Muzeul Olteniei Craiova. *Oltenia. Studii şi comunicări. Ştiinţele Naturii.* Tom. 30, No. 1/2014 ISSN 1454-6914

RADIOSENSITIVITY OF MAIZE TO GAMMA RADIATION BASED ON PHYSIOLOGICAL RESPONSES

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Abstract. The present work introduces the study of two maize lines dose-dependent radiosensitivity, based on physiological responses, namely germination, plant growth and development and content of photosynthetic pigments. The experimental material is represented by maize hybrid Turda Star and one of the parental lines. Dry seeds were exposed at doses ranging from 2 to 50 Gy. Our results showed that exposure at doses between 2 to 50 Gy resulted in a significant increase of hybrid growth parameters, expressed through the final germination percentage, speed of germination, root and shoot length. Moreover, plants derived from irradiated seeds exhibited an increased content of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) than non-irradiated ones. In parallel with this findings, the seeds belonging to the parental line showed a different pattern, respectively all studied parameters were increased at doses up to 5 Gy, while at higher doses (15-50 Gy) were significant decreased as compared with the control. The present results highlight the superiority of using maize hybrids over the parental forms. Low gamma-irradiation of maize hybrid seeds can be used to produce seedlings with improved traits. Also, it is confirmed the existence of a threshold between positive and negative effects induced by gamma radiation.

Keywords: gamma radiation, germination potential, growth parameters, maize, photosynthetic pigments.

Rezumat. Radiosensibilitatea porumbului la radiaţii gamma pe baza răspunsului fiziologic. În lucrarea de faţă este prezentat studiul privind radiosensibilitatea a două linii de porumb în funcţie de doza de iradiere. Acesta este evaluat pe baya răspunsului fiziologic, respectiv a procesului de germinare, de creştere şi dezvoltare a plantelor şi a conţinutului de pigmenţi asimilatori. Materialul experimental este reprezentat de hibridul de porumb Turda Star şi de una din formele parentale. Seminţele în stare uscată au fost expuse la o sursa de radiaţii gamma, la doze cuprinse între 2 şi 50 Gy. Pe baza rezultatelor obţinute se observă că expunerea seminţelor hibridului Turda Star la doze cuprinse între 2 şi 5 Gy a dus la o creştere semnificativă a parametrilor de creştere, exprimaţi prin procentul final de germinare, viteza de germinare şi lungimea rădăcinii şi tulpinii. Mai mult, plantele derivate din seminţele iradiate au prezentat un conţinut mai ridicat de pigmentţi asimilatori (clorofila, clorofila b, carotenoizi) faţă de cele neiradiate. În paralel cu aceste observaţii, s-a remarcat ca seminţele aparţinând liniei parentale s+au comportat diferit, mai exact toţi parametrii studiaţi au crescut până la doza de 5 Gy, în timp ce la doze mai ridicate (15-50 Gy) acesteti au fost semnificativ reduşi faţă de martor. Rezultatele obţinute evidenţiază superioritatea hibrizilor faţă de formele parentale. Expunerea seminţelor hibridului de porumb la doze joase de radiaţii gamma poate fi utilizată ca o metodă de obţinere a plantelor cu caracteristici îmbunătăţite. De asemenea, se confirmă existenţa unei doze prag între efectele pozitive şi cele negative induse de radiaţiile gamma.

Cuvinte cheie: capacitate germinativă, parametri de creştere, pigmenţi asimilatori, porumb, radiaţii gamma.

INTRODUCTION

Gamma radiation has been extensively used in plant breeding programs. As a mutagenic agent, gamma radiation can induce both beneficial and harmful effects on plants (KUMAR & KUMAR, 2009). In this regard, the hormesis term has been defined as a dose-response phenomenon characterized by a switchover from low dose stimulation to a high dose inhibition (KIM et al., 2004).

In order to evaluate the effects induced by gamma radiation, a number of parameters indicating plant response have been used. The most widely used indicators are those based on physiological changes such as seed germination and plant growth stimulation or inhibition (BORZOUEI et al., 2010). SINGH & DATTA (2010) observed that low doses of gamma radiation improved plant vigour, grain development and yield attributes of wheat. The positive effects induced by low gamma doses on plant growth and development derived from irradiated seeds have been also reported in grapevine (CHARBAJI & NABULSI, 1999) and rocket (MOUSSA, 2006). In the case of exposure at high doses, CHAOMEI & YANLIN **(**1993**)** observed that there is a reduction of the germination capacity with a corresponding decline of plant growth and development at wheat (*Triticum aesticum* L.). Exposure of tabacco (*Nicotiana tabaccum* L.) young plants at doses ranging from 30 to 50 Gy resulted in a significant decrease of the growth process, while at 70 Gy it was completely stropped (CHO et al., 2000). High doses of gamma radiation disturbed the germination process of *Echinacea purpurea* (L.) Moench, leading to a reduction of germination capacity (ICHIM et al., 2006). KIONG et al. (2008) stated that despite the diversity of gamma ray targets in plants, the photosynthetic apparatus is one of the main targets. Studies on mung bean (*Vigna radiata* (L.) R.Wilczek) (SINGH & SHARMA, 1992), alfalfa (*Medicago sativa* L.) (REJILI et al., 2008), rice (*Oryza sativa* L.) (SHEREEN et al., 2009) and okra (*Abelmoschus esculentus* Moench) (HEGAZI & HAMIDELDIN, 2010) revealed that exposure at low doses lead to an increase of the assimilatory pigments content, while at high doses it decreases significantly as compared with non-irradiated ones.

Maize (*Zea mays* L.) is one of the most important crop plants. Due to its high yield, as well as its usage in a variety of human activities, maize is one of the most cultivated species worldwide, ranking the third in cultivated area after rice and wheat, and the first as production (CRISTEA et al., 2004). Beside its economic importance, for over 100 years maize has served as a premiere model organism for biological research (LAWRENCE et al., 2008). Nowadays it is a widely used model organism in many biological investigations such as heterosis, quantitative inheritance, plant domestication, developmental physiology, genome evolution, epigenetics, pest resistance, comparative genomics and plant response to various environmental factors (STRABLE & SCANLON, 1999).

The aim of the present study is to study the dose-dependent response of two maize line following exposure at gamma radiation based on physiological responses, namely germination, plant growth and development and content of photosynthetic pigments.

MATERIALS AND METHODS

Seeds of maize triliniar hybrid Turda Star (TSh) and one of the parental line (PL), obtained from Agricultural Research and Developmental Station Turda, Romania, were used as experimental material. Dry seeds were exposed at a ^{60}Co gamma source (Gamma Chamber 900 at Faculty of Physics, Babeş-Bolyai University) at a dose rate of 5.72 Gy/h. The applied doses were 0 Gy, 2 Gy, 5 Gy, 15 Gy, 30 Gy and 50 Gy.

The irradiated and non-irradiates seeds (40 seeds/dose) were sown on moistened filter paper and then germinated in laboratory conditions (20ºC and 8h photophase). Seeds were considered germinated when the radical extension reached 2 mm in length.

In order to evaluate the effects on the germination process there were calculated two indices, namely the Final germination percentage (FGP) and the speed of germination (SG). They were calculated as described in Table 1.

Table 1. Description of parameters used to study the seed germination.

Germination index	Formula	Description	Reference source
FGP	$FGP = \frac{N_T}{N} * 100$	*N_T: proportion of germinated seeds in each treatment for the final measurement N: Number of seeds used in bioassay*	ANJUM & BAJWA 2005
SG	$GS = (N_1 * 1) + \frac{N_2 - N_1}{2} + \cdots + \frac{N_n - N_{n-1}}{n}$	*N_1, N_2, N_3, N_{n-1}, N_n: proportion of germinated seeds observed on day 1, 2, 3,…, N*	CHIAPUSIO et al (1997)

Measurements of seedlings root and shoot length, both for the control and the irradiated samples, were recorded on the 7th day after the start of the experiment.

Chlorophyll a, chlorophyll b and carotenoids content of fresh leaves was determined according to the MORAN & PORATH (1983) method. Leaves of 17 days old plants were cut into small pieces and suspended in DMF (N,N-dimethylformamide), approximately 100 mg vegetal material/2 ml DMF. The samples were left to incubate for 48h in the dark at 4ºC. The absorbance was measured with a spectrophotometer (UV/VIS SP8001 Metertech Spectrophotometer at Department of Experimental Biology and Biochemistry, Institute of Biological Research, Cluj-Napoca) at 664 nm, 647 nm and 480 nm. Chlorophyll a, chlorophyll b and carotenoids pigments were calculated according to equations of WELLBURN (1994) and expressed in µg/g fresh weight (FW).

The differences in average of all tested parameters between irradiated and non-irradiates samples was realized by subjecting the experimental data to one-analysis of variance (One-way ANOVA) with Dunnett`s post test, at a 5% level of probability. The statistical analysis was performed using GraphPad Prism (version 5.00 for Windows, GraphPad Software, San Diego, USA) and the graphics were realized with Excel program.

RESULTS AND DISCUSSIONS

Seed germination test revealed the dose-dependent response between the two maize lines. As illustrated in Fig. 1, exposure of Turda Star hybrid at 2 Gy lead to a significant increase of the FGP by 8%. At higher doses, it is observed a significant increase by 12% and 14% at 5 Gy, respectively at 15 Gy. As compared with the control, exposure at 30 Gy and 50 Gy resulted in a highly significant increase of the percentage of germinated seeds by 17% and 20%. Unlike these results, the parental line showed a different pattern. It can be noticed that at 2 Gy and 5 Gy the FGP is increased by 2% and 4%, which are no statistically significant. At doses > 5Gy, respectively at 15 Gy, 30 Gy and 50 Gy the final germination percentage was significant decreased by 8%, 12% and 16% than the control.

The speed of the germination process for TSh showed a significant increase with increasing the irradiation dose (Fig.2). Thus, comparing with the control, exposure at 2 Gy, 5 Gy, 15 Gy and 30 Gy resulted in the increase of SG by 135, 17%, 24%, respectively 29%. The highest acceleration of the germination process, by 35%, was recorded at 50 Gy. In seeds belonging to the PL there was observed an insignificant increase by 5 and 7% at 2 Gy and 5Gy. Reported at non-irradiated samples, the seeds exposed at doses ≥ 15 Gy showed a statistically significant delay in the germination process by 13-20%.

Gamma radiation had a positive significant effect on TSh root length (Table 2). Following exposure at doses ranging from 2 to 50 Gy, it was observed an increase of root length by 24-99% as compared with the control. Regarding shoot length it can be seen that at 2 Gy there was an insignificant increase by 3%, while plants derived from seeds exposed at higher doses (5-50 Gy) showed a significant increase of shoot length. Exposure of PL seeds at 2 Gy and 5 Gy resulted in an increase of root (9 and respectively 17%) and shoot (5 and 9%) length, which is not significant in statistical terms. At 15 Gy, plants showed a slightly decrease, by 21% of root length and a significant reduction of shoot length by 26%. Higher doses of gamma radiation, respectively 30 Gy and 50 Gy, significantly affected plant growth and development, the root length was decreased by 38 and respectively 61% and shoot length by 31 and 42%.

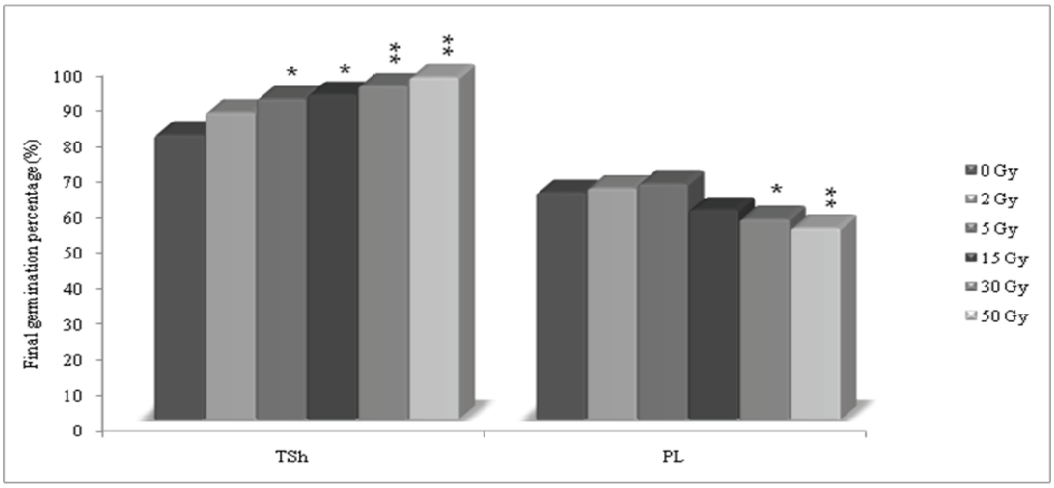

Figure 1. Effect of gamma radiation on final germination percentage

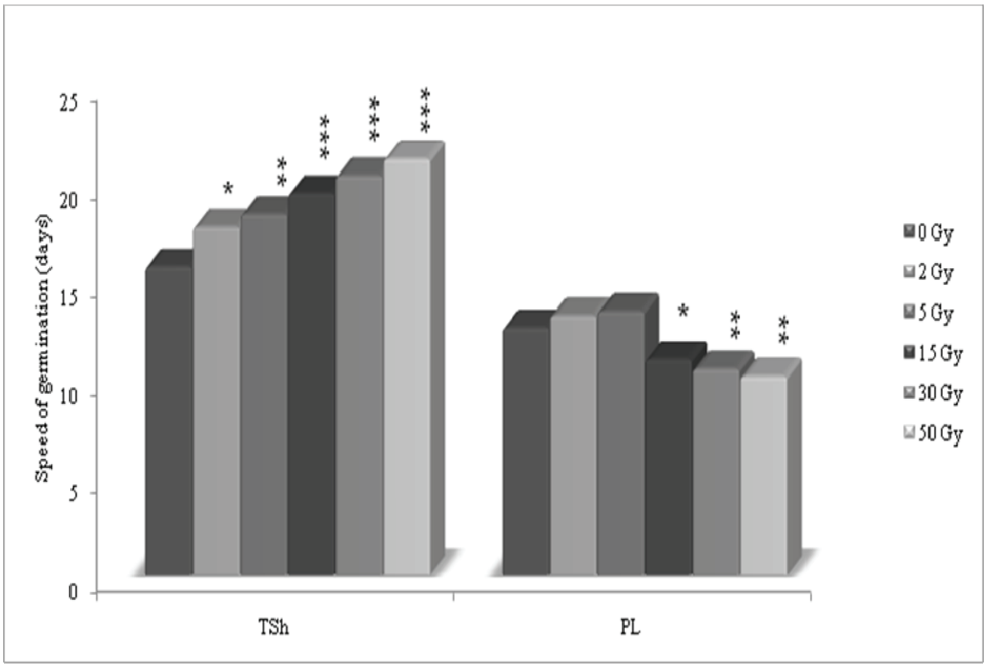

Figure 2. Effect of gamma radiation on maize speed of germination.

Table 2. Effects of gamma radiation on root and shoot length (cm).

	Turda Star Hybrid		**Parental line**	
Gamma radiation treatment (Gy)	*Root length (cm)*	*Shoot length (cm)*	*Root length (cm)*	*Shoot length (cm)*
Control (0)	1,5	0,6	0,9	0,55
2	1,8*	1,2	0,98	0,52
5	1,9**	1,5**	1,06	0,58
15	2,5***	1,8**	0,7	0,39*
30	2,8***	1,9***	0,5*	0,39*
50	2,9***	2,2***	0,3**	0,35**

The content of the assimilatory pigments in 17-days old TSh leaves is presented in Fig. 3. The statistical analysis revealed that comparing with the control the content of chlorophyll a significantly increased in plants derived from irradiated seeds, reaching the maximum increase, by 44%, at 50 Gy. Chlorophyll b, as well as the carotenoids showed a significant increase up by 105%, respectively 101%, in irradiated samples, as compared with the control.

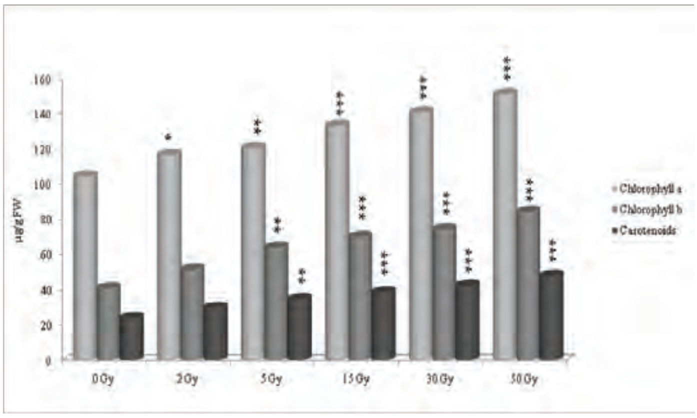

Figure 3. Effects of gamma radiation on assimilatory pigments in maize Turda Star hybrid leaves.

The analysis of the assimilatory pigments content in PL plants (Fig. 4), highlights the positive effects of doses ≤ 5 Gy, where it was recorded the maximum increase by 14% of chlorophyll a, by 38% of chlorophyll b and by 31% of carotenoids. Comparing with the control, doses between 15 and 50 Gy induced a decrease of assimilatory pigments, the minimum values being observed at 50 Gy where leaves showed a reduced content of chlorophyll a, chlorophyll b and carotenoids by 14%, 35%, respectively 32%.

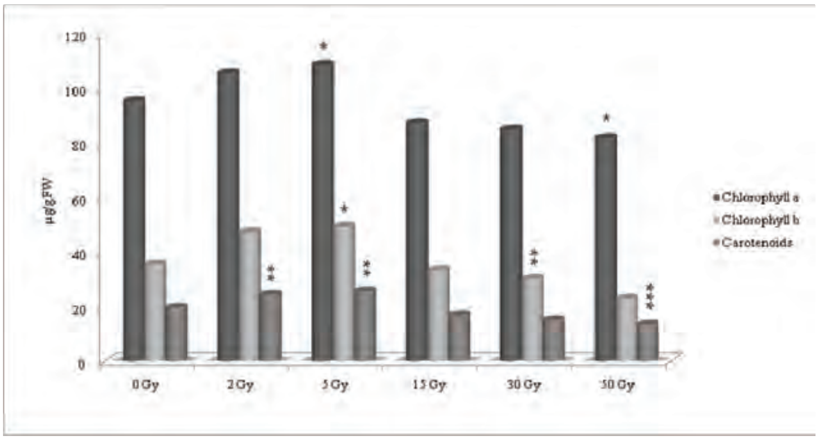

Figure 4. Effects of gamma radiation on assimilatory pigments content in maize Parental Line leaves.

Plant radiosensitivity is dependent upon several factors, some related to plant characteristics (e.g. species/cultivar/variety, plant age, physiology, tissue architecture, morphology and genome organization) and some related to radiation features (e.g. type of radiation, dose rate and time of exposure) (DE MICCO et al., 2011; JAN et al., 2012).

The results obtained in this study highlight the superiority of a maize hybrid unlike the parental form. This phenomenon is called heterosis and it can be defined as the increase in size or growth rate of hybrids over parental line, manifested in quantitative characters and expressed as increased biomass, growth rate, fertility, resistance to diseases and insects as well as tolerance to abiotic factors (CRISTEA et al., 2004).

The stimulating effects induced by gamma radiation on plant growth and development may be attributed to an increase of plant growth regulators (auxins and gibberellins) levels, which enhances the mitotic activity in the meristematic tissues by increasing the number of dividing cells (LATIF et al., 2011). ABDEL-HADY et al. (2008) stated that activation of RNA or protein synthesis during the early stage of germination in irradiated seeds may enhance the germination process. Along with the stimulation of cell division and enzymatic activity, there is an increase of mineral and water uptake, which can explain the increase of assimilatory pigments in plants derive from seeds exposed at low doses (MAJEED et al., 2010).

Gamma radiation exerts its negative effects through the increase amount of free radicals resulted from water radiolysis, which damage important cellular components and perturb vital processes (FAN & SOKORAI, 2005). Reactive oxygen species are believed to be a major contributing factor to stress injuries and cause rapid cellular damage because they are highly reactive to membrane lipids, protein and DNA (EL-BELTAGI et al., 2011). CHATURVEDI et al. (2012) stated that increased radiation doses enhanced the membrane permeability resulting in higher loss of leachates and reduced germination percentage. PREUSS & BITT (2003) attributed the decreased shoot and root length at higher doses of gamma rays to a reduced mitotic activity in meristematic tissues due to cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome. Photosynthesis, an important marker of internal plant metabolism, together with cell growth, is among the primary processes that are in general, affected by gamma radiation (SINGH et al., 2013). According to STRID et al (1990) the photosynthetic pigments can be destroyed by gamma rays with concomitant loss of photosynthetic capacity. BYUN et al. (2002) explained this by gamma radiation ability to break chlorophyll molecules apart.

CONCLUSIONS

Gamma radiation exposure of maize Turda Star triliniar hybrid at doses ranging from 2-50 Gy resulted in a increase of 8-20% of the final germination percentage, 13-35% of the speed of germination, 24-99% of the root length and 3-38% of shoot length. As compared with the control, the content of chlorophyll a increased by 11-44%, chlorophyll b by 25-105% and carotenoids 25-101. Seeds of the Parental Line submitted to exposed at doses up to 5 Gy showed an increase of the final germination percentage by 4%, of the speed of germination by 6.6%, root length by 18%, shoot length by 9.3% and the content of chlorophyll a increased by 14%, chlorophyll b by 39% and carotenoids by 31% as compared with the control. At higher doses all studied parameters were decreased, the lowest values being recorded at 50 Gy where FGP decreased by 16%, the GS by 19%, root and shoot length by 61%, respectively by 42%, chlorophyll a by 13%, chlorophyll b by 35% and carotenoids by 31%. The present results highlight the superiority o maize hybrid over the parental forms. Concerning the irradiation dose, our results confirm the conclusions of previous studies regarding the hormetic effect induced by gamma radiation. Low gamma-irradiation of maize hybrid seeds can be used to produce seedlings with improved traits.

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Received: March 29, 2014
Accepted: June 18, 2014