REGULARITY OF MITOSIS IN DIFFERENT VARIETIES OF WINTER BREAD WHEAT UNDER THE ACTION OF HERBICIDES

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Abstract. The influence of the most widespread herbicides on winter wheat in Ukraine was studied by anaphase test. Treatment with herbicides reduced the germination of the seeds and disturbed the regularity of mitosis in all varieties of wheat. The range of violations of mitosis was demonstrated by the formation of chromosomal aberrations and dysfunctions of cell cytoskeleton which occurred while processing herbicides. Varietal differences between investigated wheat by sensitivity to herbicides were discovered. The most resistant to herbicides was variety Fantasya Odesskaya, and the most sensitive – Nikoniya, while the most harmful herbicide for wheat was Napalm.

Keywords: wheat, herbicides, mutagenic activity, anaphase test

INTRODUCTION

Grain farming plays an important role in the agricultural sector of Ukraine. Cereals can be damaged by over 300-350 different types of organisms (insects, fungi, bacteria, viruses, nematodes, mites, weeds, etc.) that significantly impede implementation of the potential crop yield [17]. In order to protect crops it is necessary to use specialized chemicals. However, not all of them are safe for plants. Genetic variability under the influence of herbicides has been established for barley [25]. The most active effect revealed by hydrazides of maleic acid and chlorine-IFC, which destroyed the maturation of spindle of the cells and caused the formation of heteroployid cells, cause a reduction of plants fertility. Moreover, herbicides such as simasin, atrazine and paraquat have determined sterility which is inherited and caused increase of incidence in chlorophyll mutations. The strongest mutagenic action was set for drugs banvel-D, treflan, relatively weak – for prometryn, and in intermediate state is herbicide lentahran [8]. Plant genetic restructuring can be achieved in different ways, which can be found in the karyotype of their seeds. The criteria for violations of mitosis are the aberrations that occur in the first root sprouts mitosis: in the first place – chromatid and chromosomal fragments and bridges, pycnosis, lysis, fragmentation of chromosomes, formation of ring and dicentric chromosomes, C-mitosis, endopoliploidy, mixoploidy and other anomalies [2]. Assessment of cytogenetic variability is a highly effective method of identifying the overall impact of environmental factors on the physiological state of plants, and on the unit of heredity [1, 19, 22]. Studying the spectrum of cytogenetic violations will provide information on the mutational variability that occurs as a result of the influence of pesticides, and also establish resistance to treatment by different concentrations of the drug varieties [16]. Considered problem is especially interesting .The research is useful for the development of modern concepts of optimized use of pesticides in modern agriculture [27]. These points are needed to conduct together the physiological, biochemical and hygienic analyzes, genetic monitoring, using various test systems.

Therefore, our research has been prepared to identify potential mutagenic activity of the common and approved for use on the Ukrainian market herbicides: selefit, napalm, roundup and mistral [18] with respect to bread wheat and establish differences in the reaction of different varieties of winter bread wheat for herbicides treatment.

MATERIALS AND METHODS

The bread wheat *Triticum aestivum* L. was the biological material of the research with varieties Fantaziya odesskaya, Panna, and Odesskaya polukarlikovaya and Nikoniya. The influence of herbicides on seeds of these sorts was studied. We used herbicides of complete actions, designed for pre-processing or pregerminating application: selefit (active substance is prometryn, 500 g / l), napalm (isopropilamine salt of glyphosate, 480 g / l, in acid equivalent – 360 g / l aqueous solution) , roundup (glyphosate isopropilamine salt, 480 g / 1.48%) and mistral (active substance is metrybuzyn) in a 1:8 dilution of the recommended working concentration (working solution preparations containing the following amount of herbicide per ml of water - selefit: 0.08 mg / ml; napalm: 0.01 mg / ml; roundup: 0.012 mg / ml; mistral 0.025 mg / ml). These concentrations of herbicides modeled residual quantity for sowing and germination of seed culture. As a control we are using distilled water. The influence of herbicides on laboratory seed germination, and regularity of mitosis in wheat seedlings was assessed.

Laboratory germination was evaluated by sprouting 20 seeds in a Petri dish, and each experiment was repeated five times. The influence of herbicides on the root meristem was estimated by anaphase method [7]. The method consists in registration of chromosomal disorders under anaphase. Germinated seeds were treated alternating heat and cold, than fixed in acetic alcohol (3:1) and stained 1% acetocarmine for cytological analysis (Squash method) [21]. Each version of the experiment was viewed in at least 500 cells. Statistical analysis was performed according to Student criterion [23].

RESULTS

All herbicides produced a negative effect on seed germination of investigated wheat. In all variants of the experiment there was a significant reduction of seed germination ($P \leq 0.001$) (Table 1). But depending on preparation the degree of reduction of seed germination was also different, which indicates the individual sensitivity of studied wheat.

The strongest effect in reducing the germination capacity was selefit, followed by roundup and napalm while the smaller effect in germination capacity was recorded with mistral herbicide. Among of investigated varieties of wheat with regard to resistance to treatment with herbicides, the most cytotoxic effect in seed germination was recorded in Niconiya, while the least sensitive to the effects of herbicides was a sort of Fantaziya odesskaya.

The results of anaphase test in the application of herbicides are presented in Table 2. For all experimental smears there was significantly different ($P \leq 0.01$) share of normal anaphases.

In control samples the proportion of normal anaphases (a, b) was between 87.4 and 96.4%. Abnormal anaphases were represented mainly by anaphases with bridges (c, d) and anaphases with lag chromosomes (e, f, g, h). Anaphases with fragments were met very rarely. Treatment with herbicides significantly reduced the proportion of normal anaphase compared to control ($P \leq 0.001$). Reducing the proportion of normal anaphases was mainly attributed to the increase of anaphase with bridges. The frequency of cells with fragments was at the level of the frequency with anaphases with lagging chromosomes or lower. The reaction of wheat was different to various herbicide preparations. But there were definite regularities. For treatment with herbicides, mistral and selefit, the most regular mitosis was observed for the sort Panna and for treatment preparations napalm and roundup – variety Fantaziya odesskaya. Most sensitive to herbicide was sort Nikoniya. By treatment all four drugs in seedlings root meristem this sort had the smallest proportion of normal anaphase.

DISCUSSION

Variability in seeds germination may be caused by different permeability covers of grain and sprouts or by functioning of enzyme systems that ensure the destruction of the poison that entered. Start and functioning of protective systems associated with the signaling systems of plants cells involved in inducing under the influence of the environment. Signal net affects the cytoskeleton, and changes in the functioning of the cytoskeleton leads to activation of protective mechanisms of cells [20]. Substances inducing disorder

Table 1. Laboratory germination (%) of seeds of a winter bread wheat under the effect of herbicides

Sort	Control	Selefit	Napalm	Roundup	Mistral
Panna	88.6±3.2	47.3±5.0	57.6±4,9	42.7±4.9	66.2±4.7
Odesskaya polukarlikovaya	98.4±1.4	35.4±4.8	61.5±4,9	56.3±5.0	52.7±5.0
Nikoniya	82.5±3.8	27.2±5.7	46.8±5,0	54.5±5.0	59.3±4.9
Fantaziya odesskaya	93.4±2.5	62.7±4.8	65.3±4,8	59.7±4.9	75.2±4.3

Table 2. Frequency (%) of normal and abnormal anaphase mitosis in the root meristem seedlings of a winter wheat under the effect of herbicides

Sort	Variant	Normal anaphases, %	Abnormal anaphases with chromosomal lag, %	Abnormal anaphases with bridges, %	Abnormal anaphases with fragments, %
Panna	Control	91.2±1.6	2.3±0.9	6.5±1.4	0
	Selefit	72.3±2.5	7.3±1.5	12.2±1.9	8.2±1.6
	Napalm	62.4±2.7	11.5±1.8	21.2±2.4	5.2±1.3
	Roundup	71.1±2.6	11.2±1.8	16.1±2.1	2.0±0.8
	Mistral	75.2±2.4	6.7±1.4	11.4±1.8	7.2±1.5
Odesskaya polukarlikovaya	Control	92.6±1.5	4.2±1.2	2.2±0.8	0.5±0.4
	Selefit	70.2±2.6	11.2±1.8	16.4±2.1	2.2±0.8
	Napalm	65.1±2.7	8.0±1.6	21.6±2.4	9.3±1.7
	Roundup	69.8±2.6	10.5±1.8	17.2±2.2	2.5±0.9
	Mistral	73.5±2.5	9.2±1.7	12.4±1.9	5.4±1.3
Nikoniya	Control	87.3±1.9	7.5±1.5	3.2±1	2.1±0.8
	Selefit	64.5±2.7	11.2±1.8	19.4±2.3	5.1±1.3
	Napalm	52.8±2.8	13.3±2	20.9±2.3	12.8±1.9
	Roundup	58.7±2.8	7.4±1.5	21.5±2.4	13.2±1.9
	Mistral	71.2±2.6	0	25.1±2.5	4.2±1.2
Fantaziya odesskaya	Control	96.4±1.0	0	3.6±1.1	0
	Selefit	65.3±2.7	15.6±2.1	11.7±1.9	8.1±1.6
	Napalm	68.2±2.6	7.2±1.5	18.7±2.3	6.2±1.4
	Roundup	72.5±2.5	7.8±1.5	18.0±2.2	1.7±0.7
	Mistral	74.4±2.2	6.2±1.4	17.3±2.2	2.1±0.8

of the cytoskeleton and the process of cell division are used in agriculture as herbicides [15]. In particular, the herbicide treflan induces oxidative stress in barley. This is due to a violation of polymerization of the microtubule cytoskeleton. At the same time variety Stala barley plants are less sensitive to treflan than sorts Gonar and Dzivosny. This is evidenced by a lesser degree of damage to the microtubule of the cytoskeleton, the lack of induction of peroxidase activity and higher levels of glutathione in the cells. Differences in response to the impact of the herbicide may be due to different activity systems, and detoxification of reactive oxygen species. In herbicides resistant wheat defense mechanisms evolve rapidly [10].

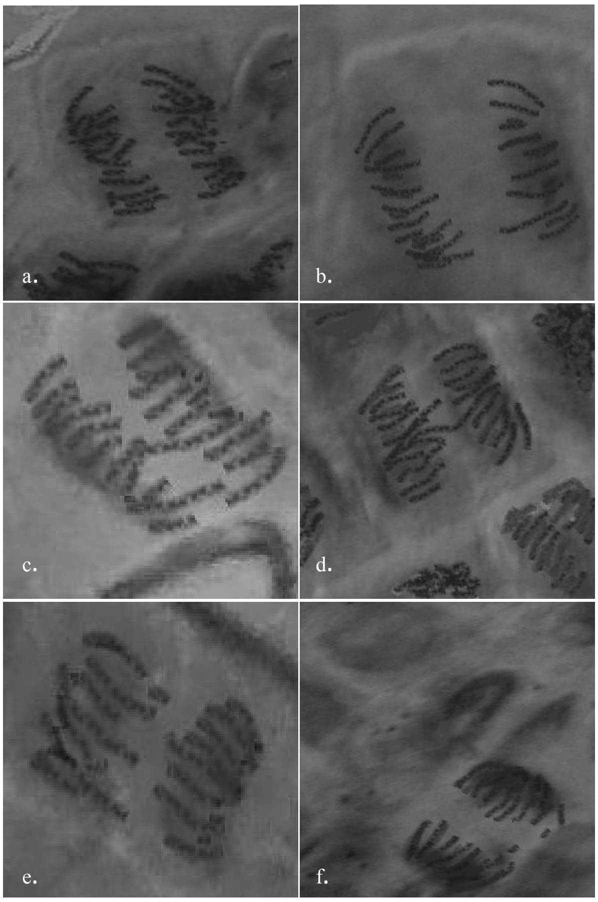

Figure 1. Representative images of different types of chromosome aberration observed in meristematic cells of bread wheat. Objective × 40; ocular lens×10; microscope- Delta Optical, Genetic Pro; camera- Nikon L23: (a, b) normal mitotic stages in untreated root meristems; (c, d) anaphase with bridge; (e, f) anaphase with fragments.

It is interesting to note that treatment with preparation roundup and preparation napalm produced different reaction in wheat although the active substance of the herbicides is the same: N-(fosfonometyl)-glycine, $C_3H_8NO_5P$. This could be explained by a reaction to the excipient drugs. The difference in results may also be explained by the fact that the same active ingredient in the original preparation and in the generic preparation was obtained by different means and in the synthesis of the last inevitably formed impurities that can change the level of toxicity.

Mutagenic effect of herbicides has been shown many times in researches on bacteria Salmonella typhimurium TA 100 [12], the bone marrow of mice [24], Drosophila [13], human blood cells [3, 5], Vicia faba [5] cotton [11], maize [9] meristem of barley [14, 6]. The present study also record mutagenic effect of herbicides in wheat, which showed results in anaphase test. Anomalies observed, indicative of spindle dysfunction, which agrees well with data about violation of cytoskeleton in barley as a result of the herbicides [15]. But the most frequent abnormality was the formation of bridges together with formation of fragments constituting major share disorders anaphase. These anomalies indicate the existence of cycles of “break-bridge-merge” [1]. Thus, herbicides act not only on cells cytoskeleton, but also directly on chromatin.

Due to the differences in the reaction of varieties to the processing of various preparations and to the ability of most herbicides to migrate in the soil, we need to understand the selection of sorts resistant to herbicides during sowing in fields with poor phytosanitary conditions. Thanks to the proper use of herbicides on resistant varieties of grain, yield was significantly increased by 20% or more compared with the economic control. When using the herbicide-sensitive sorts, crop may be reduced, so we must consider the degree of stability of sort and use herbicides on sufficiently stable sorts [4]. For sensitive sorts it is obligatory to use herbicides with specific antidotes [26].

Taking into account the parameters of seed germination and the results of anaphase test, the most resistant to the action of drugs was sort Fantaziya odesskaya. Even in the application of herbicide napalm, which showed the most aggressive action in relation to all the wheat, the percentage of seed germination and normal anaphases was significantly higher compared with other sorts. Variety Niconiya was the most sensitive to the action of herbicides. Indexes of its germination under the influence of herbicide Napalm decreased to 46,8 ± 4,7%, while the number of normal anaphases did not exceed 52.8±2,8%.

REFERENCES

[1] Alov, I.A., (1972): Cytophysiology and pathology of mitosis (In Russian), Mir, Moscow, 264 p.

[2] Bochkov, N.P., Demin, Yu.S., Luchnik, N.V., (1972): The classification and accounting methods of chromosomal aberrations in somatic cells. (In Russian), Genetika, 8: 133-141.

[3] Bolognesi, C., (2003): Genotoxicity of pesticides: a review of human biomonitoring studies, Mutation Research. 543: 251-272.

[4] Dolzhenko, A.A., (2008): Varietal resistance grain crops to the new herbicide, Agro, S.-Peterburg, 4-6: 23-24.

[5] Flores-Maya, S., Comez-Arroyo, S., Calderon-Segura, M. E., Villalobos-Pietrini, R., Waliszewski, S. V., Gómez de la Cruz, L., (2005): Promutagen activation of triazine herbicides metribuzin and ametryn through *Vicia faba*

metabolism inducing sister chromatid exchanges in human lymphocytes *in vitro* and in *V. faba* root tip meristems // Toxicology in vitro, 19: 243-251.
[6] Geraskin, S.A., Dikarjov, V.G., Spirin, E.V., Dikarjova, N.S., (2002): Influence of separation of radioactive and chemical contamination on the yield of cytogenetic damage in the intercalary meristem of spring barley. (In Russian), Radiation Biology. Radioecology, 42: 364-368.
[7] Gostimsky, S.A., Djakova, M.I., Ivanovskaya, E.V., Monakhova, M.A., (1974): Practical work on cytogenetics. (In Russian), Publishing of Moscow State University, Moscow, 172 p.
[8] Grigorenko, N.V., (1999): Cytogenetic and genetic activity of the herbicide lentagran. (In Russian), Tsitologiya i Genetika, 33: 18-22.
[9] Grigorenko, N.V., Larchenko, V.A., (1999): Cytogenetic effect of herbicides Titus on corn. (In Russian), Plant Physiology – the science of III-millennium. Materials of VI-congress Society Plant Physiologists Russia, Moscow: 557-558.
[10] Grudina, N.S., Batsmanova, L.M., Taran, N.Yu., (2010): Peroxidative homeostasis in wheat plant response reaction to diquat action. (In Russian), Physiological and biochemical basis of the production process in cultivated plants. Materials of the All-Russian symposium with the international participation devoted to 85th anniversary of V. A. Kumakov, 13-15 October 2010, Saratov: 22-23.
[11] Ibragimova, E.E., (2004): Cytogenetic and general biological action of the herbicide totril on seed cotton. (In Russian), Scientific notes of the Crimean State Engineering and Pedagogical University, 5: 85-89.
[12] Karamova, N.S., Denisov, A.P., Staszewski, Z., (2008): Assessment of mutagenic activity of pesticides: aktar, zenkor, mospilan, penkotseb, fastak in the Ames test. (In Russian), Ecological Genetics, VI: 29-33.
[13] Kopytchuk, T.E., Belokon, S.V., Sechnyak, A.L., (2011): The influence of herbicides on viability of *Drosophila melanogaster*. I. Determination of genotoxic effects of herbicides by DLM. (In Ukrainian), Bulletin of the ONU. Biology, 16(6): 47-51.
[14] Kozhuro, Yu.I., Maximova, N.P., (1999): Cytogenetic analysis of herbicides treflan and zenkor on barley plants. (In Russian), Belovezhskaya Pushcha on the border of the third millennium. Materials of the Conference, 22-24 December 1999, Kamenyuki, Brest region., Minsk, pp. 216-218.
[15] Kozhuro, Yu.I., Semenchik, E.A., Maximova, N.P., (2008): The relationship between the operation of the apparatus of cell division and development of oxidative stress in Belarusian sorts of barley. (In Russian), Genetics and Biotechnology of the XXI century. Fundamental and applied aspects. Materials of the International Scientific Conference, Publishing Center of the Belarusian State University, Minsk, pp. 14-16
[16] Lisitskaya, S.M., Ryabchenko, N.A., Mochalov, V.V., (2006): The influence of pesticides on the spectrum of chromosomal aberrations in meristematic cells of wheat. (In Russian), The I-th All-Ukrainian Congress of Ecologists, Vinnitsa, p. 141.
[17] Morton, R., Sharp, K., (2004): Agriculture of Ukraine: Guide for the manufacturer. (In Ukrainian), Naukova Dumka, Kyiv, 284 p.
[18] Novozhilova, E. V., Bilous, A. A., (2009): Comparative analysis of the list of pesticides permitted for use on cereals in the Ukrainian and international practice. (In Russian), Kyiv, 35 p.
[19] Pandey, R.M., (2008): Cytotoxic effects of pesticide in somatic cells of *Vicia faba* L., Cytology and Genetics, 42: 13-18.
[20] Papakonstanti, E.A., Vardaki, E.A., Stournaras, C., (2000): Actin cytoskeleton: a signaling sensor in cell volume regulation, Cellular Physiology and Biochemistry, 10: 257-264.
[21] Pausheva, Z.P. (1988): Practical work on cytology plants. (In Russian), Agropromizdat, Moscow: 271 p.
[22] Popova, I.S. (2009): To the use of cytologic features in environmental monitoring. (In Russian), Visnyk of Ukrainian Society of Geneticists and Breeders, 7: 242-248.
[23] Rokizkiy, P.F., (1973): Biology statistics. (In Russian), Vysheishaya shkola, Minsk, 320 p.
[24] Shukla, Y., Taneja, P., (2002): Mutagenic potential of cypermethrin in mouse dominant lethal assay, Journal of Environmental Pathology, Toxicology and Oncology, 21: 259-265.
[25] Stroev, V. S., (1988): Influence of herbicides on the genetic variability of spring barley. (In Russian), Genetika, 24: 2259-2262.
[26] Zlotnikov, A. K., Alekhin, V. T., Khryukina, E. I., Perov, N. A., Ryabchinsky, A. V., Kudryavtsev, N. A., (2008): Antidote activity of the Albite growth regulator in combination with various functional groups of pesticides. (In Russian), Agriculture, 3: 44-45.
[27] Zuza, V. S., (2006): Tolerance crop plants to herbicides. (In Russian), Agricultural Chemistry, 10: 46-51.

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