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Determination of the optimal doses of gamma irradiation for induced mutation in wheat and barley

Svetlana Glogovac* · Dragana Trkulja · Ankica Kondić-Špika · Milan Mirosavljević · Bojan Jocković · Ljiljana Brbaklić · Dragana Miladinović

Institute of Field and Vegetable Crops, Novi Sad, Serbia

*Corresponding author: svetlana.glogovac@ifvcns.ns.ac.rs

Summary: One of the major challenges that plant breeders face in the 21st century is food safety for growing human population coupled with extreme climate changes. Accordingly, the most important breeders' goal is to find appropriate methods in response to these challenges in order to create high-yielding varieties resilient to abiotic and biotic stressors. The aim of this study was to determine optimal doses of gamma irradiation in two wheat and one barley varieties and to apply the identified doses for development of mutant populations. Wheat and barley varieties showed different reactions to applied doses of gamma irradiation. Wheat varieties had germination rate over 90% at all applied doses while barley seeds showed to be more susceptible to gamma irradiation. Gamma irradiation had greater influence on seedling height which was clearly demonstrated by growth reduction with increasing radiation doses. ANOVA showed a significant difference between genotypes, applied doses of gamma radiation as well as genotype by doses interaction for seedling height. At highest dose of 600 Gy, the reduction of seedling height was 94.6%, 96.5% and 96.8% in Simonida, Rudnik and NS 40S, respectively. The irradiation doses that resulted in seedling growth reduction by 50% (GR50) were 210 Gy for barley Rudnik and wheat NS 40S, and 310 Gy for wheat variety Simonida. Identified doses were used for the irradiation of 2000 seeds of each variety in order to produce mutant populations that will be further used in a breeding program for development of varieties with increased resilience to climate change.

Keywords: barley, gamma irradiation, *Hordeum vulgare*, irradiation doses, mutant lines, physical mutagenesis, *Triticum aestivum*, wheat

Introduction

Throughout the history of breeding one of the goals that has remained constant is the development of high yielding varieties, which in the light of the growing human population and food safety issues is gaining much more importance. Meeting the growing needs for food is further hampered by the negative impact of climate change accompanied by extreme weather conditions and the reduction of available arable agricultural land (Kumar et al., 2022). Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are some of the most important cereals for human and animal nutrition worldwide. Wheat is among the most frequently produced crops in the world representing the main source of nutrients for around 40% of the world's population (Javid, 2022), while barley is mainly used for animal feed and for the production of alcoholic beverages. Productivity of these crops is increasingly threatened by global climate changes. The major yield losses are caused primarily by heat and drought (Lesk et al., 2016; Liu et al., 2016; Abhinandan et al., 2018). According to the climate change prediction models, it is estimated that wheat production losses can reach 42 Mt per °C rise in temperature (Abhinandan et al., 2018). In this global climate change scenario varieties with increased tolerance to abiotic stress are needed. Increasing stress resilience by conventional breeding is restricted by complex nature of abiotic stress tolerance and variability in plants sensitivity during their life cycle (Esmaeili et al., 2022). Finding solutions to overcome these challenges through different breeding approaches is of global importance. New genetic variations, which are of crucial importance for improvement of yield related and stress tolerance traits in breeding programs, except spontaneous occurrence, can be created also in mutation breeding by using induced mutagens.

The application of radiation in development of new plant varieties followed shortly after the first published results about radiation-induced mutations in barley (Stadler, 1928a) and maize (Stadler, 1928b), preceded by the Muller's discovery that exposure to X-rays can cause genetic mutations (Muller, 1928). Mutations can be induced by biological, chemical or physical agents. Physical mutagens, primarily gamma and X-rays are far more prevalent in plant mutation breeding than chemical because of lower costs, ease of use and non-toxicity (Ulukapi & Nasircilar, 2019). Although mutation breeding is not so common compared to other breeding methods, it could be promising for creation of varieties conferring enhanced tolerance to drought stress. The development of new crop varieties by DNA-altering techniques, especially New Genomic Techniques (NGT) that have developed rapidly in the past two decades, are the subject of numerous debates primarily for safety issues and are therefore subject to specific legislation.

Although induced mutations result in changes at the DNA level, they are not subject to NGT regulation. According to the Directive 2001/18 and Court of Justice of the European Union in July 2018 organisms obtained through conventional induced random mutagenesis (chemical or radiation) are excepted from the provisions of the Directive (Article 3 *iuncto* Annex IB) (Van Der Meer et al., 2023). It is stated that crops created by these techniques have a long history of safe utilization and do not fall into the category of organisms obtained by the new mutagenesis techniques. Based on Mutant Variety Database (MVD) (https://nucleus.iaea.org/sites/mvd) 3402 mutant varieties have been registered so far and leading regions in plant mutation breeding are Asia and Europe. The largest number of registered mutant varieties is in rice (873), and a significant number have also been registered in barley (307) and wheat (265). From varieties created by physical mutagens that were registered at this database, 63% was developed by using gamma rays (Hase et al., 2020). More information regarding mutant varieties developed so far, including name, traits that were improved by induced mutation, type of mutagens used etc., can be found at MVD database.

Different studies for testing the existing varieties to abiotic stresses have been performed at the Institute of Field and Vegetable Crops (IFVCNS), Novi Sad, Serbia. However, breeding program for active development of drought tolerant varieties does not exist. The aim of this study was to determine the optimal doses of gamma irradiation for mutation induction in two wheat and one barley variety, and to apply the identified doses in order to produce mutant populations that will be further used for selection and creation of new varieties more adapted to climate changes.

Materials and methods

As part of the activities of the project RER/5/024 "Enhancing productivity and resilience to climate change of major food crops in Europe and Central Asia" seeds of two winter wheat varieties (NS 40S and Simonida) and one winter barley variety (Rudnik) were sent to the International Atomic Energy Agency (IAEA) for irradiation treatments. The selected wheat and barley varieties are commercially used high-yielding genotypes in the production of Serbia. Beside many positive traits they already possess, their further improvement related to the better climate resilience is needed. Because of that, they were chosen for the production of mutant populations which will be further evaluated for potentially improved mutant lines with better climate adaptation characteristics. The radio-sensitivity test was carried out according to the FAO/IAEA Manual on Mutation Breeding (Spencer-Lopes et al., 2018). Dried seeds of all genotypes were exposed to 75, 150, 300, 450 and 600 Gy gamma irradiation. After induced mutation performed by IAEA, the obtained irradiated seeds were used in the experiment conducted in a greenhouse at IFVCNS, Serbia. The treatments and non-treated control were sown in trays filled with soil in rows with 20 seeds each. Three replicates were performed per assay, one tray per replicate. Plants were cultivated under natural daylight conditions, maintaining a temperature of approximately 20°C (± 2°C). The irrigation schedule involved watering the plants once or twice a week. After two weeks of cultivation, germination rate and seedling height were evaluated.

Analysis of Variance (ANOVA) was performed for seedling height in statistical software StatSoft, Inc. (2013) STATISTICA v. 12. Growth Reduction Value 50 (GR50) was determined for each genotype as mutagen dose that leads to seedling growth reduction by 50% (Riviello-Flores et al., 2022). The determined GR50 values were used for irradiation of larger seed quantities and production of mutant populations for the selected wheat and barley varieties.

Results and discussion

The results showed different reactions of treated wheat and barley seeds to applied doses of gamma irradiation, which is demonstrated by differences in germination percentage (Fig. 1). Wheat varieties showed germination above 90% at all applied doses of irradiation.

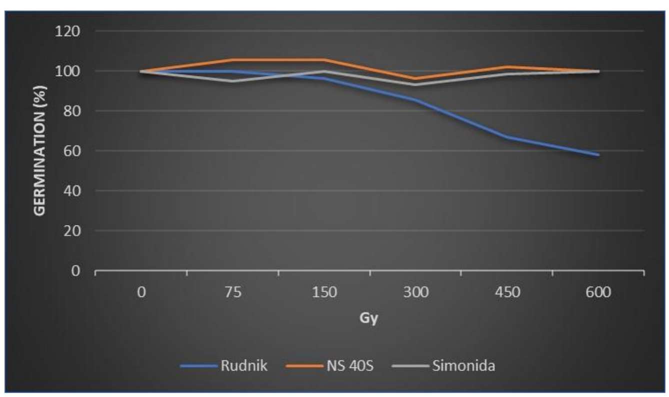

Figure 1. Germination percentage of wheat and barley varieties at different doses of gamma irradiation

These results are in accordance with Borzouei et al. (2010) and Melki & Marouani (2009). In their studies, there was no significant difference in germination of wheat seeds exposed to gamma irradiation and control. Seeds of barley variety Rudnik were more susceptible to gamma irradiation than wheat varieties which is in accordance with the findings of Franco et al. (2015) and Mohamed et al. (2021) that the effect of gamma radiation and degree of sensitivity depends on the species, variety and developmental stage of the embryo at the moment of radiation. Germination rate of barley seeds at 300, 450 and 600 Gy was reduced by 14.2, 33.2 and 42.1%, respectively, compared to control. Rozman (2014) also pointed out that barley seeds treated by the highest dose of irradiation (400 Gy) had significantly lower percentage of germination compared to lower doses (100 Gy and 200 Gy) and to control. A negative effect of higher doses or concentrations of mutagens on germination percentage in rice was determined by Gowthami et al. (2017) citing disruptions at the cellular level (physiological or physical level) and chromosomal damage as possible causes. A similar finding was also made by Naibaho et al. (2021), who stated that the highest level of rice germination occurred at radiation doses of 100-400 Gy and the lowest at a dose of 700 Gy, while there was no germination at doses of 800 Gy and above. The results showed that gamma irradiation had greater impact on seedling growth compared to germination process. Seedling height decreased with increasing irradiation doses (Tab. 1).

Table 1. Average seedling height, percentage of height over control (C) and percentage of height reduction (R) for three analysed varieties under different doses of radiation

Variety	Seedling height	Irradiation doses					
		0	75 Gy	150 Gy	300 Gy	450 Gy	600 Gy
Rudnik	Average (mm)	198.2	198.7	129.9	67.6	27.1	7.0
	% C	100.0	100.3	65.5	34.1	13.7	3.5
	% R	0.0	-0.3	34.5	65.9	86.3	96.5
NS 40S	Average (mm)	216.8	215.9	160.0	41.9	8.8	6.9
	% C	100.0	99.6	73.8	19.3	4.1	3.2
	% R	0.0	0.4	26.2	80.7	95.9	96.8
Simonida	Average (mm)	215.6	215.8	198.7	128.8	22.2	11.6
	% C	100	99.9	92.0	59.6	10.3	5.4
	% R	0.0	0.1	8.0	40.4	89.7	94.6

The decrease in the seedling growth with increasing radiation doses was clearly observed (Fig. 2). Reduction of seedling height at the highest dose of 600 Gy was 94.6%, 96.5% and 96.8% in Simonida, Rudnik and NS 40S, respectively. Growth reduction with increasing radiation dose was also determined in research by other authors on wheat and other plant species. Kiani (2022) showed reduction of wheat seedling length and fresh and dry weight with increasing gamma irradiation doses with reduction by 30% at the dose of 200-300 Gy. This reduction of growth could be explained by the effects of irradiation on the signalling pathway of growth regulatory factors and cell oxidation at the highest irradiation doses (Wi et al., 2007). Naibaho et al. (2021) also stated that plant height decreased with higher irradiation doses with reduction up to 46.51% at 600 Gy.

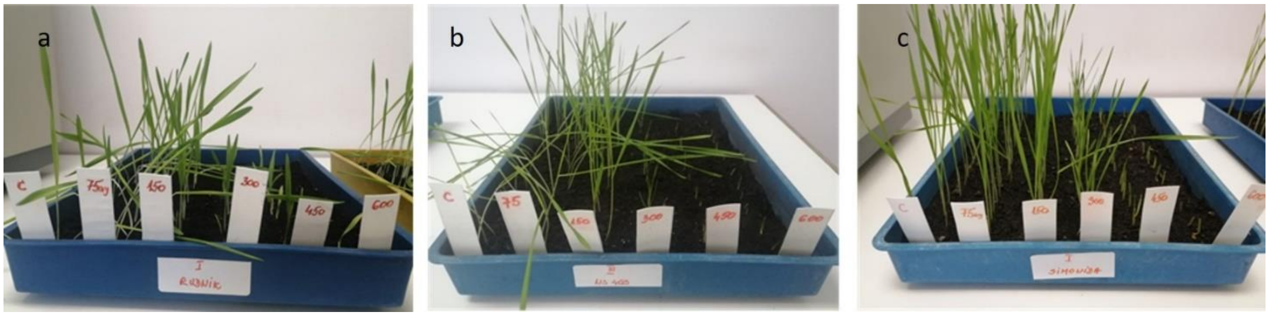

Figure 2. Seedling growth at different gamma irradiation doses: a) barley variety Rudnik; b) wheat variety NS 40S; c) wheat variety Simonida

The efficiency of mutation breeding will be higher if the optimal dose of radiation is determined, which will lead to an increase mutation frequency and will not significantly threaten the survival and reproduction of plants (Hong et al., 2022). A significant difference between genotypes, applied doses of gamma radiation as well as genotype by dose interaction was determined (Tab. 2). On the other hand, there is no significant difference in seedling height due to replication, the interaction genotype by replication, and the interaction between replication and applied doses. The sum of square of dose accounted for the highest percentage of the total sums of squares indicating that dose had the most significant impact on seedling height variation. Also, a greater sum of squares for genotype by dose interaction compared to sum of squares for genotype showed differences in the sensitivity of varieties to different doses of radiation.

Table 2. ANOVA for seedling height of three analysed genotypes and five different doses of gamma irradiation

Source of variation	SS	df	MS	F	p
Genotype	7954.4	2	3977.22	35.7551	0.000000
Replication	188.6	2	94.29	0.8477	0.443230
Dose	379376.1	5	75875.21	682.1172	0.000000
Genotype × replication	516.5	4	129.13	1.1609	0.357528
Genotype × dose	12961.4	10	1296.14	11.6523	0.000003
Replication × dose	576.3	10	57.63	0.5181	0.857474
Error	2224.7	20	111.23		

SS – sum of squares; df- degrees of freedom; MS- means squares; F- ratio; p - probability

Radiosensitivity curve is constructed to determine the most effective mutagen dose which reduces plant height for 50 percent (GR50). For varieties Rudnik and NS 40S the dose of 210 Gy was identified as GR50, while 310 Gy was determined for Simonida (Fig. 3).

This result is in agreement with Dwinanda et al. (2020), who exposed wheat seeds to doses of 100 to 1000 Gy, and found that the effective dose of irradiation for creating high genetic variability with minimal physical damage was from 200 to 400 Gy.

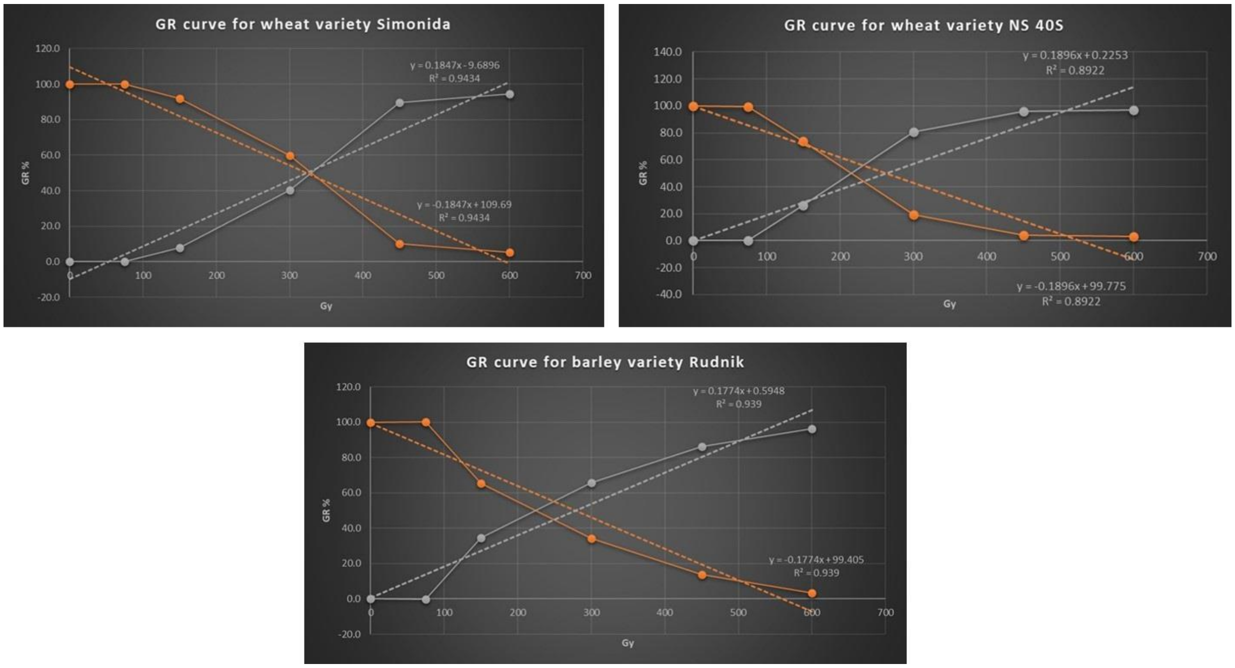

Figure 3. Radiosensitivity curve of seedling height for two wheat and one barley variety

Conclusions

Results of the study showed that gamma irradiation had negative effect on seed germination and growth in wheat and barley varieties with different reactions of varieties to applied doses. For each variety, GR50 values were identified and used for production of mutant lines. The obtained populations will be used in wheat and barley breeding programs for creating varieties with increased resilience to drought stress caused by ongoing climate change.

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References

Abhinandan, K., Skori, L., Stanic, M., Hickerson, N. M. N, Jamshed, M., & Samuel, M. A. (2018). Abiotic Stress Signaling in Wheat – An Inclusive Overview of Hormonal Interactions During Abiotic Stress Responses in Wheat. *Frontiers in Plant Science, 9*, 734. https://doi.10.3389/fpls.2018.00734

Borzouei, A., Kafi, M., Khazaei, H., Naseriyan, B., & Majdabadi, A. (2010). Effects of gamma radiation on germination and physiological aspects of wheat (*Triticum aestivum* L.) seedlings. *Pakistan Journal of Botany, 42*(4), 2281-2290.

Dwinanda, P., Syukur, S., & Suliansyah, I. (2020). Induction of mutations with gamma ray radiation to improve the characteristics of wheat (*Triticum aestivum* L.) genotype IS-Jarissa. IOP Conf. Series. *Earth and Environmental*

Science, 497, 012013. https://doi.org/10.1088/1755-1315/497/1/012013

Esmaeili, N., Shen, G., & Zhang, H. (2022). Genetic manipulation for abiotic stress resistance traits in crops. *Frontiers in Plant Science, 13*, 1011985. https://doi.10.3389/fpls.2022.1011985

Franco, J. G., Franco, S. S. H., Franco, C. H., Arthur, V., Arthur, P. B., & Villavicencio, A. L. (2015). Effects of Gamma Radiation in Soybean. *International Nuclear Atlantic Conference - INAC* 2015, São Paulo, S.P., Brazil, 4-9 October 2015

Gowthami, R., Vanniarajan, C., Souframanien, J., & Pillai, M.A. (2107). Comparison of radiosensitivity of two rice (*Oryza sativa* L.) varieties to gamma rays and electron beam in M1 generation. *Electronic Journal of Plant Breeding, 8*(3), 732-741. https://doi.org/10.5958/0975-928X.2017.00111.9

Hase, Y., Satoh, K., Seito, H., & Oono, Y. (2020). Genetic Consequences of Acute/Chronic Gamma and Carbon Ion Irradiation of Arabidopsis thaliana. *Frontiers in Plant Science, 11*, 336. https://doi.10.3389/fpls.2020.00336

Hong, M. J., Kim, D. Y., Jo, Y. D., Choi, H. I., Ahn, J.W., Kwon, S. J., Kim, S. H., Seo, Y. W., & Kim, J. B. (2022). Biological Effect of Gamma Rays According to Exposure Time on Germination and Plant Growth in Wheat. *Applied Sciences (Switzerland)*, *12*(6), 3208. https://doi.org/10.3390/app12063208

Javid I. M., Shams, N., & Fatima, K. (2022). Nutritional Quality of Wheat. In M. R. Ansari (Ed.), *Wheat - Recent Advances.* IntechOpen. https://doi.org/10.5772/intechopen.104659

Kiani, D., Borzouei, A., Ramezanpour, S., Soltanloo, H., & Saadati, S. (2022). Application of gamma irradiation on morphological, biochemical, and molecular aspects of wheat (*Triticum aestivum* L.) under different seed moisture contents. *Scientific Reports, 12*, 11082. https://doi.org/10.1038/s41598-022-14949-6

Kumar, L., Chhogyel, N., Gopalakrishnan, T., Hasan, M. K., Jayasinghe, S. L., Kariyawasam, C. S., Kogo, B. K., & Ratnayake, S. (2022). Climate change and future of agri-food production. In Rajeev Bhat (Ed.), *Future Foods*, 49-79. https://doi.org/10.1016/B978-0-323-91001-9.00009-8

Lesk, C., Rowhani, P., & Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. *Nature*, *529* (7584), 84-87. https://doi.org/10.1038/nature16467

Liu, B., Asseng, S., Müller, C., Ewert, F., Elliott J, et al. (2016). Similar estimates of temperature impacts on global wheat yield by three independent methods. *Nature Climate Change, 6*(12), 1130-1136. https://doi.org/10.1038/nclimate3115

Melki, M., & Marouani, A. (2009). Effects of gamma rays irradiation on seed germination and growth of hard wheat. *Environmental Chemistry Letters, 8,* 307-310. https://doi.10.1007/s10311-009-0222-1

Mohamed, E. A., Osama, E., Manal, E., Samah, A., Salah, G., Hazem, K. M., Jacek, W., & Nabil, E. (2021). Impact of gamma irradiation pretreatment on biochemical and molecular responses of potato growing under salt stress. *Chemical and Biological Technologies in Agriculture, 8*, 35. https://doi.org/10.1186/s40538-021-00233-8

Muller, H.J. (1928). The measurement of gene mutation rate in *Drosophila*, its high variability, and its dependence upon temperature. *Genetics, 13*(4), 279–357. https://doi.10.1093/genetics/13.4.279

Naibaho, D., Purba, E., Hanafiah, D. S., & Hasibuan, S. (2021). Radiosensitivity and effect of gamma ray irradiation on upland rice CV. Sidikalang IOP Conf. Series. *Earth and Environmental Science*, *782*, 032068. https://doi.org/10.1088/1755-1315/782/3/032068

Riviello-Flores, M. L., Cadena-Iñiguez, J., Ruiz-Posadas, L. M., Arévalo-Galarza, M. L., Castillo-Juárez, I., Soto H,, M., & Castillo-Martínez, C. R. (2022). Use of Gamma Radiation for the Genetic Improvement of Underutilized Plant Varieties. *Plants, 11,* 1161. https://doi.org/10.3390/plants11091161

Rozman, L. (2014). The effect of gamma radiation on seed germination of barley (*Hordeum vulgare* L.) *Acta agriculturae Slovenica*, *103*(2), 307-311. https://doi.org/10.14720/aas.2014.103.2.15

Spencer-Lopes, M. M., Forster, B. P., & Jankuloski, L. (2018). Manual on Mutation Breeding (3rd ed.). Food and Agriculture Organization of the United Nations (FAO).

Stadler, L.J. (1928a). Mutations in Barley induced by x-rays and radium. *Science, 68*, 186-187. https://doi.10.1126/science.68.1756.186

Stadler, L.J. (1928b). Genetic effects of x-rays in maize. *Proceedings of the National Academy of Sciences, 14*, 69-75. https://doi.10.1073/pnas.14.1.69

Ulukapi, K., & Nasircilar, G. A. (2019). Induced Mutation. Creating Genetic Diversity in Plants. In Mohamed A. El-Esawi (Ed.), *Genetic Diversity in Plant Species - Characterization and Conservation.* IntechOpen. https://doi.10.5772/intechopen.81296

Van Der Meer, P., Angenon, G., Bergmans, H., Buhk, H. J., Callebaut, S., Chamon, M., Eriksson, D., Gheysen, G., Harwood, W., Hundleby, P., Kearns, P., Mcloughlin, T., & Zimny, T. (2023). The Status under EU Law of Organisms Developed through Novel Genomic Techniques. *European Journal of Risk Regulation, 14*(1), 93-112. https://doi.10.1017/err.2020.105

Wi, S. G., Chung, B. Y., Kim, J. S., Kim, J. H., Baek, M. H., Lee, J. W., & Kim, Y. S. (2007). Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron, 38*(6), 553-64. https://doi.org/10.1016/j.micron.2006.11.002

Određivanje optimalnih doza gama zračenja kod indukovanih mutacija pšenice i ječma

Svetlana Glogovac · Dragana Trkulja · Ankica Kondić-Špika · Milan Mirosavljević · Bojan Jocković · Ljiljana Brbaklić · Dragana Miladinović

Sažetak: Jedan od najvećih izazova za oplemenjivače u 21. veku je da se osigura dovoljna količina hrane za rastuću ljudsku populaciju u ekstremnim vremenskim uslovima izazvanim klimatskim promenama. Shodno tome, najvažniji cilj za proces oplemenjivanja je pronalaženje odgovarajućih metoda za stvaranje visokoprinosnih sorti tolerantnih na abiotičke i biotičke faktore stresa. Cilj ovog istraživanja bio je da se utvrde optimalne doze gama zračenja za dve sorte pšenice i jednu sortu ječma i da se identifikovane doze primene za razvoj mutantnih populacija. Sorte pšenice i ječma su ispoljile različite reakcije na primenjene doze gama zračenja. Sorte pšenice su imale klijavost preko 90% pri svim primenjenim dozama, dok je za ječam utvrđena veća osetljivost na gama zračenje. Gama zračenje je imalo veći uticaj na visinu klijanaca, pri čemu je redukcija rasta bila izraženija sa povećanjem doze zračenja. Analiza varijanse (ANOVA) je pokazala značajnu razliku u visini klijanaca između genotipova, primenjenih doza gama zračenja kao i interakcije genotip × doza. Pri najvećoj dozi od 600 Gy smanjenje visine klijanaca iznosilo je 94,6%, 96,5% i 96,8% kod Simonide, Rudnika i NS 40S, redom. Doze zračenja pri kojima je redukcija rasta klijanaca bila 50% (GR50) iznosile su 210 Gy za sorte Rudnik i NS 40S dok je za sortu pšenice Simonida bila 310 Gy. Identifikovanim dozama je zračeno 2000 semena svake sorte za proizvodnju mutantnih populacija koje bi se u budućim programima oplemenjivanja mogle koristiti za stvaranje sorti tolerantnih na izmenjene klimatske uslove.

Ključne reči: doze zračenja, fizička mutageneza, gama zračenje, *Hordeum vulgare*, ječam, mutantne linije, pšenica, *Triticum aestivum*