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CHANGE OF PHYSIOLOGICAL-BIOCHEMICAL AND GENETIC PARAMETERS OF SEEDLINGS OF MEDICINAL PLANTS UNDER THE INFLUENCE OF MILLIMETER RADIATION AFTER *EX SITU* CONSERVATION

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În urma aplicării iradierii milimetrice cu lungimea de undă de 5,6 mm, densitatea puterii de 6,6-10,0 mWt/cm^2 și expoziția de 2,8 și 30 min asupra semințelor de ricin (*Ricinus communis* L.), echinacea (*Echinacea purpurea*) și ciumăfae (*Datura stramonium*) din colecția Băncii de Gene din Moldova s-a stabilit o accelerare substanțială a proceselor de creștere după parametrii energiei germinative și germinației semințelor, conținutului enzimei o-AIA și al sumei proteinelor ușor solubile în rădăcioarele germenilor, de rând cu micșorarea aberațiilor cromozomiale în meristemele rădăcioarelor embrionare. Metoda prelucrării semințelor cu iradierea milimetrică în cazul conservării materialului genetic *ex situ* poate fi recomandată pentru mărirea viabilității lor și ar putea servi ca procedeu perspectiv de păstrare efectivă a resurselor genetice vegetale.

Key words: *genetic resources, medicinal plants, castor-oil plant, echinacea, datura, millimeter radiation, germination power, germinability, IAA-oxidase, freely soluble proteins, chromosome aberrations.*

At current stage of civilization development, conservation of biological diversity of plants is one of the important and rather difficult problems. Agricultural biodiversity is a necessary condition for development of sustainable agriculture both at present and in the future. Its conservation is a solid guarantee for implementation of various food programs. Significant work on conservation of agrobiodiversity is performed by gene banks in many countries worldwide. Long-term conservation of genetic resources, verification of their assured compliance with the original, first of all in terms of genetic integrity, are the matters of great importance [1, 10].

Laboratory of plant genetic resources of the *Institute of Genetics, Physiology and Plant Protection* of the *Academy of Sciences of Moldova* conducts for more than 10 years diversified studies on the increase of viability of collection accessions of various agricultural crops under the conditions of *ex situ* conservation, since as a result of long-term storage seeds loss their germinability due to the aging processes [3, 8-13].

In order to increase viability of such seeds different chemical and physical factors are applied. In solving this task, which is very important for gene banks, one of the promising physical factors is millimeter radiation (MMR) that is already used for a long time in general biology, medicine, microbiology and less often – in plant cultivation, especially for the purposes of conservation of plant genetic resources. Millimeter radiation is characterized by regulating and information action on biological objects [6] and exerts favorable effect on seed viability. Therefore it is reasonable to use this factor for stimulation of germination processes in old seeds whose viability is decreased due to long-term storage. We conducted a number of experiments with the seeds of grain crops, cereals, pulses, vegetables, and industrial crops [3, 8-13]. These studies were dedicated to medicinal plants.

MATERIALS AND METHODS

Study objects included seeds of castor-oil plant (*Ricinus communis*), spurge family (*Euphorbiaceae*); echinacea (*Echinacea purpurea*), sunflower family (*Asteraceae*) and datura (*Datura stramonium*), nigtshades (*Solanaceae*). Following long-term storage, seeds were treated with millimeter radiation with the wavelength of 5.6 mm, power density of 6,6-10,0 mW/cm^2 and exposures to radiation of 2, 8 and 30 min, then seeds were sprouted in Petri dishes in distilled water in thermostat at 25^{o}C. Number of seeds in each variant was 200-300 seeds. Germination power (GP) and germinability (G) were determined according to ISTA International Methods [2]. Activity of IAA-oxidase enzyme [2] and sum of freely soluble proteins (FSL) [4] were determined in rootlets of seedlings. Incidence and range of chromosome aberrations (ICA) were determined in apical meristem of embryo roots [14].

RESULTS AND DISCUSSION

When seeds of castor-oil plant were treated with MMR, stimulation of GP was observed after all exposures, the best ones were 2 and 30-min exposures. Level of stimulation of seed germination was 38,1 and 9,6%, respectively. With regard to germinability of seeds, maximal stimulation effect was 11.4% with 30-min exposure (Table 1). At that, maximal growth of rootlets was observed with 2- and 8-min exposures to radiation (it exceeded the control by 23,0 and 45,0%, respectively).

Analysis of content of IAA-oxidase enzyme in rootlets of seedlings of castor-oil plant showed that there were no differences when 30-min exposure was compared with the control. Sum of freely soluble proteins in seedling roots in this variant exceeded the control by 41,0 μg/g of crude substance.

It is known that natural mutation process begins in seeds during long-term storage that leads to increase of incidence of chromosome aberrations up to 8-10%. This incidence is about 3% in the control (fresh seeds) [7]. It is assumed that millimeter radiation can induce modification of genetic effect in old seeds. Cytological analysis demonstrated that the number of cells with chromosome abnormalities (aberrations) was 6,6% in the control (Table 1).

Table 1. *Physiological-biochemical and genetic parameters of seeds and seedlings of castor-oil plant under the influence of MMR*

Parameters	Exposures to MMR, min			
	0	2	8	30
GP, %	21.8 ± 8.13	59.9±1.23***	27.2±1.41	31.4±2.05**
G, %	84.8 ± 4.81	83.8 ± 3.60	91.4 ± 2.11	96.2 ± 1.20*
IAA-oxidase, c.u.	0.0306	0.1163	0.0566	0.0305
FSP, μg/g of crude substance	526	390	390	567
ICA, %	6.6 ± 1.4	5.2 ± 1.4	3.2±0.8***	2.4 ± 0.5*

Note: in tables 1-3 *, **, *** mean that difference as compared to control is significant with p≤0.95; 0.99; 0.999

Treatment of castor-oil plant seeds with MMR with 2-min exposure resulted in insignificant decrease of this parameter (up to 5,2%), but with 8- and 30-min exposures this decrease was significant (3,2 and 2,4%, respectively). The range of studied chromosome aberrations was less wide and represented mostly by single and double chromosome bridges and chromosome lagging.

With regard to echinacea seeds (Table 2) it was revealed that MMR exerts significant stimulating effect on germinating power of seeds with all exposures to radiation – 2.8 and 30 min (it exceeded the control by 68,4; 55,1 and 73,5%, respectively). Seed germinability also increased with the use of all three exposures, significant exceedance over control was observed with 2-min exposure to MMR.

Content of IAA-oxidase enzyme in seedling rootlets decreased after irradiation in all tested variants, especially with 30-min exposure (it decreased by 5,6 times as compared to control). As to the sum of freely soluble proteins, variant of 2-min exposure was the most prominent (increase of 44,4% as compared to control).

It was revealed that treatment of seeds of echinacea with MMR resulted in significant decrease of chromosome aberrations in the variant with 8-min exposure (ICA decreased by 3,9 times as compared to control). Also in this variant the least number of single and double chromosome bridges was recorded (24 and 9 – in control, 8 and 4 – in experiment). It should be noted that in contrast to castor-oil plant and datura, totally 4 triple chromosome bridges and 3 chromatid bridges in all variants were found only in ana-telophase of meristem of echinacea. Chromosome lagging was lower with 8 - and 30-min exposures to radiation as compared to control.

In experiments with old datura seeds the significant increase of germination power and germinability was found with 2- and 8-min exposures (Table 3). Germination power of seeds in these variants exceeded the control by 10,7 and 9,1%, seed germinability – by 14,3 and 10,1%, respectively. With maximal 30-min exposure of seeds to MMR stimulating effect on parameters – germination power and germinability of seeds was minor and insignificant.

Table 2. *Influence of MMR on physiological-biochemical and genetic parameters of seeds and seedlings of echinacea*

Parameters	Exposures to MMR, min			
	Control	2	8	30
GP, %	9,8	16,5**	15,2*	17,0**
G, %	52,1	62,0 *	59,0	58,1
IAA-oxidase, c.u.	0.2831	0.1015	0.1023	0.0502
FSP, μg/g of crude substance	270	390	210	150
ICA, %	12,4 ±1,8	12,0 ± 1,9	3,2±0.60***	11,8 ± 1,6

Biochemical analysis of enzyme content in datura roots showed that content of IAA-oxidase decreases as compared to control with 2- and 8-min exposures by 2,4 and 8,9 times, respectively, and with 30-min exposure it increases by 2,4 times. These data clearly demonstrate stimulation of growth activity of radiated seeds in the first case (2- and 8-min exposures) or its inhibition – in the second case (30-min exposure) and correlate with GP and G of seeds. Sum of freely soluble proteins in rootlets as compared to control also increased with 2- and 8-min exposures (by 9,0 and 50,0 μg/g of crude substance).

Table 3. *Physiological-biochemical and genetic parameters of seeds and seedlings of datura under the influence of MMR*

Parameters	Exposures to MMR, min			
	Control	2	8	30
GP, %	21,3 ± 1,72	32,0 ± 1,31*	30,4 ± 1,00*	25,6 ± 1,01
G, %	29,9 ± 3,42	44,2 ± 1,60*	40,0 ± 1,18***	34,3 ± 1,96
IAA-oxidase, c.u.	0.1756	0.0730	0.0196	0.4151
FSP, μg/g of crude substance	100	109	150	75
ICA, %	7,4 ± 1,35	6,2 ± 1,56	3,8 ± 0,63***	6,0 ± 1,26

Incidence of chromosome aberrations decreased significantly after 8-min exposure and insignificantly- after 2- and 30-min exposures (Table 3). In case of 8-min exposure to MMR, incidence of

chromosome aberrations decreased almost 2-fold. Range of chromosome aberrations is rather narrow, mainly these are single and double bridges and chromosome lagging.

Thus, treatment of old seeds of medicinal plants with millimeter radiation leads to stimulation of primary metabolic processes in seeds and seedlings. Treatment of seeds with MMR after their long-term storage results in decrease of chromosome aberrations that, apparently, promotes faster elimination of cells with chromosome abnormalities, along with the increase of normally dividing cells. Thereby, the ability of millimeter radiation to restore normal state of seeds, viability of which was decreased after their long-term storage, was demonstrated even on the genetic level. During stimulation of growth activity of seedlings the content of IAA-oxidase enzyme decreases, that is confirmed by our data on germination energy and germinability of seeds. With that, activation of protein synthesis occurs in seedling rootlets, that is a very favorable factor for germination of seeds under the conditions of *ex situ* conservation.

CONCLUSIONS:

1. Significant acceleration of growth processes by physiological-biochemical and genetic parameters of seeds and seedlings was found under the influence of millimeter radiation with the wavelength of 5,6 mm, power density 6,6-10,0 mW/cm^2 and exposures of 2-, 8- and 30-min on castor-oil plant, echinacea and datura seeds.
2. Millimeter radiation can be recommended for increasing viability of seeds of medicinal plants after their long-term storage in gene bank.

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