EFFECTS OF PLANT GROWTH REGULATORS ON SEEDLINGS ELONGATION AND ON CYTOGENETIC PARAMETERS IN *HORDEUM VULGARE* L. CV *MADALIN*

ELENA TRUŢĂ[1*], ZENOVIA OLTEANU[2], LĂCRĂMIOARA OPRICĂ[2], ŞTEFANIA SURDU[1], MARIA MAGDALENA ZAMFIRACHE[2], GABRIELA CĂPRARU[1], MARIA CRAITA ROSU[1]

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Abstract: The treatments of barley seeds with plant growth regulators induced effects whose amplitude, analyzed in the dynamics of the three quantitative determinations, was in relation with the type and concentration of tested compound and also with the seedlings age. Auxin slightly stimulated seedlings growth, kinetin and 2,4-D+Kin mixture inhibited their elongation in all tested concentrations, while the treatment with gibberellic acid generally expressed in increase of barley seedlings lenght. Mitotic index had no significant variations comparatively to control. Ana-teophase chromosome aberrations were present in a percentage that surpassed the control average in several variants subjected to the treatment with growth regulators: 10 mg l^{-1} and 25 mg l^{-1} 2,4-D; 1 mg l^{-1} gibberellic acid; 1 mg l^{-1} 2,4-D+ 1 mg l^{-1} kinetin.

INTRODUCTION

The phytohormones have essential roles in regulation of plant physiological processes, because they serve as integrators and inducers of multicellular organisms differentiation and play a major role in hereditary information expression, so determining the various correlation types in plant organisms. Plant ontogeny phases are the result of dynamic equilibrium established between hormones with stimulative effects and those having inhibitive action. The growth and developmental rates of organs also depend on endogenous hormone level. The physiological state, the correlation degree between characters, ontogenetic phase, gene epistasy and pleiotropy, the environmental conditions are factors which modulate the quantitative modifications of plant regulators (DUCA et al., 2003). In literature are cited sevceral examples confirming the effects of some plant growth regulators on genetic material in plants and animals. In some species, like *Nicotiana tabacum*, *Lens caulinaris*, *Heliantus tuberosus*, *Cucumis sativus,* the action of growth regulators resulted in the modification of labelled timidine incorporation in DNA, with the alteration of nucleic acid synthesis (LERNER, 1999). Also, the addition of phytohormones in *Cymbidium* tissue cultures induced DNA modifications. Thus, auxins determined increases of A+T rich fraction, gibberellic acid increased G+C fraction, while the cytokinins had the lowest effect (NAGL and RICKER, 1976, citaţi de LERNER, 1999). The cytotoxic and mutagenic effects of 2,4-D synthetic auxin were observed both in animals (on hamster fibroblasts, for example) and in root apical meristems (PAVLICA et al. 1991), where it determined modifications of cell cycle and mitosis, changes in cromosome and chromatin structure, especially at concentrations higher 5 µg/ml. At small doses, 2,4-D behaves like a systemic herbicide, having carcinogenetic, mutagenic, clastogen or neurotoxic effects. MUSTONEN et al. (1986) evidenced a significant increase of aberrations in *in vitro* human lymphocytes subjected to 2,4-D action (0.125 – 1.250 mM).

MATERIAL AND METHOD

Biological material was represented by *Hordeum vulgare* L. cv. *Mădălin* from Podu Iloaie Agricultural Research Center. They were subjected to 4 hours treatments by immersion in growth regulators solutions: **2,4-D (A)** (1 mg l^{-1}; 10 mg l^{-1}; 25 mg l^{-1}; 50 mg l^{-1}), **kinetin (B)** (1 mg l^{-1}; 10 mg l^{-1}; 25 mg l^{-1}; 50 mg l^{-1}), **gibberellic acid (C)** (1 mg l^{-1}; 10 mg l^{-1}; 25 mg l^{-1}; 50 mg l^{-1}), **2,4-D + Kin mixture (D)** (1 mg l^{-1} 2,4-D+ 1 mg l^{-1} kinetin; 10 mg l^{-1} 2,4-D+ 10 mg l^{-1} kinetin; 25 mg l^{-1} 2,4-D+ 25 mg l^{-1} kinetin; 50 mg l^{-1} 2,4-D+ 50 mg l^{-1} kinetin).

For cytogenetic analysis, the microscopic preparations were obtained by squash method and analyzed at a Nikon Eclipse 600 light microscope. The photos were realized at 100x objective, with a Cool Pix Nikon digital camera, 1600x1200 dpi resolution, the images being processed in Adobe Photoshop programme. Five preparations/variant were scored, and ten microscopic fields/preparation, to estimate mitotic index and chromosome aberrations.

RESULTS AND DISCUSSIONS

1. Influence of hormone treatments on seedlings growth in early ontogeny. The effects of treatments with exogenous hormone regulators were in relation with the type and the concentration of tested solution and also with the seedlings age (Table 1; Figure 1). The treatment duration was the same for all variants.

Concerning the 3 days old seedlings, the differences face to control are no significant in spite of some fluctuations. 2,4-D and GA_3 determined, at 10 mg l^{-1}, a slight stimulation of elongation seedlings, more visible for auxin. The other values are for all A, B and C variants closed to control (slightly superior to control, for auxin, slightly inferior to control average, for kinetin and gibberellic acid).

The variants treated with auxin – cytokinin mixture registered more important increases comparatively to control average, for all tested concentrations, although it is obvious that for D4 variant, the increased hormone adding becomes to exert a negative influence on 3 days old seedlings, although the average slightly surpasses that of control.

In 10 days old barley seedlings, a slight stimulation of growth appears in 2,4-D treatments (the plantlets are higher than control with 5 – 10 %), but an inhibition in all tested concentration for kinetin and 2,4-D + kinetin mixture was observed (the strongest effect was noted for 50 mg l^{-1} kinetin, variant having the plantlets height with 68% smaller than control: 38.80±3.08 mm, face to 120.24±4.04 mm).

In barley and wheat seeds, gibberellic acid induces expression of α-amilase coding gene. This enzyme is necessary to starch mobilization that contributes to postgerminative embryo growth. This could be an explanation for generally higher values, comparatively both to control and the other tested hormones, especially in 6 days old seedlings (C1=68.71±2.43, C2=64.2±3.86; C3=61.6±5.35; C4=68.83±4.61, face to control average, 58.15±3.81 mm), and even at the age of 10 days. Gibberellic acid determines the seedlings elongation, both by stimulation of this process and by activation of cell division.

Therefore, considering especially the results for the last two measurements which offer informations on final trend for the expression of effects of hormone treatments, we can sustain that auxin determined a slight stimulation of plant elongation, kinetin and 2,4-D+Kin mixture inhibited the growth, while the barley seed treatment with gibberellic acid generally reflected in stimulation of growth in early ontogenetic stages. The inhibitive effect was stronger at maximum tested concentrations of kinetin and hormone mixture.

Table 1. Dynamics of growth of barley seedlings in early ontogeny, after the seed treatments with growth regulators

Variant		Seedlings age					
		3 days		6 days		10 days	
		x±Sx	STDEV	x±Sx	STDEV	x±Sx	STDEV
Control		7.85±0.59	4.26	58.15±3.81	22.56	120.24±4.04	28.22
A	A1 – 1 mg l^{-1} 2,4-D	8.08±0.53	3.77	55.71±4.56	26.63	137.81±3.05	21.14
	A2 – 10 mg l^{-1} 2,4 D	10.18±0.57	3.58	58.12±0.51	18.63	125.11±3.22	23.79
	A3 – 25 mg l^{-1} 2,4-D	7.56±0.56	4.14	53.51±3.47	20.54	122.29±3.85	25.80
	A4 – 50 mg l^{-1} 2,4-D	8.22±0.43	3.07	57.11±4.36	25.79	136.51±2.68	17.16
B	B1 – 1 mg l^{-1} kinetin	7.10±0.37	2.62	54.28±3.49	20.95	105.62±5.34	34.63
	B2 – 10 mg l^{-1} kinetin	7.10±0.41	2.88	53.89±2.52	14.90	86.80±3.85	28.54
	B3 – 25 mg l^{-1} kinetin	7.34±0.39	2.78	60.31±3.34	19.77	86.11±3.85	25.83
	B4 – 50 mg l^{-1} kinetin	6.74±0.35	2.44	31.83±1.95	11.54	38.80±3.08	20.67
C	C1 – 1 mg l^{-1} GA3	7.28±0.85	6.03	68.71±2.43	14.39	115.64±5.57	37.35
	C2 – 10 mg l^{-1} GA3	8.23±0.71	5.01	64.2±3.86	22.86	129.66±6.56	43.50
	C3 – 25 mg l^{-1} GA3	6.98±0.47	3.33	61.6±5.35	31.68	119.33±8.39	54.37
	C4 – 50 mg l^{-1} GA3	6.72±0.54	3.81	68.83±4.61	27.67	159.33±7.41	46.27
D	D1 - 1 mg l^{-1} 2,4-D + 1 mg l^{-1} Kin	9.85±0.61	4.30	49.58±4.26	25.55	119.83±4.03	29.07
	D2 - 10 mg l^{-1} 2,4-D + 10 mg l^{-1} kin	10.13±0.64	4.53	56.86±3.55	21.04	115.31±4.06	28.42
	D3 - 25 mg l^{-1} 2,4-D + 25 mg l^{-1} Kin	10.04±0.56	3.98	52.97±3.11	18.93	94.78±2.85	19.98
	D4 – 50 mg l^{-1} 2,4-D + 50 mg l^{-1} Kin	8.47±0.52	3.65	37.86±0.52	14.15	76.68±3.11	23.48

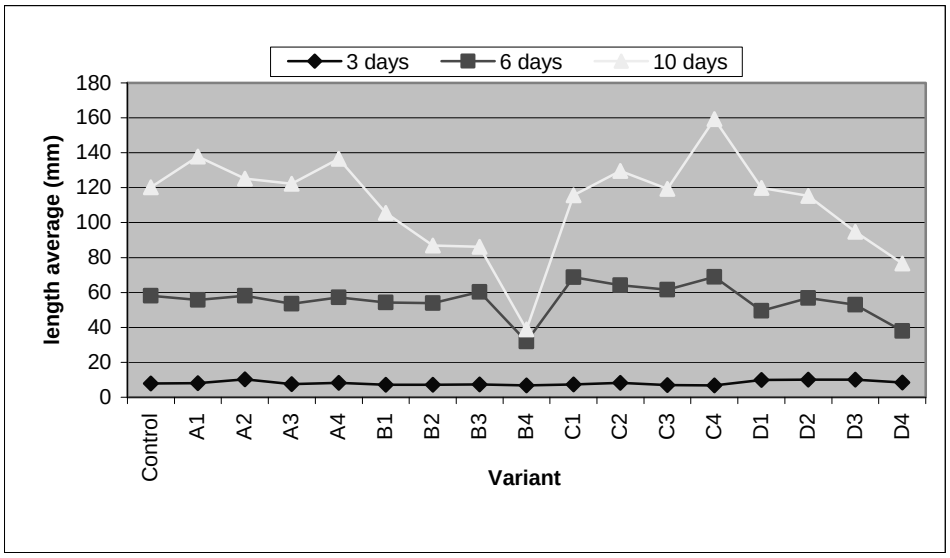

Figure 1. Influence of hormone treatments on dynamics of barley seedlings growth in early ontogeny

2. Influence of hormone treatments of barley seeds on cytogenetic parameters. Mitotic index not displayed significant variations comparatively to control (Table 2; Figure 2). The lowest values of percentage of dividing cells were registered for kinetin treated variants (3.96% for B1, 4.19% for B4, comparatively to control - 5.45%). B4 is also the variant having the highest degree of growth inhibition in barley seedlings.

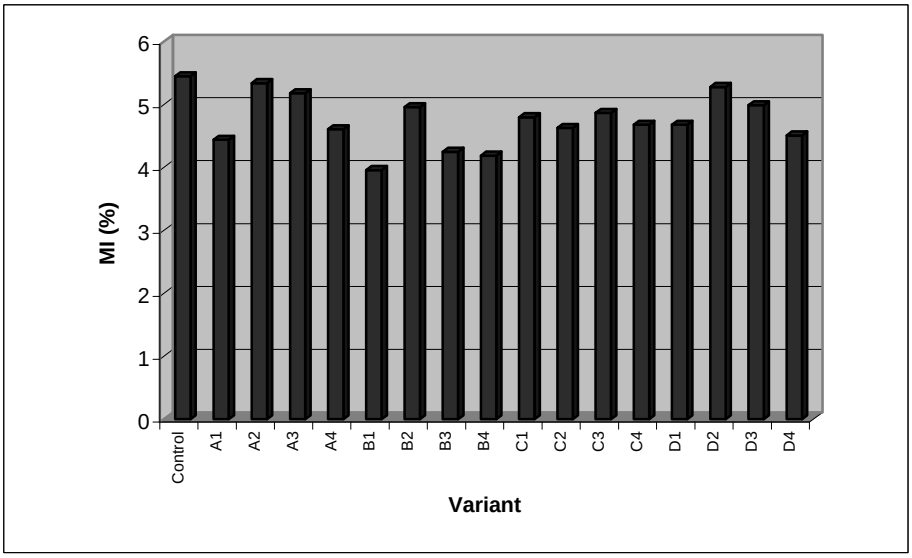

Figure 2. Evolution of mitotic index in *Hordeum vulgare* cv. *Mădălin*, after hormone treatment

Ana-telophase chromosome aberrations (Figure 3-4) were present in a percentage surpassing control average in some of variants subjected to exogenous treatment with growth regulators. 2,4-D auxin induced structural and numerical chromosome modifications at 10 mg l^{-1} and 25 mg l^{-1}. Thus, as numerical modification is the tetraploidy state (2n=4x=28) evidenced in 10 mg/l 2,4-D treated variant. As aberration types, the most numerous were chromosome bridges, followed by expulsed chromosomes and multipolar ana-telophases. At maximum 2,4-D tested concentrations – 25 and 50 mg l^{-1} – were evidenced too lagging chromosomes.

In kinetin treated variants, the incidence of chromosome aberrations was inferior to control average, indifferently to tested concentration, but the lysis of chromatin material and a big number of like apoptotic cells were visible.

Gibberellic acid and 2,4-D+kinetin mixture determined a high frequency of chromosome aberrations at the minimum tested concentration (1 mg l^{-1}). This result is in accordance with data from literature (GERASKIN et al., 2006) concerning the increased ability of small hormone concentrations to induce chromosome aberrations.

Table 2. Effects induced by exogenous plant regulators on cell division, number and types of chromosome aberrations in barley

Variant	Total analyzed cells	Dividing cells	Mitotic index (%)	Faze ale diviziunii mitotice								Abnormal ana-telophases		Types of chromosome aberrations							
				Prophase		Metaphase		Anaphase		Telophase				Bridges		Lagging chromosomes		Expulsed chromosomes		Multipolar ana-telophases	
				Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
Control	8707	475	5.45	220	2.52	118	1.35	60	0.68	77	0.88	45	32.44	23	16.58	2	1.44	13	9.37	4	2.88
A1	6846	304	4.44	118	1.72	82	1.19	58	0.84	46	0.67	29	27.88	18	17.30	0	0	1	0.96	6	5.76
A2	5199	278	5.34	137	2.63	62	1.19	37	0.71	42	0.80	28	35.44	12	15.18	0	0	9	11.39	7	8.86
A3	5184	269	5.18	130	2.50	63	1.21	37	0.71	39	0.75	28	36.84	15	19.73	3	3.96	7	9.21	2	2.63
A4	5784	267	4.61	136	2.34	51	0.88	48	0.82	32	0.55	25	30.58	17	20.79	1	1.22	2	2.44	4	4.88
B1	5448	216	3.96	70	1.28	63	1.15	43	0.78	40	0.73	20	24.09	12	14.45	0	0	6	7.22	1	1.20
B2	4233	210	4.96	87	2.05	36	0.85	36	0.85	51	1.20	23	26.43	8	9.19	0	0	5	5.74	9	10.34
B3	6204	264	4.25	107	1.72	57	0.91	49	0.78	51	0.82	31	31.00	16	16	0	0	10	10	4	4
B4	6105	256	4.19	94	1.53	62	1.01	37	0.60	63	1.03	27	27.00	11	11	2	2	5	5	4	4
C1	4973	239	4.80	104	2.08	61	1.22	26	0.52	48	0.96	29	39.18	15	20.26	1	1.35	8	10.80	2	2.70
C2	4897	227	4.63	90	1.83	57	1.16	50	1.01	30	0.61	24	30.00	8	10	1	1.25	7	8.75	5	6.25
C3	5905	288	4.87	112	1.89	63	1.06	61	1.03	52	0.88	34	30.08	23	20.34	1	0.88	3	2.64	6	5.28
C4	5505	258	4.68	112	2.03	49	0.89	51	0.92	46	0.83	31	31.95	18	18.55	0	0	6	6.18	5	5.15
D1	7132	334	4.68	153	2.14	71	0.99	60	0.84	50	0.70	52	47.27	33	29.99	14	12.72	2	1.81	3	2.72
D2	5677	300	5.28	121	2.13	77	1.35	69	1.21	33	0.58	29	28.43	18	17.64	0	0	10	9.80	1	0.98
D3	4162	208	4.99	77	1.84	39	0.93	47	1.12	45	1.08	29	31.52	14	15.21	1	1.08	6	6.52	8	8.69
D4	3502	158	4.51	62	1.77	37	1.05	29	0.82	30	0.85	15	25.42	11	18.64	2	3.38	3	5.08	1	1.69

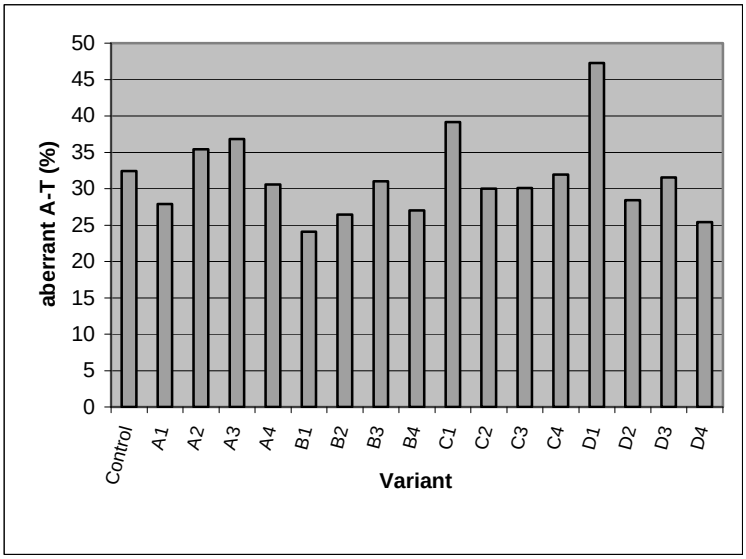

Figure 3. Incidence of aberrant ana-telophases after barley seed treatments with growth regulators

The most encountered ana-telophase abnormalities were bridges, lagging chromosomes, expulsed chromosomes and multipolar ana-telophases (Figures 4-7).

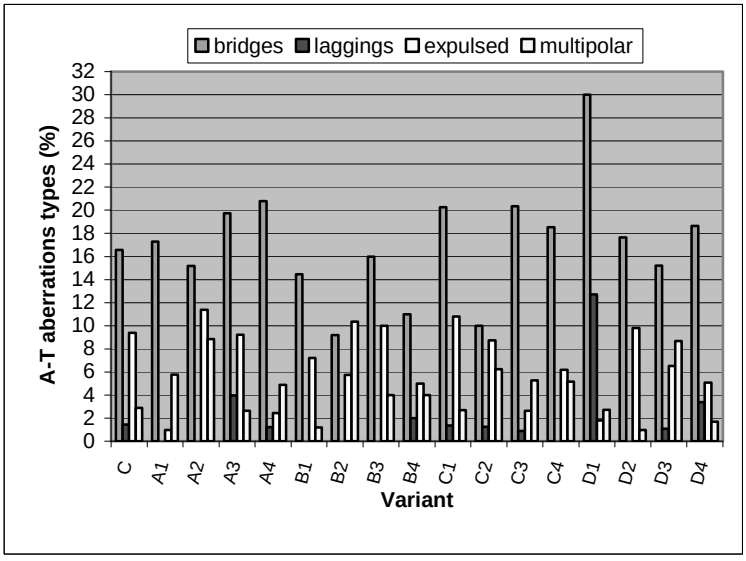

Figure 4. Incidence of types of chromosome aberrations in ana-telophase after barley seed treatments with growth regulators

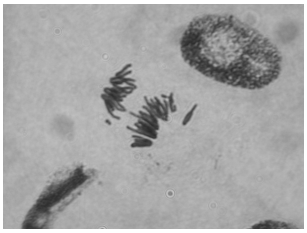

Figure 5. Multipolar anaphase with expulsed chromosomes - 25 mg l^{-1} GA3

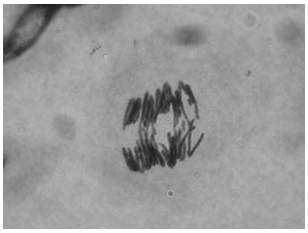

Figure 6. Multipolar anaphase with bridges – 50 mg l^{-1} 2,4-D

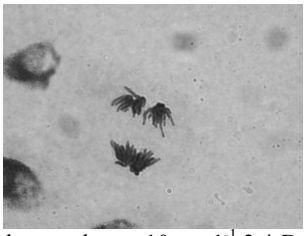

Figure 7. Tripolar anaphase - 10 mg l^{-1} 2,4-D+ 10 mg l^{-1} kin

The big proportion of chromosome aberrations can be considered as a consequence of oxidative stress induced by hormone treatment. The reactive oxygen species and their intermediates can interfere with genetic material and, in this way, can alter the nucleic acid macromolecules, by their depolimerization. The seed treatments with growth regulators also influenced the chromosome bahaviour in mitotic metaphases (Fig. 8).

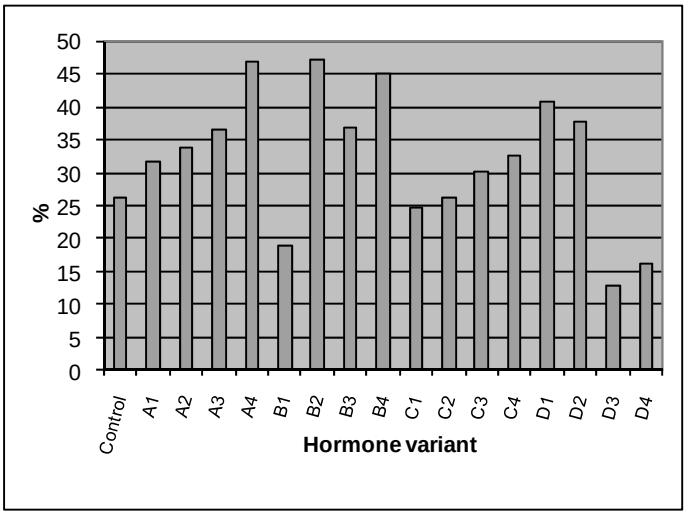

Figure 8. Incidence of abnormal metaphases after barley seed treatments with growth regulators

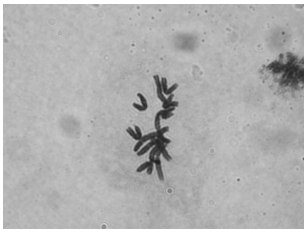

Figure 9. Metaphase with expulsed chromosomes 10 mg l^{-1} 2,4-D+ 10 mg l^{-1} kinetin

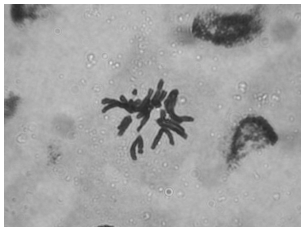

Figure 10. Fragmented metaphase, with expulsed chromosomes – 25 mg l^{-1} kinetin

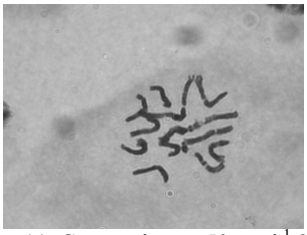

Figure 11. C-metaphase - 50 mg l^{-1} 2,4-D

Metaphases with expulsed chromosomes from equatorial plate, sticky chromosomes, fragmented metaphases and C-metaphases were the abnormalities encountered in this mitosis phase (Figures 9-11). For 2,4-D and gibberellic acid, the number of modified metaphases increased in direct relation with auxin concentration. The kinetin variants which have a relatively low number of ana-telophase aberrations, have – except B1 variant – an increased value of metaphase abnormalities.

In variants treated with hormone mixture, the percentage of abnormal metaphases was higher than control at 1 and 10 mg l^{-1} and inferior to control at 25 and 50 mg l^{-1} concentrations.

CONCLUSIONS

The treatments of barley seeds with plant growth regulators induced effects whose amplitude was in relation with the type and concentration of tested compound.

The inhibitive effect on seedling growth was stronger at the maximum tested concentrations of kinetin and 2,4-D+Kin mixture.

Mitotic index had no significant variations comparatively to control.

The percentage of ana-telophase aberrations surpassed the control average in variants: 10 mg l^{-1} and 25 mg l^{-1} 2,4-D; 1 mg l^{-1} gibberellic acid; 1 mg l^{-1} 2,4-D+ 1 mg l^{-1} kinetin.

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1 – Biological Research Institute Iaşi

2 – University "Alexandru Ioan Cuza", Iaşi, Faculty of Biology

* - email: *trutaelena@yahoo.com*