УДК 633.16: 576.354.4: 632.954

A. L.Sechnyak, **T. E.Kopytchuk**

Odesa National Mechnykov I. I. University, Department of Genetics and Molecular Biology,

2, Dvoryanska Str., Odesa, 65082, Ukraine, e-mail: sechnyak@ukr.net, tatyana_kopytchuk@i.ua

INFLUENCE OF HERBICIDES ON SPRING BARLEY MEIOSIS

The effect of the herbicides “Selefit”, “Napalm”, “Roundup” and “Mistral” to regularity of spring barley meiosis was studied. The difference in cytogenetic activity and individual varieties response to the preparations was found. Spectrum of the meiotic aberrations shows an advantageous effect of the preparations to the division spindle, while there were observed sufficient herbicide activities towards the chromosomes.

Key words: meiosis regularity; herbicides; barley

It is known that cytoskeletal components involved in the plant adaptation syndrome formation [1]. However, active substances of many herbicides are active against the microtubules and have high affinity to plant tubulin [2]. Investigations of the effects of dinitroaniline herbicide Treflan on root cells of different barley varieties was proved that the herbicide induces and increased activity of peroxidase enzyme complex and this process correlates with the microtubule cytoskeleton polymerization violation [3]. Other herbicides also have shown different negative effects toward nucleospindle. [4] However, all these studies were related to the mitotic apparatus of plants. The question of how common the investigated regularities require similar researches regarding the regularity of meiosis influenced by the herbicides. The purpose of this investigation is to research the regularity of spring barley meiosis influenced by the herbicides with different active ingredients.

Materials and Methods

As a material of trial five different varieties of spring barley from the National Center of Seeds and Sorts Studies - Selection and Genetics Institute

(Odessa, Ukraine) were chosen. These were Galaktik, Eney, Vodogray, Stalker and Helios varieties of spring barley.

For the cultural treatment used herbicides with a continuous action, intended for preplanting or presowing treatment: Selefit (active agent - prometrin, 500 g/l), Napalm (active agent - izopropilamine glyphosate salt, 480 g/l), Roundup (active agent - izopropilamine glyphosate salt, 480 g/l) and Mistral (active agent-metrybuzyn (700g/kg). Plants were treated during the early stage of the stem elongation by work concentration of the investigated drugs

To evaluate the influence of the drug on the regularity of meiosis unbearded ears were fixed in acetic alcohol, then carried out the preprocessing by 4% solution of ferric alum and stained with 1% acetocarmine [5]. The frequency of normal and defective tetrads of microspores quantified. For each variant of the experiment counted 400 tetrads (including polyades). Statistical analysis was performed using Student's t test [6].

Results and Discussion

Plant growth suppressed moderately. However, in all investigated barley varieties meiotic index was significantly decreased ($R \leq 0{,}001$) compared with control (Table. 1). At the same time differences were found for both cytogenetic activity of drugs and for the reaction of the varieties on the treatment.

Most significant action was demonstrated by Napalm. Meiotic index in conditions of herbicide treatment averaged 51.6 + 1.1% (range, depending on the variety, composed 40.0-60.0%). Relatively mild effects were demonstrated by Mistral (meiotic index 62.9 + 1.0%, interval – 59.3-68.3%) and Selefit (meiotic index - 62.6 + 1.1%, interval – 51.3-71.3%). Interim cytogenetic activity was demonstrated by herbicide Roundup (mean - 58.7 + 1.1%, interval – 54.3-65.3%).

Varieties reactions were also significantly different and depended on the herbicide that was used. The most sensitive to herbicide treatment were Galactic and Eney varieties. The average value of meiotic index after the herbicides treatment constituted 53.5 + 1.2% + 1.2 and 55.2%, respectively. The least

sensitive varieties were Stalker (mean meiotic index was 63.2 + 1.2%, interval - 59,3-71,3%) and Vodogray (meiotic index 64.3 + 1.2%, interval – 58.5-68.5%). Helios sensitivity to the action of the herbicides was relatively moderate toward other groups. The average value of meiotic index was 59.2 + 1.2%, and the interval – 52.3-68.3%.

Table 1

The share of normal and defective products of meiosis in barley treated by herbicides and control (%)

Variety	Variant of the experiment	Normal tetrades	Tetrades with micronucleous	Nontypical tetrades	Polyades
Galactic	Control	88,0±1,6	5,8±1,2	4,5±1,0	1,8±0,7
	Selefit	51,3±2,5	24,8±2,2	14,5±1,8	9,5±1,5
	Napalm	40,0±2,4	36,3±2,4	18,3±1,9	5,5±1,1
	Roundup	56,5±2,5	20,7±2,0	19,8±2,0	4,5±1,0
	Mistral	66,0±2,4	8,5±1,4	14,0±1,7	11,5±1,6
Eney	Control	84,0±1,8	7,3±1,3	6,5±1,2	2,3±0,7
	Selefit	60,0±2,4	18,8±2,0	15,3±1,8	6,0±1,2
	Napalm	47,0±2,5	18,3±1,9	24,3±2,1	10,5±1,5
	Roundup	54,3±2,5	22,3±2,1	14,3±1,8	9,3±1,5
	Mistral	59,5±2,5	19,3±2,0	12,3±1,6	9,5±1,5
Vodogray	Control	92,8±1,3	4,3±1,0	2,5±0,8	0,5±0,4
	Selefit	68,5±2,3	13,3±1,7	9,8±1,5	8,5±1,4
	Napalm	58,5±2,5	17,8±1,9	16,3±1,8	7,3±1,3
	Roundup	65,3±2,4	17,3±1,9	13,5±1,7	4,0±1,0
	Mistral	61,3±2,4	16,8±1,9	11,5±1,6	10,5±1,5
Stalker	Control	92,5±1,3	4,3±1,0	3,3±0,9	0
	Selefit	71,3±2,3	12,3±1,6	9,3±1,5	6,8±1,3
	Napalm	60,0±2,4	11,8±1,6	19,5±2,0	8,8±1,4
	Roundup	62,3±2,4	18,8±2,0	11,5±1,6	10,5±1,5
	Mistral	59,3±2,5	21,0±2,0	10,3±1,5	9,5±1,5
Gelios	Control	95,5±1,0	2,3±0,7	2,0±0,7	0,3±0,3
	Selefit	61,3±2,4	18,5±1,9	11,5±1,6	8,8±1,4
	Napalm	52,3±2,5	20,5±2,0	18,5±1,9	8,8±1,4
	Roundup	55,0±2,5	22,3±2,1	15,5±1,8	7,3±1,3
	Mistral	68,3±2,3	11,3±1,6	12,0±1,6	8,5±1,4

Among the defective meiotic products tetrads with micronuclei, nontypical tetrads and polyades were observed (Fig. 1). Obviously, the reason of the formation of tetrads with micronuclei is chromosomal rearrangements and the cycle "gap - fusion - bridge - gap", while the formation of polyades and nontypical tetrads associated with spindle apparatus dysregulation [7].

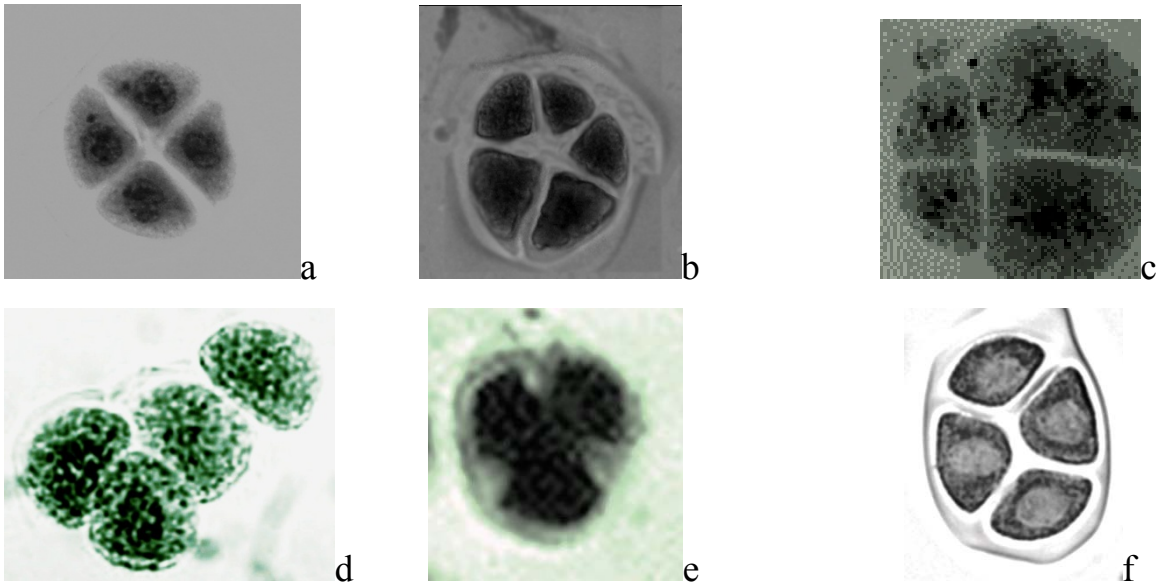

Fig. 1. Defective meiosis products triggered by the herbicides. The lens × 40, ocular - × 10 camera Nikon L23: a - tetrad with micronuclei; b - pentad; nontypical tetrad: c – nongomolographic, d - T-shaped, e - tetrahedral, f - rhombic

Among the offtype tetrads observed nongomolographic (Fig. 1c), linear and T-shaped tetrads (Fig. 1d). There were also tetrahedral (Fig. 1d) and rhombic (Fig. 1e) tetrads. Whereas nongomolographic tetrad is a result of abnormal formation typical for cereals, isobilateral and other types of tetrads, is not usual for cereals [5]. The share of the offtype tetrades triggered by the herbicides ranged from 11.5 to 24.3%. Formation offtype meiotic tetrads defined orientation poles division.

The share of polyades preferably tetrads, was 4,0-11,5%. Considered that the incidence of polyades is determined by lag and disorientation of chromosomes at the anaphase stage I [9]. There are data that sometimes polyades arise regarding the "correct" flow of meiosis, for example by fragmentation of young nuclei in telophase II. Nuclear fission occurs in a manner that resembles amitosis. Perhaps

this is caused by the disorders of the formation of cell walls and phragmoplast between the four newly formed nuclei [10].

The formation of atypical tetrads may be conditioned by a violation of spindle orientation and sharply irregular – chromosomes disjunction in anaphase I [9]. There are many substances that can both inversely and irreversibly affect the polymerization-depolymerization of a spindle protein and consequently on the spindle functioning [8].

Analysis of meiosis spectrum of disorders depending on the applicable herbicide showed that the percentage of polyades and atypical tetrads averaged 22.9 + 0.5%, whereas the share of tetrads with micronuclei was significantly ($R \leq 0.001$) less -18.4+ 0.4. Thus, herbicides that were tested had effect mostly on the spindle, even though significant activity against chromosomes was observed.

Conclusions

1. Herbicides interrupted the normal flow of meiosis. Most harmful action the herbicide Napalm had. Selefit and Mistral had relatively soft action.

2. Varieties reaction on the herbicides treatment was significantly different and depended on the applied drug.

3. The investigated herbicides influenced mostly on the spindle, even though had significant activity against chromosomes.

References

1. Kozhuro Yu. I., Semenchik E. A., Maksimova N. P. (2010) Varietal differences in the stress response of barley (Hordeum vulgare) on the effects of the herbicide treflan ["Sortovye razlichiya stress-reaktsii rasteniy yachmenya (Hordeum vulgare) na vozdeystvie gerbitsida treflana"], Vestsi natsiyanaln. akad. nav. BelarusI. Ser. biyal. Nav, № 4, pp. 101–106
2. Konstantinov A. V. (1971) Meiosis [Meyoz], Minsk: Izd-vo BGU, 180 p.
3. Kopyitchuk T. E., Sechniak A. L. (2012) Influence of residual doses of herbicides on cytogenetic stability of barley seedlings ["Vliyanie

ostatochnykh doz gerbitsidov na tsitogeneticheskuyu stabil'nost' prorostkov yachmenya”] Faktori eksperimental'noï evolyutsiï organizmiv: Zb. nauk. pr, V. 4, pp 237–241.

4. Pausheva Z. P. (1988) Practical work on Plant Cytology [Praktikum po tsitologii rasteniy]. Moskva: Agropromizdat 1988, p 271.
5. Poddubnaia-Arnoldi V. A. (1976) Cytoembryology angiosperms. Fundamentals and prospects [Tsitoembriologiya pokrytosemennykh rasteniy. Osnovy i perspektivy], Moskva: Nauka, p 507.
6. Popova I. S. (2009) To a question about the use of cytological features for monitoring the environment [K voprosu ob ispol'zovanii tsitologicheskikh priznakov pri monitoringe okruzhayushchey sredy] Visnik Ukraiinskoho tovaristva henetikiv ta selektsioneriv, N 2, pp 74–82.
7. Rokitskii P. F. (1973) Biological statistics [Biologicheskaya statistika], Minsk: Vyisheyshaya shkola, p 319.
8. Fulton A. (1987) The cytoskeleton. Architecture and choreography cells [Tsitoskelet. Arkhitektura i khoreografiya kletki], Moskva: Mir, p. 120.
9. Shamina N. V. (2006) Diagnosticum plant meiotic abnormalities in its products [“Diagnostikum anomaliy rastitel'nogo meyoza po ego produktam”], Cytology, V. 48, N 6. pp. 486–494.
10. Papakonstanti E. A., Vardaki E. A., Stournaras C. (2000) Actin cytoskeleton: a signaling sensor in cell volume regulation, Cell Physiol. Biochem, V. 10, N 5/6, pp 257–264.
11. Vaughn K. C., Lehnen L. P. (1991) Mitotic Disrupter Herbicides, Weed Science, V. 39, N 3, pp. 450–457.