Bangladesh J. Nuclear Agric, 36(1): 121-128, 2022 *ISSN 0258-7130*

Short communication

GAMMA RAY INDUCED RADIOSENSITIVITY OF BLACK CUMIN GENOTYPE

S. Tasmin[1*], M.R. Islam[1], M.N.H. Mehedi[1], K.T. Akter[1] and M.A. Tarafder[2]

Mutagenesis using ionizing radiation has been widely used for the genetic variability with novel characteristics. To know the optimum dose in black cumin an experiment was carried out at Bangladesh Institute of Nuclear Agriculture, HQs farm, Mymensingh. Collected three germplasm (BC10861, BC10863 and BC10864) of black cumin seeds were irradiated with different doses of gamma radiation as 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy and 300 Gy to determine the LD_{50} for development of desirable mutants. Maximum seed germination (94.5-97.1%) was found from the lowest dose (50 Gy) of gamma radiation, while the minimum germination (18.2-22.0%) was obtained from the highest dose (300 Gy) among the three germplasm BC10861, BC10863 and BC10864. The seedlings survivability was 45-49% at 150 Gy. After one month, in the later growth stage none of the seedlings survived at the dose of 250 Gy and 300 Gy. At lower dose of 50 Gy maximum numbers of leaves (6.12-7), number of roots per plant (7.13-8.05), root length (5.34-7.1 cm), shoots length (5.3-7.1 cm) and fresh weight per plant (0.25-0.29 g) were observed among the germplasm. The seed germination and seedlings survival decreased with the radiation dose increased. The seedling growth parameters like number of leaves, root length, shoot length and fresh weight per plant reduced around 50% at 150 Gy dose of gamma radiation. Among the six doses of irradiation the LD_{50} was close to 150 Gy which caused around 55-51% death of seedlings, 45-49% seedlings survival and around 50% reduction in plant growth. Hence, it is concluded that 150 Gy may be the optimum dose for creating useful mutation induction in black cumin.

Key words: Black cumin, gamma ray, irradiation, LD_{50}, mutagenesis.

Nigella sativa L., known as black cumin, belongs to the Ranunculaceae family and the order Ranales (Malhotra, 2004). Seed of black cumin contains about 21% protein, 35% carbohydrates and 35-38% plant fats and oils (Preussa *et al*., 2003). It contains all essential amino acids and rich source of vitamins and minerals (Sarada *et al*., 2015, Sastry *et al*., 2013). The seeds and its essential oil have been widely used in functional foods, nutraceutical and pharmaceutical products to treat different diseases. However, the production is not sufficient in accordance with demand in Bangladesh.

The physical mutagens like gamma rays are known to be the most popular mutagen because of their simple use, high penetration, reproducibility, high mutation frequency and less disposal problems (Chahal & Ghosal, 2002). After the discovery that physical mutagens can induce mutation, many plant breeders and geneticists started observing the use of radiation induced mutations for altering plant characters (Ahloowalia *et al*., 2004). During

[1]Horticulture division, Bangladesh Institute of Nuclear Agriculture, BAU Campus, Mymensingh-2202
[2]Soil Science Division, Bangladesh Institute of Nuclear Agriculture, BAU Campus, Mymensingh-2202
[*]Corresponding authors' email: stshorna_5@yahoo.com

the last seventy years, more than 3402 mutant varieties have been officially released (Mutant Variety Database, IAEA, 2021). Mutation induced by radiation was the most commonly used method to develop direct mutant varieties (89%). Although mutations are beneficial for producing variability in populations, the treatments themselves can be detrimental and can cause a reduction in germination, growth rate, plant vigour and pollen and ovule fertility in a plant (Micke and Donini, 1993). If the variability is low in the population/germplasm, then it is necessary to create variability for crop improvement. The variability is created through recombination techniques in general, but it's difficult in black cumin, due to small flower size, lack of genetic variability and non availability of any type of sterility (Sastry and Anandaraj, 2013). There is immense need of high yielding varieties, which is not possible without generating variability in black cumin and also in other seeds species. Mutagenic effectiveness is a measure of the mutations induced per unit dose of a mutagen (time×concentration/dose), while mutagenic efficiency gives an idea of genetic damage (mutation) in relation to the total biological damage caused in M_1 generation (Ambavane *et al*., 2015). In addition response of plants to gamma radiation may vary with the genetic makeup of the crops, the tissue exposed, and the dose of irradiation (Sakamoto 2006). While the mutagenic effectiveness decreased with the increase in dose of mutagen that indicates negative relationship between effectiveness and number of mutagens.

However, for initiating mutation breeding programme in any crop to create the desired variability, the knowledge of optimum dose of mutagen is essential. The growth parameters including percent seed germination, seedling growth are some of the usually used criteria for determining mutagenic sensitivity in a particular crop and variety (Lal *et al*., 2009). Therefore, this experiment was conducted to determine the optimum dose of radiation for LD_{50} in black cumin.

The experiment was carried out at BINA HQs farm, Mymensingh. Dry black cumin seeds (200 no.) were irradiated with gamma rays at the doses of 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy and 300 Gy by ^{60}Co source. The irradiated seeds along with non irradiated seeds were placed on blotting paper for percent seed germination. Following parameters related to determine LD_{50} were recorded:

Percent seed germination: Seed germination was recorded treatment wise on the day (14 day) when the control showed 100 percent germination. The emergence of plumule and radicle was considered as germination.

$$\text{Percent seed germination} = \frac{\text{No. of germinated seeds}}{\text{Total no. of seed}} \times 100$$

Percent seedling survival: The percent survival of seedling was calculated after 30 DAS using formula given below:

$$\text{Percent survival} = \frac{\text{No. of plant survived}}{\text{No. of seed sown}} \times 100$$

Seedling height (cm): Seedling height was recorded from15 randomly selected seedlings per replication after 30DAS.

Root length (cm): Root length of 15 randomly selected seedlings was recorded at 30 DAS for each replication.

Shoot length (cm): Shoot length was recorded from 15randomly selected seedlings per replication after 30 DAS.

Fresh weight of plants (g): Freshly uprooted seedlings were placed on blotting paper to soak the surface water and then weighted on an electronic balance and mean value was calculated for each treatment.

Number of Leaf per seedling: Leaves were counted manually from randomly selected 15 selected seedlings at 30 DAS for each replication.

Number of root per seedling: Roots were counted manually from randomly selected 15 selected seedlings at 30 DAS for each replication

Experiment was carried out in completely randomized design and each treatment was replicated thrice. All data were analyzed using the statistics following Gomez and Gomez (1984). The means were separated by Duncan Multiple Range Test (DMRT) at 5% level of significance.

The data pertaining to seed germination and seedling survival is presented in Figure 1 which revealed that as the irradiation doses increased, both percentage of seed germination and seedling survival decreased. The seed germination was 100% in non irradiated seeds, while after imposing irradiation the percent germination were 97 in 50 Gy, 96 in 100 Gy, 91 in 150 Gy, 76 in 200 Gy, 49 in 250 Gy and 19 in 300 Gy for BC10861 germplasm, 95 in 50 Gy, 93 in 100 Gy, 93 in 150 Gy, 73 in 200 Gy, 54 in 250 Gy and 18 in 300 Gy for BC10863 germplasm and 96 in 50 Gy, 97 in 100 Gy, 94 in 150 Gy, 76 in 200 Gy, 50 in 250 Gy and 22 in 300 Gy for BC10864 germplasm (Fig 1). Among the doses of irradiation, 250 Gy and 300 Gy were more lethal. The plumule and radical emerged at the dose 250 Gy and 300 Gy but in later growth stages failed to transform into complete seedling.

With regards to the survival of seedlings, the survival of seedlings decreased with increase in dose of gamma radiation. In control the 100% seedlings were survived but the irradiated seedlings reduced in its survivality after germination. The seedlings survival rate was 85.0 in 50 Gy, 65.0 in 100 Gy, 47.0 in 150 Gy, 38.0 in 200 Gy, 20.0 in 250 Gy and 5.0 in 300 Gy for BC10861 germplasm, 77.0 in 50 Gy, 73.0 in 100 Gy, 49.0 in 150 Gy, 37.0 in 200 Gy, 15.0 in 250 Gy and 3.0 in 300 Gy for BC10863 germplasm and 81.0 in 50 Gy, 67.0 in 100 Gy, 45.0 in 150 Gy, 33.0 in 200 Gy, 18.0 in 250 Gy and 2.5 in 300 Gy for BC10864 germplasm (Fig 1).

The decrease in seed germination induced by mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009). Micco *et al*., (2011) have correlated seed germination with abnormalities in mitotic cycles and in metabolic pathways of the cells. The reduction in germination and survival may be due to absorption of ionizing radiation in biological materials, acting directly on critical targets in the cell (Kovacs and Keresztes, 2002). Bashir *et al*., (2013) also reported that the seed germination percentage and percent survival decreased with an increase in dose of the gamma irradiation. They concluded that lower treatment of gamma irradiation has influenced less biological damage and would be suitable for inducing desirable mutations. The present findings are in agreement with the above mentioned reports. Similar findings were also reported where in, higher doses of gamma radiation reduced germination percentage and survival in fennel (Verma *et al*., 2017) and coriander (Sarada *et al*., 2015).

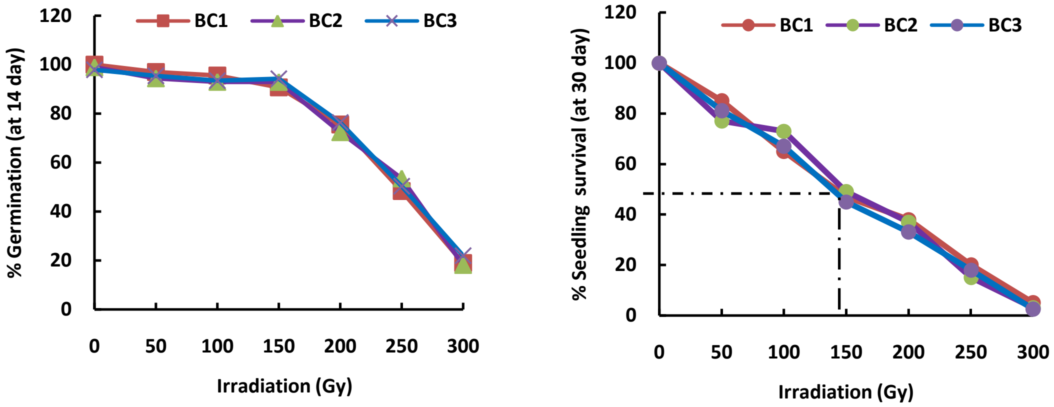

Fig. 1. Mutagenic effect of gamma rays on percent germination and percent seedling survival in black cumin (BC1 = BC10861, BC2 = BC10863 & BC3 = BC10864)

Fig. 2(a): Effect of different doses of gamma irradiation on growth of black cumin of BC-10861 germplasm

Fig. 2(b): Effect of different doses of gamma irradiation on growth of black cumin of BC-10863 germplasm

Fig. 2(c): Effect of different doses of gamma irradiation on growth of black cumin of BC-10864 germplasm

Table 1. Effect of acute exposure of different doses of gamma rays on growth characters of seedlings

Germplasm	BC10861					BC10863					BC10864				
Dose (Gy)	No. of leaves	No. of roots plant^{-1}	Root length (cm)	Shoot length (cm)	Fresh wt. plant^{-1} (g)	No. of leaves	No. of roots plant^{-1}	Root length (cm)	Shoot length (cm)	Fresh wt. plant^{-1} (g)	No. of leaves	No. of roots plant^{-1}	Root length (cm)	Shoot length (cm)	Fresh wt. plant^{-1} (g)
0 (Control)	7.53a	8.25a	7.31a	9.07a	0.37a	7.34a	7.53a	6.52a	8.70a	0.31a	6.87a	8.01a	6.43a	8.63a	0.33a
50	7.00ab	8.05a	7.10a	8.62ab	0.29b	6.94a	7.13a	5.54b	7.91b	0.25b	6.12b	7.52ab	5.34b	7.31b	0.28a
100	6.87b	7.57b	5.74b	7.93b	0.21c	5.85b	6.54b	5.33b	7.53b	0.24b	5.95bc	7.35b	5.11b	6.23c	0.21b
150	5.32c	6.43c	4.64c	5.72c	0.13d	5.35b	5.38c	3.51c	5.76c	0.14c	5.35c	5.95c	3.89c	4.93d	0.15c
200	4.24d	2.45d	3.24d	4.52d	0.07e	3.24c	2.19d	1.70d	3.74d	0.05d	3.62d	2.54d	2.60d	3.52e	0.08d
250	0.00e	0.00e	0.00e	0.00e	0.00f	0.00d	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e	0.00f	0.00e
300	0.00e	0.00e	0.00e	0.00e	0.00f	0.00d	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e	0.00f	0.00e
CV (%)	4.43	3.78	4.45	4.99	9.96	5.53	3.98	5.97	4.64	12.5	6.42	4.34	6.74	4.89	11.65
SE (±)	0.16	0.14	0.15	0.21	0.01	0.19	0.13	0.15	0.18	0.01	0.21	0.16	0.12	0.17	0.02

Highly significant differences were observed among the lower and higher doses of irradiation on number of leaves, root length, shoot length and fresh weight per plant in Fig. 2 (a, b and c). From the Table 1, The maximum number of leaves (7), number of roots per plant (8.05), root length (7.10 cm), shoots length (8.62 cm) and fresh weight per plant (0.29 g) were observed at lower dose 50 Gy and the minimum number of leaves (4.24), number of roots per plant (2.45), root length (3.24 cm), shoots length (4.52 cm) and fresh weight per plant (0.07 g) were observed at higher dose 200 Gy in BC10861 germplasm.

Again the maximum number of leaves (6.94), number of roots per plant (7.13), root length (5.54 cm), shoots length (7.91 cm) and fresh weight per plant (0.25 g) were observed at lower dose 50 Gy and the minimum number of leaves (3.24), number of roots per plant (2.19), root length (1.70 cm), shoots length (3.74 cm) and fresh weight per plant (0.05 g) were observed at higher dose 200 Gy in BC10863 germplasm.

Furthermore, the maximum number of leaves (6.12), number of roots per plant (7.52), root length (5.34 cm), shoots length (7.31 cm) and fresh weight per plant (0.28 g) were observed at lower dose 50 Gy and the minimum number of leaves (3.62), number of roots per plant (2.54), root length (2.60 cm), shoots length (3.52 cm) and fresh weight per plant (0.08 g) were observed at higher dose 200 Gy in BC10864 germplasm. On the other hand, no survivality was observed in case of all germinated seedlings at the gamma radiation doses 250 and 300 Gy (Table. 1). The high dose irradiation that caused growth inhibition could be described to the cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome as reported by Preussa and Britta (2003). Similar results were obtained by Yadav and Ramkrishna, (2013) in *Cuminum cyminum*.

The results of the experiment indicated that high dose of gamma radiation reduced germination percentage, seedling survival, shoot length, root length, number of leaves and fresh weight of plant drastically. The high doses of 250 to 300 Gy gamma rays were more lethal. The 150 Gy of gamma radiation was found to cause near about 50% seedling survival and 50% reduction in root length, shoot length and fresh weight per plant. It is expected that 150 Gy of gamma ray would be the optimal dose for inducing useful mutation in black cumin which will help to develop desirable mutants.

References

Ahloowalia, B.S., Maluszynski, M. and Nichterlein, K. 2004. Global impact of mutation-derived varieties. *Euphytica*135: 187-204.

Ambavane, A.R., Sawardekar, S.V., Sawantdesai, S.A. and Gokhale, N.B. 2015. Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (*Eleusine coracana* L. Gaertn). *Journal of Radiation Research and Applied Sciences* 8: 120-125.

Bashir, S., Wani, A.A. and Nawchoo, I.A. 2013. Mutagenic sensitivity of gamma rays, EMS and sodium azide in *Trigonella foenum-graecum* L. *Science ResearchReporter* 3(1): 20-26.

Chahal, G.S. and Gosal, S.S. 2002. *Principles and procedures of Plant Breeding*. Oxford: Alpha Science International Ltd. pp. 399-412.

Gomez, K.A. and Gomez, A.A. 1984. Statistical procedures for agricultural research. John Wiley and Sons, USA.

Khan, S. and Goyal, S. 2009. Improvement of mungbean varieties through induced mutations. *African Journal of Plant Science* 3: 174-180.

Kovacs, E. and Keresztes, A. 2002. Effect of gamma and UV-B/C radiation on plant cells. *Micron* 33: 199- 210.

Lal, G.M., Toms, B. and Lal, S.S. 2009. Mutagenic sensitivity in early generation in black gram. *Asian Journal of Agricultural Science* 1: 9-11.

Malhotra SK 2004. In: Handbook of Herbs and Spices, Woodhead Publishing Series in Food Science, Technology and Nutrition. 2: 206-214.

Micco, V.D., Arena, C., Dand, P. and Durante, M. 2011. Effects of sparsely and densely ionizing radiation on plants. *Radiation and Environmental Biophysics* 50: 1-19.

Micke, A. and Donini, B. 1993. Induced mutations. In Hayward, M.D., Bosemark, N.O. and Romagosa, I. (edited) Plant breeding principles and prospects. Chapman and Hall, London. pp. 52-62.

Mutant Variety Database, IAEA. 2021. https:// mvd.iaea.org.

Preussa, S.B. and Britta, A.B. 2003. DNA-damage induced cell cycles check point in Arabidopsis. *Genetics* 164: 323-334.

Sakamoto, W. 2006. Protein degradation machineries in plastids. Annual Review of Plant Biology, 57, 599-621

Sarada, C., Jyothi, K.U.V., Rao, S. and Reddy, P.V. 2015. Mutagenic sensitivity of gamma rays, EMS and their combinations on germination and seedling vigour in coriander (*Coriandrum sativum* L). *International Journal of Advances in Pharmacy, Biology and Chemistry* 4(2): 430-438.

Sastry, E.V.D. and Anandaraj, M. 2013. Cumin, Fennel and Fenugreek". Soils, plant growth and crop production. Encyclopedia of Life Support Systems (EOLSS). Pp. 1-10.

Verma, A.K., Sharma, S., Kakani, R.K., Meena, R.D. and Choudhary, S. 2017. Gamma radiation effects seed germination, plant growth and yield attributing characters of fennel (*Foeniculum vulgare* Mill.). *International Journal of Current Microbiology andApplied* 6: 2448-2458.

Yadav, S.K. and Ramkrishna, R. 2013. Effectiveness and efficiency of physical and chemical mutagens on cumin (*Cuminum cyminum* L.). *Vegetos* 26: 44-49.