COBISS Code 1.01

DOI: 10.14720/aas.2014.103.2.15

Agrovoc descriptors: hordeum vulgare,barley,varieties,gamma radiation,germinability,seeds,temperature,greenhouses,induced mutation,genetics

Agris category code: f30,f62

The effect of gamma radiation on seed germination of barley (*Hordeum vulgare* L.)

Ludvik ROZMAN[1]

Received September 12, 2014; accepted September 24, 2014.
Delo je prispelo 12. septembra 2014, sprejeto 24. septembra 2014.

ABSTRACT

The aim of the study was to determine the effect of gamma radiation on barley (Hordeum vulgare L.) seed germination and changes of seed viability associated with aging. The seed samples of the variety 'Astor' were irradiated at the Jožef Štefan Institute, in the Reactor Centre at Podgorica near Ljubljana, in 2006. The samples were irradiated with three different doses: 0.1, 0.2 and 0.4 kGy. After the irradiation, the seeds were stored in a refrigerator at 4 °C. The percentage of seed germination was tested each year after irradiation until 2014, except in the second and the third year. The experiments were based on the Latin square design with 4 replications of each treatment, and were conducted in a greenhouse under controlled temperature at 20 °C. In all years, the samples irradiated with the highest dose (0.4 kGy) exhibited a significantly lower percentage of germination. The germination rate of the samples irradiated with 0.2 and 0.4 kGy decreased significantly with aging, when compared to the control and the samples irradiated with 0.1 kGy. In all years of testing, the percentage of germination of seeds irradiated with 0.1 kGy did not differ from the control, and in the fifth year after irradiation, it was even significantly higher than the control.

Key words: gamma radiation, *Hordeum vulgare* L., seed germination, induced mutations

IZVLEČEK

VPLIV OBSEVANJA Z GAMA ŽARKI NA KALIVOST JEČMENA (*Hordeum vulgare* L.)

Cilj raziskave je bil ugotoviti vpliv obsevanja zrnja ječmena z gama žarki na kalivost ter na spremembo kalivosti s staranjem zrnja po obsevanju. Vzorci zrnja ječmena sorte 'Astor' so bili obsevani na Inštitutu Jožef Stefan, Ljubljana v Reaktorskem centru Podgorica v letu 2006. Vzorci so bili obsevani s tremi različnimi dozami: 0,1, 0,2 in 0,4 kGy ter po obsevanju hranjeni v hladilni omari pri temperaturi 4 °C. Po obsevanju smo vsako leto, razen v 2. in 3. letu, do l. 2014, v rastlinjaku preizkušali kalivost obsevanih vzorcev. Poskus je bil postavljen po metodiki latinskega kvadrata v 4 ponovitvah, poleg treh različnih doz obsevanja še kontrola z neobsevanim zrnjem. Vzorec z največjo dozo obsevanja (0,4 kGy) je v vseh letih preizkušanja kalivosti imel statistično značilno najmanjšo kalivost. Pri dozi obsevanja 0,2 in 0,4 kGy se je kalivost s staranjem zrnja statistično značilno zmanjšala glede na kontrolo in dozo sevanja 0,1 kGy. Seme obsevano z dozo 0,1 kGy je v vseh letih preizkušanja imelo enako kalivost kot kontrola, v 5. letu po obsevanju pa celo značilno večjo kalivost kot kontrola.

Ključne besede: gamma žarki, *Hordeum vulgare* L., kalivost zrnja, inducirane mutacije

1 INTRODUCTION

In plant breeding, mutations play an important role in the development of genetic variations and new varieties. Despite of a large number of undesirable mutants, the use of induced mutations has been found to be very useful not only in practical plant breeding but also in theoretical genetic research. The indications are numerous 'mutant' varieties which are very popular and have high economic impact on agriculture and food production (Ahloowalia et all, 2004). As reported by Kurowska et al. (2012) more than 3000 'mutant' varieties belonging to 200 species have been

[1] Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, e-mail: ludvik.rozman@bf.uni-lj.si

documented and officially released. All officially released 'mutant' varieties are documented in the FAO/IAEA Mutant Varieties Database (http://mvgs.iaea.org/ Search. aspx). Besides the artificially induced mutants, in agriculture production there are also some well-known spontaneous mutants such as sweet maize (Zea mays saccharata L.), pumpkins with non-lignified seed coat (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Grebensc.) and various dwarf genotypes in many plant species. For the induction of mutations, the most frequently used are gamma and X radiation, fast neutrons and some chemical agents like ethyl methane-sulphonate and methyl- or ethyl-nitroso urea. The radiation treatments became very popular in late 1920s when Stadler (1928) discovered and described some of the mutagenic effects of X-rays on barley and maize.

The aim of this study is to investigate the effect of gamma radiation (three different doses) on the percentage of seed germination, and changes of seed viability associated with aging (i.e., how seed germination changes in the years after irradiation).

2 MATERIAL AND METHODS

The study included barley (*Hordeum vulgare* L.), the variety 'Astor', seeds treated with gamma radiation in 2006, in the Jožef Štefan Institute, the Reactor Centre at Podgorica near Ljubljana. Three samples of barley seeds, 0.5 kg each, were treated with gamma rays using three different doses: 0,1, 0.2 and 0.4 kGy. As a control, we used a sample which was not irradiated.

After the treatment, the samples were stored in plastic bags in a refrigerator under the temperature 4-6 °C. Such conditions are required for the storage of the seed gene bank material (seed germination can be retained up to 20 years). The germination tests were conducted repeatedly each year after the irradiation. Each time, after the seeds had been taken for the experiment, the remaining seeds were put immediately back to the refrigerator.

The experiments were conducted in a greenhouse under the controlled temperature of 20 °C and were based on the Latin square design with 4 replications of each treatment. On each of the plots, 100 seeds were sown. Seeds of each treatment were sown in a special plateau filled with special germination substrate. To enable normal germination, regular watering was provided. Ten days after sowing we counted the number of all the seedlings that germinated. The same procedure was repeated each year, except in the second and the third year, until 2014.

The data were statistically analysed. For the analysis of variance the program Statgraphics Centurion XV.II and Microsoft Excel 2010 was used. The differences among the mean values of all treatments (doses, years) were compared using the Duncan's multiple rang test.

3 RESULTS

The analysis of variance showed that there were statistically significant differences in seed germination among years and among different doses of gamma radiation (Table 1). Considering all years, only the seeds treated with 0.4 kGy had a significant lower germination rate than other three treatments (control, 0,1 and 0.2 kGy). These treatments showed statistically equal seed germination (Table 2). Different percentage of seed germination was also found in different years after the treatment.

Regardless the irradiation dose, the lowest percentage of seed germination was found in the seventh and the ninth year after the irradiation, while the percentage of seed germination tested in the eighth year after the irradiation was not statistically different from rest of the years.

Table 1: The analysis of variance for the germination of seeds treated with different doses of gamma radiation, and in different years after irradiation

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-value
Doses	7186.38	3	2395.46	42.71	0.0000
Year	1407.67	6	234.61	4.18	0.0009
Row	101.59	3	33.86	0.60	0.6142
Column	62.32	3	20.77	0.37	0.7745
Residual	5159.44	92	56.08		
Total	13788.1	107			

Table 2: The percentage of germinated seeds treated with different irradiation doses

Irradiation dosses (kGy)	Seed germination (%)
0.4	70.5 a*
0.2	86.8 b
0.1	90.6 b
Control	90.5 b

* - the same letter indicate the non-significant difference at p = 0.05

Table 3: The percentage of germinated seeds after different number of years after irradiation

Years after irradiation	Seed germination (%)
9	77.6 a*
7	81.3 ab
8	84.3 bc
4	84.9 bc
5	86.6 bc
1	88.6 c
6	88.7 c

* - the same letter indicate the non-significant difference at p = 0.05

For the irradiation dose of 0,1 kGy there were significant differences in germination only between the sixth and the ninth year after the treatment (Table 4). For the irradiation dose of 0.2 kGy only in the ninth year after the treatment the germination rate was significantly different from the rest of the years (among which there were no significant differences).

Similar results were obtained at the highest dose of irradiation (0.4 kGy, Table 4). The lowest percentage of germinated seeds was found in the ninth year after the treatment. Regarding the control (without irradiation), there were significant differences in germination only between the first (the highest percentage of seed germination) and the fifth year (the lowest percentage of seed germination) after the treatment. However, there was no significant difference between the first and the ninth year after irradiation.

Table 4: The percentage of germinated seeds treated with different irradiation doses in different years after treatment

Years of testing	Irradiation doses (kGy) 0.1	0.2	0.4	Control
1	90.5 ab	90.0 b	80.0 c	94.0 b
4	93.2 ab	90.8 b	64.5 b	91.3 ab
5	90.7 ab	89.2 b	80.9 c	85.8 a
6	96.1 b	89.6 b	77.2 bc	93.1 ab
7	90.1 ab	85.0 b	68.3 bc	92.5 ab
8	89.6 ab	86.4 b	72.0 bc	89.0 ab
9	84.3 a	75.6 a	46.3 a	87.8 ab

* - the same letter indicate the non-significant difference at $p = 0.05$

In all years, only seeds treated by the highest dose of irradiation exhibited significantly lower germination rate than other doses and control. In the first, fourth, eighth, and ninth year after the irradiation no significant differences among irradiation doses of 0.1 and 0.2 kGy) and control were found. In the seventh year after irradiation, the non-irradiated seeds (control) germinated significantly better than irradiated with 0.2 kGy, while in the fifth year, the germination of the seeds of the control was lower than those irradiated with 0.1 kGy (Table 5). In the last two years after treatment, all the irradiation doses resulted in obvious decreasing percentage of germination (Fig. 1).

Table 5: The percentage of seed germination in different years after treatment at different irradiation doses

Irradiation doses (kGy)	Year of germinating test after irradiation 1st	4th	5th	6th	7th	8th	9th
0.1	90.5 b	93.2 b	90.7 c	98.8 c	90.2 c	89.6 b	84.3 b
0.2	90.0 b	90.8 b	89.2 bc	89.6 b	85.0 b	86.4 b	74.4 b
0.4	80.0 a	64.5 a	80.9 a	77.3 a	68.4 a	72.0 a	48.6 a
Control	94.0 b	91.3 b	85.8 b	93.1 bc	92.5 c	89.0 b	87.8 b

* - the same letter indicate the non-significant difference at $p = 0.05$

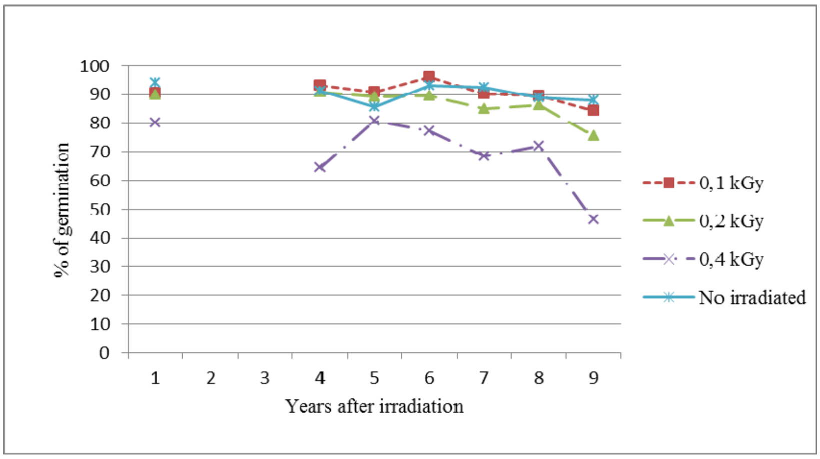

Figure 1: Germination of barley seeds in different years after treatment with three different doses of gamma rays

4 DISCUSION AND CONCLUSIONS

In plant breeding the variability of the genetic material is very important. Higher variability may also be created by mutagenesis. There are numerous mutagenic agents such as irradiation, treatments with various chemical substances, temperature, etc. Recently, very popular are also treatments with the laser radiation. The mutagenic treatment should not be drastic because it may cause lethal damages of the organism. It is very important to determine the right dose. As reported by several authors (Rybinski, 2002; Gladyszewska, 2009; Hernandez-Aguilar et al., 2009; Sacala et al., 2012) the laser irradiation leads to a lower frequency of very severe or milder injuries. Serious injuries can be prevented by various, mostly low doses of irradiation (Qing-he Li et al., 2012). In literature, it is possible to find data about the effects of different doses of irradiation. Wang et al. (2010) reported that low-dose laser irradiation induced low-frequency but significant alterations in DNA methylation level and pattern in sorghum plants, while Horn and Shimelis (2013) found that the effects of different doses of gamma radiation depended also on genotypes. The percentage of cowpea germination dropped from 100 % (at 0 Gy) to 0 % when applying 0.3 and 0.4 kGy on the genotypes 'Nakare' and 'Shindimba', but the germination of the genotype 'Bira' eposed to 0.6 kGy was 47 %. In our study, we established that the maximum dose of irradiated barley (0.4 kGy) has a strong influence on the reduced germination rate and rapid aging of seeds (i.e., lower germination in later years). The lowest irradiation dose (0.1 kGy) did not exhibit lower germination than the control, even more, in the fifth year after irradiation it had a significantly higher germination rate than the control. The seeds treated with higher doses (0.2 and 0.4 kGy) exhibited fast decrease of germination rate in last two years and this may be very important for plant breeders and especially for the maintainers of the gene banks material. Irradiated seeds should not be stored for a long time.

5 ACKNOWLEDGEMENTS

We thank colleagues of Reactor center Jozef Stefan Institute in Podgorica for the irradiation of samples of barley without which we could not carry out this investigation.

6 REFERENCES

Ahloowalia B.S., Maluszynski M., Nichterlein K. 2004. Global impact of mutation derived varieties. Euphytica 135: 187–204. DOI: 10.1023/B:EUPH.0000014914.85465.4f

Gladyszewska B. 2011. Estimation of a laser biostimulation dose. Int. Agrophysics 25: 403-405.

Hernandez-Aguilar C., Dominigues-Pacheco A., Cruz-Orea A., Ivanov R., Carballo-Carballo A., Zepeda-Bautista R., Galindo Soria L. 2009. Laser irradiation effects on field performance of maize seed genotypes. Int. Agrophysics 23: 327-332.

Horn, L., Shimelis, H., 2013. Radio-sensitivity of selected cowpea (*Vigna unguiculata*) genotypes to varying gamma irradiation doses. Scientific Research and Essays, Vol. 8(40): 1991-1997.

FAO/IAEA Mutant Varieties Database http://mvgs.iaea.org/Search.aspx (9.9.2014).

Kurowska, M., Labocha-Pawłowska, A., Gnizda, D., Maluszynski M., Szarejko I. 2012. Molecular analysis of point mutations in a barley genome exposed to MNU and gamma rays. Mutation Research 738–739: 52–70. DOI: 10.1016/j.mrfmmm.2012.08.008

Li, Q. H., Wang, S. X., Zhao, Y. M., Xu, J., Gao, T. T., Ren, W. J. 2012. Irradiation dose and effect on germination and growth of desert shrub *Nitraria tangutorum* Bobr. with two gamma irradiation modes. *Pak. J. Bot.*, 44(2): 661-666.

Rybiñski, W. 2001. Influence of laser beams combined with chemomutagen (MNU) on the variability of traits and mutation frequency in spring barley. Int. Agrophysics 15: 115-119.

Sacala, E., Demczuk, A., Grzyoe, E., Prooeba-Bialczyk, U., Szajsner, H. 2012. Impact of presowing laser irradiation of seeds on sugar beet properties. Int. Agrophysics 26: 295-300. DOI: 10.2478/v10247-012-0042-6

Stadler L. J. 1928. Mutations in barley induced by X-rays and radium. Science 68: 186–187. DOI: 10.1126/science.68.1756.186

Wang, H. C., Feng Q. B., Zhang M. A., Yang C. A., Sha W. C., Liu, B. 2010. Alteration of DNA methylation level and pattern in sorghum (Sorghum bicolor L.) pure-lines and inter-line F1 hybrids following low-dose laser irradiation. Journal of Photochemistry and Photobiology B: Biology 99: 150–153. DOI: 10.1016/j.jphotobiol.2010.03.011