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CYTOGENETIC VARIABILITY UNDER THE ACTION OF THE EPIMUTAGEN TRITON-305X

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Epimutagens are an important tool in the genetic improvement of plants due to their ability to significantly increase the frequency of beneficial hereditary changes. They do not belong to genotoxic compounds, i.e. those that cause a significant number of negative genetic changes in cells.

Usually, these substances can induce epimutations in various protein bases of plant chromosomes with high efficiency, but their use has its drawbacks.

The aim was to reveal the specificity of action on the parameters of cytogenetic variability according to the frequencies and spectra of chromosomal aberrations in winter wheat cells depending on the concentration and variety, to show the main parameters by which the process of variability at the cellular level of organization can be modeled.

We used the chemical epimutagen Triton-305X, here and hereinafter referred to as TX-305, which belongs to the type of chemicals that are capable of leading to significant changes in histones in the chromosome complex and, thus, to a change in gene expression. Seeds of bread winter wheat varieties Perspektyva Odeska, Sonata Poltavska, Shpalivka and MIP Lada were treated with an aqueous solution of TX-305 in concentrations of 0.01%, 0.05%, 0.1% and 0.5%, water was the control. 1000 grains of winter wheat were taken for each treatment. Exposure to the mutagen was 24 hours.

Chromosomal aberrations were analyzed by light microscopy on preparations of mitotic apices of primary roots of winter wheat varieties at the late stage of metaphase and early anaphase. After TX-305 treatment, parts of the root apices were cultivated in Petri dishes on filter paper with distilled water in a thermostat at a temperature of + 20-22°C. After that, a part of the samples with a length of 0.8-1.0 cm was cut and fixed for 24 hours in Clark's solution, which consists of 3 parts of 96% ethyl alcohol and 1 part of ophthalmic acid. About 25-30 roots were prepared for each variant. Cytological studies were provided with temporary preparations stained with acetocarmine. The samples were evaluated using a Micromed XS-3330 light microscope (multiplication 600 times) with a 5M camera. Each variant contains approximately 1000 plant cells at the corresponding stages.

Statistical analysis of the dates was performed using the Statistica 10.0 program. Differences between the selections were determined using one-way analysis of variance (ANOVA) and were considered reliable at $P < 0.05$. Differences between the samples were evaluated using the Tukey HSD test.

The total number of rearrangements was slightly influenced by the variety factor, but the increase in the concentration of TX-305 was significant and reliable. Individual varieties differed significantly in pairwise comparison. This applies to the MIP Lada variety, which turned out to be less tolerant than others (higher frequency of aberrations).

The number of chromosomal aberrations varied from 1.29% (Perspektiva Odeska) to 2.99% (MIP Lada) under the action of TX-305, 0.01%, under the action of TX-305, 0.05% from 2.59% (Shpalivka) to 4.27% (MIP Lada), under the action of TX-305, 0.1% from 3.97% (Sonata Poltavska) to 5.49% (MIP Lada), at the concentration of TX-305, 0.5% from 4.90% (Sonata Poltavska) to 7.16% (MIP Lada). Thus, the cytogenetic variability caused by this factor was quite high, but only for the MIP Lada variety was it comparable to the effect of the least harmful chemical mutagens.

According to the spectrum of chromosomal rearrangements, such indicators as fragments (single and double, which are generally more characteristic of the action of chemical supermutagens), bridges (also single – chromatid – and double – chromosome), micronuclei, lagging chromosomes (others) were studied. Cells with multiple chromosomal aberrations (complex) were taken into account.

For the total frequency of fragments, no significant difference was found by the genotype factor, and the difference by the concentration factor was also unreliable. The number varied from 7 (Perspektyva Odeska) to 13 (Sonata Poltavska and MIP Lada) under the action of TX-305, 0.01%, under the action of TX-305, 0.05% from 11 (Shpalivka) to 20 (Perspektyva Odeska and MIP Lada), under the action of TX-305, 0.1% from 19 (Sonata Poltavska) to 25 (Perspektyva Odeska), at the concentration of TX-305, 0.5% from 21 (Shpalivka) to 28 (MIP Lada).

For the case with bridges, no significant difference was found for the genotype factor, but for the concentration factor, the difference was significant. In general, the number of bridges varied from 5 (Perspektiva Odeska) to 10 (MIP Lada) under the action of TX-305, 0.01%, under the action of TX-305, 0.05% from 8 (Perspektiva Odeska) to 14 (MIP Lada), under the action of TX-305, 0.1% from 10 (Sonata Poltavska) to 19 (varietal MIP Lada), at the concentration of TX-305, 0.5% from 12 (Sonata Poltavska) to 27 (MIP Lada). As for other types of chromosomal rearrangements (lagging chromosomes and micronuclei), for them the variety factor also turned out to be insignificant, and the reaction to increasing concentration was also statistically unreliable. The number of other aberrations varied from 1 (Perspektyva Odeska) to 7 (MIP Lada) under the action

of TX-305, 0.01%, under the action of TX-305, 0.05% from 5 (Perspektyva Odeska) to 9 (varietal MIP Lada), under the action of TX-305, 0.1% from 7 (Sonata Poltavska and Shpalivka) to 15 (MIP Lada), at the concentration of TX-305, 0.5% from 2 (Perspektyva Odeska) to 8 (MIP Lada).

The influence of the variety on the induction of complex aberrations is insignificant, increasing the concentration leads to a significant increase in the frequency of complex changes. The number varied from 0 (Shpalivka) to 2 (MIP Lada) under the action of TX-305, 0.01%, under the action of TX-305, 0.05% from 1 (Shpalivka) to 42 (Sonata Poltavska), under the action of TX-305, 0.1% from 3 (three varieties) to 7 (MIP Lada), at the concentration of TX-305, 0.5% from 2 (Perspektiva Odeska) to 8 (MIP Lada).

Factor analysis showed that the increase in the concentration of TX-305 was significant for the total frequency and number of rare changes, while the genotype affected only the number of bridges.

To determine the nature of the influence of cytogenetic activity depending on the factors of the genotype of the object of influence and the concentration of the mutagen, a discriminant analysis was conducted. As can be seen, in the case of genotype, discriminant analysis showed the significance for the genotype of only one parameter of the model – the number of bridges.

In general, the results of the analysis are atypical for the cytogenetic activity of chemical mutagens (among the model features, only frequency and number of bridges are present as indicators of the strength of action).

That is, the differentiating ability is sufficient only for these parameters. This is not enough to identify more vulnerable forms of forms (MIP Lada, all others are approximately at the same level). However, according to the analysis of centroid distances, it is inappropriate to use simultaneously concentrations of TX-305 0.01 and 0.05% and TX-305 0.1 and 0.5%, respectively. Variants TX-305 0.01% and TX-305 0.1% should be removed.

Analysis of the action of TX-305 as an epimutagen showed that for this factor, when studying cytogenetic activity, such an indicator as the number of bridges acquires greater weight in the factor space of the genotype. With increasing concentration, there is mainly a gradual increase, but not always with significant transitions between individual variants, the difference between TX-305 0.01 and 0.05% and TX-305 0.1 and 0.5%, respectively, is not always significant.

The variety MIP Lada showed a higher genetic affinity to the action of TX-305 due to its higher vulnerability to the consequences of the action due to the increase in cytogenetic variability, especially in the indicators of the total frequency of rearrangements, the number of bridges. The behavior of the other three varieties does not differ significantly, they are less vulnerable, the reaction is approximately at the same level. The higher concentrations used should be attributed to the range of moderate cytogenetic activity.

The theses material is written on the basis of the authors' research.