APPLICATION OF MILLIMETER RADIATION TO INCREASE THE VIABILITY OF LENTIL (*LENS CULINARIS* L.) SEEDS DURING *EX SITU* CONSERVATION

APLICAREA RADIAȚIEI MILIMETRICE PENTRU SPORIREA VIABILITĂȚII SEMINȚELOR DE LINTE (*LENS CULINARIS* L.) ÎN TIMPUL CONSERVĂRII *EX SITU*

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Liudmila CORLATEANU
ORCID: 0000-0002-1018-1832
E-mail: liudmila.corlateanu@sti.usm
Moldova State University, Chisinau, Republic of Moldova

Anatolie GANEA
ORCID: 0000-0002-8658-6879
E-mail: anatolie.ganea@sti.usm.md
Moldova State University, Chisinau, Republic of Moldova

Serghei MASLOBROD
ORCID: 0000-0002-2678-7847
Moldova State University, Chisinau, Republic of Moldova

Sumar. *S-a demonstrat că, în condiții de conservare ex situ, expunerea semințelor de linte, ca reprezentantă a culturilor leguminoase, la radiații milimetrice (MMI) cu o lungimea de undă de 5,6 mm, densitatea puterii de 6,6 mW/cm2 și expoziția de 30 de minute are ca rezultat cea mai mare stimulare a proceselor de germinare. Această concluzie a fost obținută pe baza parametrilor morfofiziologici (energia germinativă și germinarea semințelor), biochimici (activitatea enzimei o-AIA, suma proteinelor ușor solubile din rădăcinile semințelor) și genetici (frecvența și spectrul aberațiilor cromozomiale din meristemul rădăcinii) ai semințelor și plantulelor. S-a constatat că stimularea proceselor metabolice primare ale semințelor și plantulelor de linte are loc atunci când se utilizează expuneri mai lungi (30 min) pe semințe decât cele în experimentele asupra semințelor de legume, cereale și culturi industriale (expuneri de 2…8 min). În consecință, prin intermediul radiațiilor milimetrice este posibilă creșterea viabilității semințelor de linte în timpul conservării ex situ, ceea ce este extrem de important și necesar pentru menținerea genofondului vegetal.*

Cuvinte-cheie: *iradierea milimetrică, semințe, lintea, energia germinativă, capacitatea de germinare, enzima o-AIA, suma proteinelor ușor solubile, frecvența aberațiilor cromozomiale,Colecții active, banca de gene, conservarea ex situ, expuneri la iradiere.*

INTRODUCTION

The main objective of plant genetic banks is to provide breeders with high quality

seed material for breeding purposes while developing higher yielding and more resistant varieties and hybrids. There is also an urgent need to conserve plant populations that are on the verge of extinction until they can be reintroduced to their former natural environment [1]. The role of plant genetic resources in the development of sustainable agricultural development, food production and environmental protection is enormous. During *ex situ* conservation, complex physiological and biochemical aging processes occur in seeds, which eventually lead to loss of germination under field conditions. Seed aging has a negative impact on obtaining genetically homogeneous material. Therefore, it is necessary to apply various methods of exogenous influence on old seeds to increase their viability. In recent decades, various chemical and physical methods are often used to improve seed viability during *ex situ* conservation. Weak electromagnetic fields of millimeter range are widely used in medicine, biology and crop production [2, 3]. Millimeter radiation is one of the promising physical factors and, being absorbed in the treated plant, unlike chemical methods of treatment, has a direct effect on the vitality of the plant. Positive is the fact that the treatment of seeds with MMI does not introduce any harmful substances into the plant object or the environment, which is of great importance for agriculture [4].

In recent years, we have studied the possibility of using millimeter radiation on seeds of various taxonomic groups during *ex situ* conservation in the plant genetic bank. As a result of numerous experiments, many different data were obtained confirming the feasibility of using this method to activate the primary metabolic processes of seeds of various agricultural plant species [5, 6, 7, 8, 9]. The application of millimeter radiation on plant objects is more promising and effective compared to other physical factors (strong electric and magnetic fields, high and low temperature).

The aim of this work was to study the possibility of millimeter radiation application on lentil seeds to increase seed viability during *ex situ* conservation in the plant genetic bank.

MATERIALS AND METHODS

The article presents experimental data on the effect of millimeter radiation on seeds of grain legume plants, in particular lentils (*Verzuie* variety), after 9 years of storage in the plant genetic bank in order to increase their viability. Dry lentil seeds were irradiated by MMI with a wavelength of 5.6 mm, power density of 6.6 mW/cm^2 at 3 exposures: 2, 8 and 30 minutes. In each variant of the experiment 300 seeds were irradiated in 3-fold repetition, in the control - seeds without irradiation. After treatment, seeds were germinated in Petri dishes in a thermostat at 25°C. After germination of lentil seeds, the following morphophysiological, biochemical and genetic parameters of seeds and seedlings were studied: germination energy (GE) and germination capacity (GC) of seeds [10], activity of the enzyme o-IAA [11], the sum of readily soluble proteins (SRPP) in seeds and seedling roots [12], and the number of chromosomal aberrations (NCA) in the cells of primary seedling roots [13]. The data were processed using the *Statistica 7* program package.

RESULTS AND DISCUSSION

Significant stimulation of germination energy of lentil seeds was found at 30-min exposure to MMI irradiation (11.5% higher than the control), and significant inhibition of GE - at 2-min exposure (11.7% decrease of the parameter) (fig.). This peculiarity was also manifested when conducting similar experiments on chickpea seeds. It should be noted that stimulation of germination energy on seeds of other plant species was detected only at short MMI exposures (2 and 8 min). A similar pattern was maintained when determining seed germination, which was maximum at 30-min exposure (89.0%), germination exceeded the control by 11.4%. At 30-min stimulation exposure of MMI a lower content of the enzyme o-IAA (0.555u.u.) in seedlings compared to the control (0.435u.u.) was observed, at inhibitory 2-min exposure the opposite dependence was revealed, which corresponds to the known pattern - inverse correlation between these parameters [14].

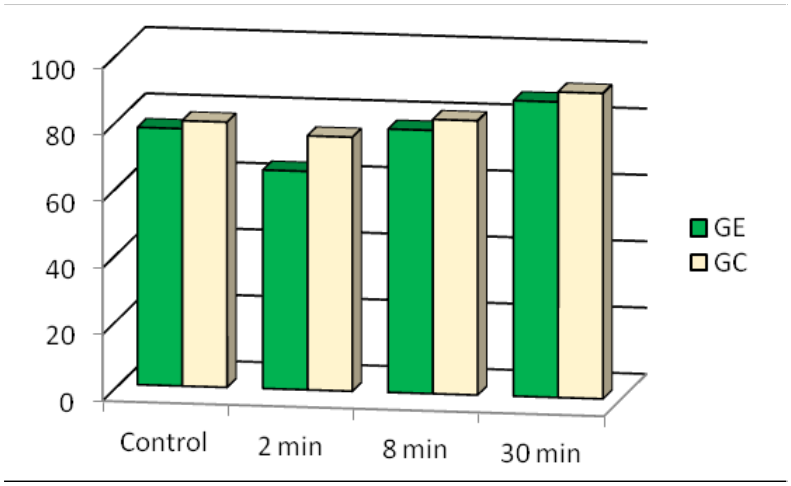


Figure. Germination energy and germination capacity (%) of lentil seeds under millimeter radiation

The obtained data indicate the stimulation of growth activity of lentil seedlings at 30 min MMI exposure and correlate with germination energy and seed germination.

Enhancement of growth activity of lentil seedlings after prolonged seed storage under 30-minute stimulation exposure to millimeter radiation was accompanied by an increase in protein synthesis in both seeds and roots compared to the control. The excess amounted to 150 and 170 μg/g of crude matter, respectively. When 2-minute inhibitory exposure of seeds to MMI irradiation was used, protein synthesis decreased in seeds and roots (by 60 and 35 μg/g of crude matter, respectively) compared to the control (table 1).

Table 1. Some biochemical parameters of lentil seeds and seedlings when seeds are exposed to millimeter radiation

No. n/a	Variants MMI exposures, min	o-IAA, c. u.	SRPP, µg/g crude matter	
			roots	seeds
1	Control	0,555	180	250
2	2	0,910	120	215
3	8	0,670	225	370
4	30	0,435	330	420

After chromosome analysis in the cells of seedling roots as a result of irradiation of lentil seeds after long-term storage with millimeter radiation, a decrease in the number of chromosomal aberrations was detected compared to the control at exposures of 8 and 30 minutes (by 3.5 and 3.2%, respectively, control - 7.5%) (table 2).

It is known that the number of chromosomal abnormalities in seedling root cells increases during long-term seed storage, and with the help of millimeter radiation it is possible to restore the normal genetic state of seeds. Differences in the types of chromosomal aberrations were also found. In stimulation variants using MMI irradiation exposures of 8 and 30 minutes, the number of single and double chromosomal bridges was sharply reduced and triple bridges were completely absent. In the same variants, the number of single chromosome bridges decreased from 14,0 in the control to 6,0 and 7,0 in the experiment, respectively, and double bridges to 4,0 and 3,0, respectively (control – 6,0). Chromosome lagging in the cells of lentil seedling roots in the variant with 30 min exposure to MMI irradiation was observed 2 times less frequently compared to the control.

Table 2. Effect of millimeter radiation on the frequency and spectrum of chromosomal aberrations in the meristem of lentil seedling roots

No. n/a	Variants of exposures MMI, min	NCA, %	Types of aberrations			
			Chromosomal bridges			Lagging chromosomes
			X	XX	XXX	
1	Control	7,5±1,25	14,0	6,0	3,0	6
2	2	6,8±1,19	9,0	8,0	4,0	8
3	8	4,0±0,55*	6,0	4,0	-	4
4	30	4,3±0,65*	7,0	3,0	-	3

X, XX, XXX - single, double and triple bridges, respectively
** - differences are significant compared to control ($p<0.05$)*

Consequently, such a physical factor as millimeter radiation contributes to the strengthening of repair processes in plant cells. In this case, there is a faster elimination of cells with chromosomal abnormalities and an increase in the number of normally di-

viding cells. Thus, the ability of millimeter radiation to restore the normal state of cells, the viability of which decreases after long-term storage of seeds in the genetic bank of plants, was shown at the genetic level.

CONCLUSIONS

1. The effect of millimeter irradiation (wavelength 5.6 mm, power density 6.6 mW/cm^2, exposure 30 minutes) on lentil seeds as a representative of leguminous crops during *ex situ* conservation has a positive stimulating effect on seeds and activates the primary metabolic processes of seeds and seedlings.

2. Based on the studied morphophysiological, biochemical and genetic parameters of seeds and seedlings it is reasonable to propose the use of millimeter radiation to increase the viability of lentil seeds after their long-term storage in the genetic bank of plants.

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