridges and fragments, both in terms of a more complete reflection of the role of the source material, and in terms of combining both factors for analysis. Usually, for this type of cytogenetic analysis, this parameter does not carry particularly significant information.

As for the indicator of the presence of two or more chromosomal rearrangements in one dividing cell (at the corresponding stages of mitosis), in this situation, the increase in the number of such rearrangements was significantly affected by both the increase in concentration ($P = 1.2*10^{-9}$; $F = 16.06$; $F_{0.05} = 2.86$) and the genotype of the target of action ($P = 0.002$; $F = 5.53$; $F_{0.05} = 2.71$). However, the Tukey test showed that there were no significant differences in the increase in concentrations for the variants when pairwise comparison was made between the control and TX-305 0.01%, TX-305 0.01% and TX-305 0.05%, TX-305 0.1% and TX-305 0.5%, and significant differences exist in the initial variant for the impact only for the Lyrik variety in relation to the varieties of the first group (Spivanka, Altigo, Podolyanka). Thus, this indicator is the least promising both for analysis by concentrations and for analysis by the nature of the influence of the starting material, which is new – in previous cases it was at least within the model when it came to increasing the dose. The number of cells with two or more rearrangements still increases with increasing concentration ($r = 0.78$).

The normal trend is the complete absence of cells with two or more rearrangements in the control in all cases. Under the action of a TX-305 concentration of 0.01%, we see that they appeared in significant amounts in varieties Altigo, Lyrik, and Flamenko ($P = 0.00023$; $F = 5.71$; $F_{0.05} = 2.44$), for the rest, more or less without significant differences from control ($P = 0.07$; $F = 2.06$; $F_{0.05} = 2.44$). For the second concentration of TX-305 0.05%, the Spivanka variety did not differ from the previous concentration ($P = 0.08$; $F = 2.01$; $F_{0.05} = 2.76$), but was higher than that of the control ($P = 1.3*10^{-8}$; $F = 8.19$; $F_{0.05} = 2.78$), while in the variety Courtot it increased compared to the control ($P = 1.9*10^{-9}$; $F = 9.14$; $F_{0.05} = 2.99$), but did not significantly differ from the first concentration ($P = 0.09$; $F = 1.87$; $F_{0.05} = 2.44$). The remaining genotypes increased significantly compared to the previous concentration and control ($P = 0.00029$; $F = 7.66$; $F_{0.05} = 2.57$). A concentration of TX-305 of 0.1% showed that in all varieties it caused a significant increase in the number of cells with two or more aberrations compared to all previous variants ($P = 1.9*10^{-9}$; $F = 8.19$; $F_{0.05} = 2.74$). The extreme concentration of TX-305 of 0.5% did not lead to a significant increase in the indicator in the Courtot and Podolyanka varieties ($P = 0.06$; $F = 2.01$; $F_{0.05} = 2.55$), in the Spivanka variety it even decreased and significantly differed only from the control ($P = 2.4*10^{-16}$; $F = 12.12$; $F_{0.05} = 2.99$), remaining unchanged from the index for all previous concentrations ($P = 0.11$; $F = 1.16$; $F_{0.05} = 2.99$). In other varieties, this parameter increased under the action of TX-305 0.5% ($P = 1.7*10^{-9}$; $F = 7.84$; $F_{0.05} = 3.11$).

The conducted discriminant analysis and reverse identification of acting factors (Tables 5, 6 and 7, Fig. 1) confirmed the established regularities and made it possible to identify model variables from those studied in the cytogenetic analysis.

Table 5
Factor loadings (unrotated)

Parameter	Concentration	Genotype
Pollen fertility	0.879*	0.822*
General rates	0.811*	0.611
Fragments	0.885*	0.613
Bridges	–0.623	–0.251
Other abnormalities	–0.857	0.869*
Double and more	–0.411	0.633
Explanation variants	4.492	1.893
Non-explanation	0.453	0.107

Table 6
Discriminant Function

Parameter	Wilks' - Lambda	F_{remove} (4,11)	p-value
Pollen fertility	0.022	16.82	< 0.01
General rates	0.021	13.63	< 0.01
Fragments	0.018	8.17	< 0.01
Bridges	0.009	2.96	0.16
Other	0.018	9.03	< 0.01
Double and more	0.011	2.47	0.11

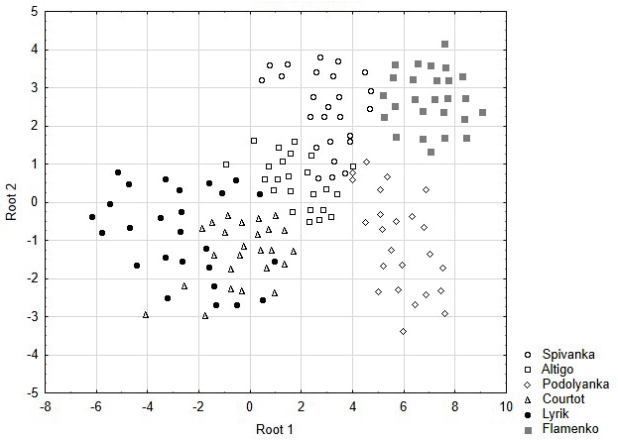

Fig. 1. Classification by canonical roots

Thus, it can be seen that the parameters of pollen fertility, the general rate of chromosomal rearrangements, the presence of fragments and double fragments were the key parameters for the analysis of the impact concentration. The remaining indicators are not in the model and may not be analyzed for this epimutagen. It was extremely difficult, due to the weakness of the effect of the factor at the level of the chromosome apparatus, to isolate the model parameters for changing the characteristics of the source material, however, it was still possible. Such indicators turned out to be pollen fertility and, which is fundamentally new, the presence of micronucleus rearrangements and lagging chromosomes. Completely inconsistent and excluded when constructing location models in the factor space (Fig. 1) were the parameters for the presence of bridges and, which also turned out to be fundamentally new, the number of cells with two or more aberrations, which is clearly associated with the low genotoxicity of the studied agent.

Table 7
Results of classification for genotypes (share of objects by parameters from previous table in model for such genotype)

Genotype	Objects in model, %
Spivanka	86.7
Altigo	86.7
Podolyanka	93.3
Courtot	73.3
Lyrik	73.3
Flamenko	100.0
Total	85.6

In the constructed model, the varieties Flamenko and Podolyanka are most clearly distinguished (Fig. 1, Table 6), the other varieties are partially mixed in the factor space and, thus, have a significantly lower resolution when studying this set of parameters. It should also be noted that for the first time indicators in the study of damaging effects at the cell level in our studies turned out to be less effective than indicators of growth and development at the previous stage of research. As a rule, the opposite is true – cytological studies are much more accurate for monitoring undesirable effects. At the same time, it is also obvious that in this case the focus of action is more shifted to changes that do not affect the DNA of the cell.

Discussion

The key issue in the induction of valuable forms in the study of the formative process of the action of ecogenetic factors has always been the ratio between the level of damage that led to a number of negative, up to lethal, effects and a sample that can be subjected to further monitoring in order to identify valuable features and forms (Shu et al., 2013). In fact, within the framework of ecological genetics, the task of optimizing the process of reducing the first, clearly negative trend is constantly being performed, while reducing the variability of the resulting material is unacceptable. In fact, having come to the optimal ratio by establishing the desired dose or concentration and varying the starting material for processing, further changes are possible only by changing one of the components of the system - namely the active substance (Nazarenko, 2016). This leads to a constant search for new agents with a fundamentally different mechanism of influence on hereditary variability (Nazarenko, 2018; Pramanik et al., 2018; Oney-Birol & Balkan, 2019).

Negative trends in solving these optimization problems are primarily associated with direct DNA damage with weak possibilities for regulating its scale. Although it has long been shown that small changes in large quantities are much more promising, it is not possible to limit the scale of damage in any way. This process in chemicals is, albeit less obvious, still sufficient to lead to permanent adverse effects, even lethal. First of all, various cytogenetic studies are used to test for such features, as they are faster than any field monitoring and more reliable, carried the definition of depression in other ways (Spencer-Lopes et al., 2018).

Previously, it was quite widely noted that exposure to mutagens leads to a significant and sharp decrease in pollen fertility, while this was gradual in nature up to a certain dose (concentration), followed by a sharp increase in sterility. In this situation, however, it is not observed for all genotypes; moreover, in most cases it is much milder (Oney-Birol & Balkan, 2019). This once again indicates that the changes caused by this substance are rather small, less traumatic. In addition, the second point is that they clearly depend on the genetically determined mechanism of the adaptive response, which, in turn, differs even at the level of individual groups of varieties (Nazarenko & Izhboldin, 2017; Nazarenko, 2018).

Such indicators as the general rate of chromosomal aberrations and various rates of individual changes in the spectrum were of key importance for determining the nature of the impact on the chromosomal apparatus. Thus, such indicators as the presence of double fragments, bridges, the ratio of fragments and bridges (in terms of the nature of the mutagenic factor), and the presence of complex chromosomal rearrangements were of great importance for the identification of a mutagenic factor at the cell level (Nazarenko, 2016; Siahpoosh et al., 2020). However, the presence of such a number of micronuclei and lagging chromosomes with such a small presence of cells with complex aberrations has never been observed under the action of any chemical substance (Nikolova et al., 2015). Usually, this was typical for the action of just high doses of gamma-rays or chemical supermutagens (Verma & Khah, 2016), however, in this case, the survival of the material in this experiment is significantly higher than under the action of semi-lethal and sublethal doses or concentrations of mutagens (Nazarenko & Izhboldin, 2017). Thus, we can count on the emergence of new forms, of which this type of change acts as a cytological marker (Yali & Mitiku, 2022).

At the same time, a number of similar parameters should also be noted. Under the action of other chemicals, fragments predominate, although they do not always completely prevail in the spectrum, but, in fact, the ratio with bridges under the action of any concentration is always in favour of fragments and double fragments (Fathin et al., 2021). Two genotypes clearly demonstrate the dynamics in terms of pollen sterility and, although less pronounced, in terms of the spectrum of chromosomal aberrations, which is quite typical for a slightly weaker effect of the same nitrosoureas. However, the number of chromosomal disorders is still much less than it should be under the action of other mutagens, taking into account the degree of pollen sterility (Verma & Khah, 2016).

An analysis of the features of this effect indicates that this agent at the cell level demonstrates fundamentally new mechanisms of genotype-mutagenic interaction, apparently associated, firstly, with a less damaging effect on cell DNA and, secondly, with fundamentally different applied damage, which was expressed in the specific frequency of individual types of changes for chromosomal rearrangements (Pramanik et al., 2018; Spencer-Lopes et al., 2018). Moreover, the latter is characteristic of all genotypes without exception, regardless of whether they belong to local or foreign forms. Given that at least three possibilities have already been shown for these genotypes in terms of response to other indicators (Palmieri et al., 2016), this certainly indicates a different mechanism of action (Nader et al., 2022). Moreover, other researchers did not observe a similar effect with classical physical or chemical mutagens, so you can be sure that in this case we are talking about the impact of an epimutagenic nature, albeit indirect confirmation of this fact based on the behaviour of the chromosomal apparatus of the nucleus (Nazarenko & Beiko, 2022).

The parameters for monitoring cytogenetic activity have changed somewhat; the possibilities have even somewhat expanded due to more balanced types of changes in the specific proportion of the total number of rearrangements, which is clearly shown by the analysis. However, the previously significant indicators still remained in the model, therefore, in general, the possibilities for identifying and determining the specifics of the concentration by exposure remained within the framework of the already developed protocol (Nurmansyah et al., 2018; Mangi et al., 2021). What is new is that it can be used in this case as well, and the activity of the epimutagen is still accompanied by traditional effects that are strong enough to be registered, albeit weakened to varying degrees depending on the genotype (Shu et al., 2013).

The epimutagen also showed a rather high site-specificity – in particular, with respect to at least one type of genotypes, it clearly has a higher damaging effect than the other two, that is, either it has a higher degree of affinity due to genetically determined features of the genome, or it is associated with a specific system of adaptive response to this type of impact (Pramanik et al., 2018). In any case, this is an extremely significant genotype-mutagenic effect, which allows one to better understand the nature of the impact on the chromosome apparatus (Spencer-Lopes et al., 2018). This, together with the above differences, allows us to hope for qualitatively different changes in the heredity of the processed material (Nazarenko & Beiko, 2022). It is possible that the changes will also affect the frequency of variability for the already studied trait and the features of the genetic mechanism for the occurrence of the desired traits, transferring a number of forms from limited or difficult to use for induction of biodiversity into a more practical value formation.

Conclusion

The use of new substances, more complex in their action, instead of classical chemical supermutagens, which are more prone to less severe small changes and, as a result, have significantly lower genotoxicity – in any case, this is what the cytogenetic test of these agents shows, allows us to conclude that they have great potential, and also to make a primary

conclusion about the nature of their action. It seems that the activity of these substances, although not completely associated with changes at the DNA level, nevertheless interacts more with the epigenetic mechanisms of regulation of hereditary activity and variability. This allows us to hope that, with lower genotoxicity, these classes of substances will show a significantly higher efficiency in the induction of useful types of biodiversity, which can significantly affect the rate of induction of new valuable forms. In the future, it is planned to conduct research just at this level, highlighting precisely such families and lines with the help of thorough phenology, biometric and biochemical studies at the plant level, thus establishing the relationship between what happens at the cell level and how it affects the body in general, on its heritable variability. At the same time, classical methods for studying the cytogenetic activity of this type of compound still show their ability to identify both a particular agent and the magnitude of its application.

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