

Electron beam effects and determination of GR50 dose in tropical field corn

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Abstract: The maize improvement aimed at enhancing its productivity to feed the increasing world population and is largely dependent on genetic variability present in the breeding material. In the present study, additional genetic variation was induced by a physical mutagen, electron beam (EB). The experiments were conducted for determining the best dose for a 50 per cent reduction in growth (GR 50). The seeds of maize inbred line, PML 93, treated with 10 different doses (50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, and 700 Gy) of EB, were tested for seedling growth parameters using pot culture method. Data on various seedling growth parameters such as per cent germination, seedling length, root length, fresh and dry weight, vigor index I and II were recorded. Significant variation was found among the treatments and 200 Gy of the electron beam was identified as the best GR 50 dose for the inbred line PML 93. The cellulase and pectolyase enzymes were deployed for the root tip cell wall digestion and karyotyping was performed to understand the effect of mutation EB doses on chromosomes. Compared to control, the mutagens with a dose of 200 Gy, induced chromosomal breakage at one or two places. These

aberrations may lead to heritable variations that can be used for crop improvement in the future.

Keywords: Inbred line · Electron beam · GR 50 · Karyotype · Vigour index

Introduction

Maize is an important cereal crop having an array of diverse uses, as food, feed, and raw material for agro-based industries. Though maize crop shows high genetic variation, genetic enhancement of specific traits needs specialized methods to create novel variation. Mutagenesis is one such option in maize breeding that enables heritable variations (Saif-u-Malook *et al.*, 2015). Several mutagens have been used for mutagenesis in crop plants. They are broadly classified as physical and chemical mutagens. Further, physical mutagens are classified as classical radiation mutagen, particle mutagen, and space radiation mutagen (Ma *et al.*, 2021). The selection of suitable mutagen and the procedure to handle mutant generations to isolate desirable mutants depends on the breeding objective and the target trait (Shrivastava *et al.*, 2021). Apart from the conventional electromagnetic radiations like X-rays and gamma rays, the electron beam is now an alternative source of energy to induce mutations in plants (Mondal *et al.*, 2017; Sao *et al.*, 2020). Unlike gamma irradiators, electron beam accelerators have prompt radiation which can be stopped by switching off the source of electrons and radiofrequency power (Mittal, 2012). However, the use of electron beams in mutation breeding was limited due to the availability of the source and particularly the problem of getting low-dose delivery units for mutation breeding applications. However, a linear

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electron accelerator-based facility presently operational at Raja Ramanna Centre for Advanced Technology (RRCAT), Indore, India, overcomes all limitations (Mondal *et al.*, 2017). The same facility was used to induce the mutation in stable maize inbred line, PML 93. It is a conventionally derived inbred line, has excellent general combining ability and high yield *per se* (3.5 t/ha). It has all the required desirable characteristics to be used as female parental lines in the hybrid breeding program. However, the kernel size of the inbred line falls into the small category, as per the maize DUS descriptor (Das *et al.*, 2006.), which becomes a limitation in economical hybrid seed production and fetching farmers' preference when PML 93 is a seed parent. The mutagenesis is expected to create variability for seed size in PML 93 while retaining its high combining ability.

An understanding of the radio-sensitivity of the genotype is required beforehand for selecting the optimal dose for a specific genotype i.e., the dose that might produce the desired mutant (a mutant trait in low mutational load genetic background) at a frequency that can be detected in the mutagenized population (Bado *et al.*, 2016). Limited studies have documented the mutagenic potential of the electron beam in maize. It is important to have a dose that makes a trade-off between radiation-induced damage/lethality and an effective number of mutants induced by them (Ahloowalia *et al.*, 2004). Doses of mutagen that lead to 50 per cent lethality or 50 per cent

growth reduction are considered as LD50 or GR50, respectively, beyond which isolation of useful and effective mutant is difficult (Viana *et al.*, 2019). Hence, the present investigation was conducted to determine the GR 50 dose required to induce desirable mutation in maize crop using inbred line, PML 93.

Materials and methods

Mutagen treatment

The seeds of test inbred line viz., PML-93 were selected from the two different lots (Lot-I and Lot-II), maintained during post rainy season, 2020 at two different locations (ICAR-Indian Agricultural Research Institute, New Delhi and ICAR-IARI, Regional Research Centre, Dharwad). Germination test of unirradiated seeds was carried out using the standard paper towel method, in which Lot-II showed 100 per cent germination. A total of 100 seeds from the lot- II were irradiated with ten different doses of electron beam viz., 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, and 700 Gy at Raja Rammana Centre for Advanced Technology, Indore, India. Unirradiated seed samples were used as the control for the comparative analysis. The control and irradiated seeds were pre-treated with Bavistin (2 g/kg) before seeding in the pots to prevent fungal infections.

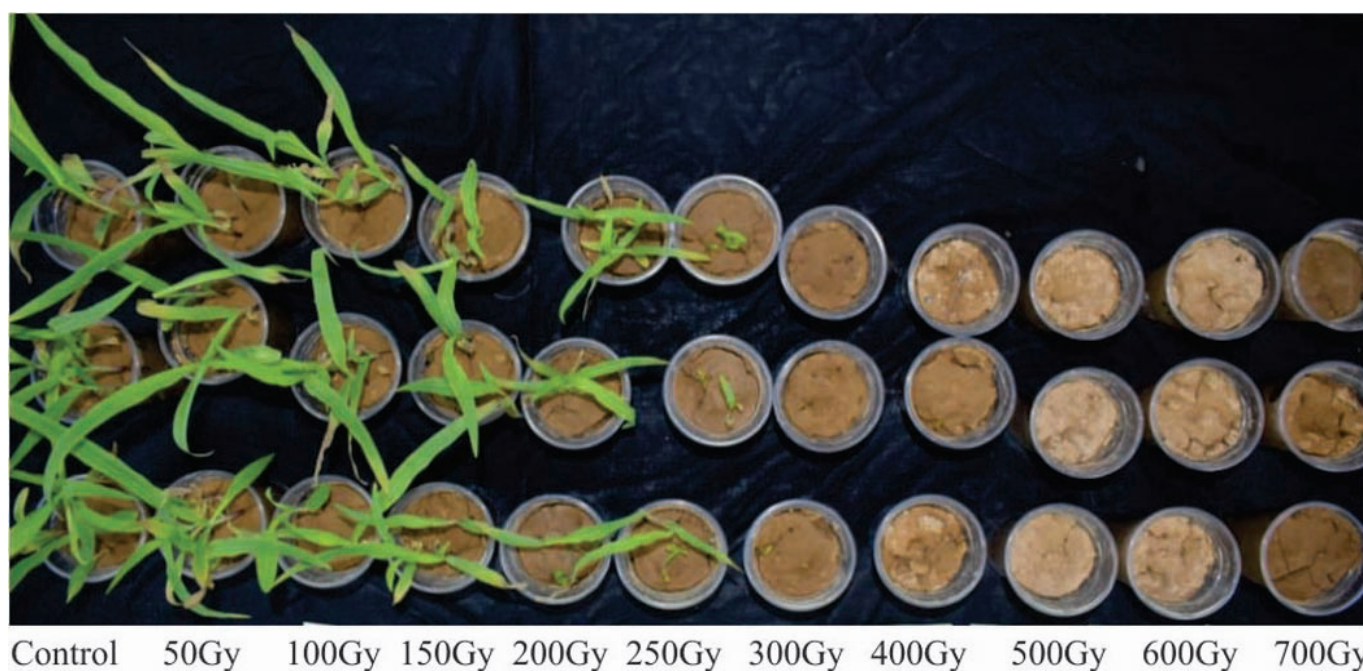


Figure 1. Effect of EB irradiation on the seedling growth parameters of inbred line, PML 93

Pot experiment

A total of 30 seeds each for all ten dosages of EB were sown in the pot (6-inch diameter) containing solarized soil medium. Total three biological replications were placed in a completely randomized design under natural conditions. Pots were irrigated as and when required, with potable water. The seedling growth parameters were recorded 10 days after germination of the control seed. The growth parameters viz., per cent germination (number of germinated seeds / total number of seeds) $\times$ 100), seedling length (root and shoot length), seedling fresh weight and dry weight (oven-dry weight), vigor index I [(mean root length + mean shoot length)] $\times$ per cent seed germination) and vigor index II [(mean root dry weight + mean shoot dry weight)] $\times$ per cent seed germination) were recorded on individual seedlings and the mean value of replications was used for statistical analysis using SAS 9.3 v (SSCNARS, IASRI, New Delhi).

Karyotyping

To assess the effects of EB on chromosomes, the meristematic region of roots was studied using an enzyme-based method proposed by Snowdon *et al.* (1997) Young growing root tips of 1–2 cm length (10 days after germination) were fixed at the metaphase I stage in Carnoy's solution (cold ethanol: glacial acetic acid (3:1)). At first, the root tips were rinsed (2 $\times$ 10 minutes) in double-distilled water and were incubated in citrate solution (0.01M, pH 4.5) for 15 minutes. Root tips were treated with enzyme solution containing 5:1 cellulase (Onozuka R-10 cellulase, Yakult Honsha Co. Ltd., Japan) and pectolyase (Y23 pectolyase, Seishin Pharmaceutical Ltd., Japan) prepared in citrate solution (0.01M, pH 4.5) and incubated for 1 hour 5 minutes at 37°C. Treated root tips were scrambled on glass slides in Carnoy's solution and covered with a coverslip of width about 1 cm and

visualized under a microscope (LEICA DM 750, Germany) at 100 $\times$ (100 $\times$ /1.25 oil) and images were captured through a high-resolution camera (LEICA DFC 3000 Germany) attached with it.

Results and discussion

Analysis of variance indicated the presence of significant differences for all the studied parameters viz., root length, shoot length, root dry weight, shoot dry weight, vigor index-I, and vigor index-II among different doses of EB (Table 1).

The effect of different doses of the electron beam on studied growth parameters are presented in Table 2 and Figure 2. The highest per cent seed germination was observed in the control seed (91.7 per cent) followed by seed treated with 50 Gy EB (76.7 per cent). The germination percentage was reduced to half for the seeds treated with 250 Gy of EB and it reached zero when the dosage was 500 Gy or more. Compared to control (17.8 cm), the mean root length (MRL) was found to decrease from 16.7 to 0.43 cm when the radiation dose was increased from 50 Gy to 400 Gy and further increase in the dose showed a lethal effect.

Upon exposure to different doses varying from 50 Gy to 700 Gy, the observed shoot lengths were 19.9 cm, 19.7 cm, 16.3 cm, 15.4 cm, 7.9 cm, 1.76 cm, and 1.43 cm at 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, and 400 Gy, respectively. The doses higher than 400 Gy were lethal.

The mean root dry weight (MRDW) ranged from 0.02 (unirradiated) to 0.00 (300Gy). The 50 per cent or more reduction in MRDW in comparison to control was observed from the dose 150 Gy and it reached zero when the dose was raised to 300 Gy. A similar trend of decreasing mean shoot dry weight (MSDW) with increasing electron beam dosage was noticed that ranged from 0.05 g (unirradiated) to 0.00 (400 Gy). The growth reduction with respect to MSDW started at 150 Gy.

Table 1. Analysis of variance for growth parameters of PML 93 under different concentrations of electron beam irradiation

Sources of variation	MSS						
	df	RL	SL	RDW	SDW	VI- I	VI- II
Replication	2	1.62	0.24	0.00	0.00	46.54	0.46
Treatment	10	157.19**	253.32**	0.0002**	0.0014**	3704.51**	17.99**
Error	20	1.24	1.35	0.00	0.00	28.53	0.42

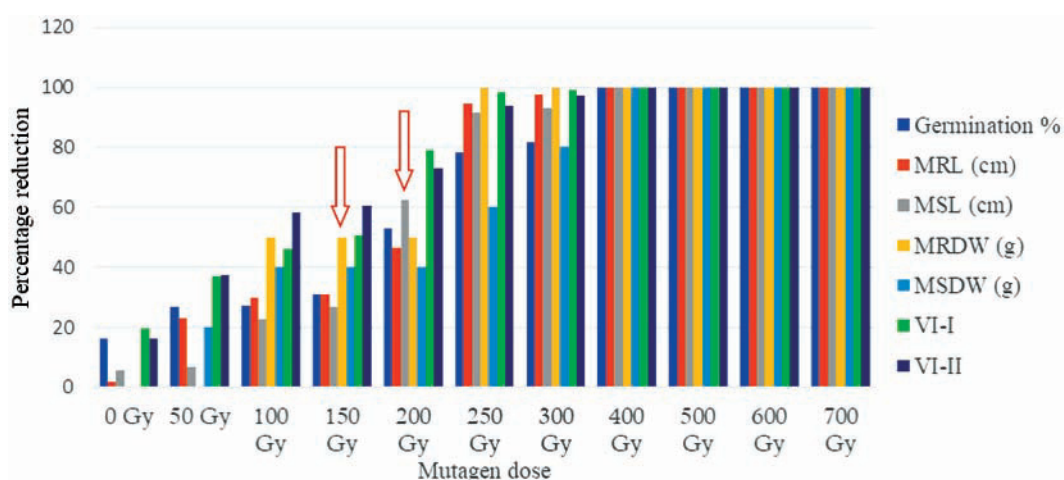
** : Significant at 1% probability, Abbreviations: PT- Paper Towel, RL- Root Length, SL- Shoot Length, RDW- Root Dry Weight, SDW- Seed Dry Weight, VI- I- Vigour Index I, VI- II- Vigour Index II.

Table 2. Effect of Electron beam irradiation on maize seedling growth parameters

S.No.	Treatment	Germination %	MRL (cm)	MSL (cm)	MRDW (g)	MSDW (g)	VI-I	VI-II
1	0 Gy	91.7	17.0	21.1	0.02	0.05	3493.8	6.42
2	50 Gy	76.7	16.7	19.9	0.02	0.05	2807.2	5.37
3	100 Gy	67.0	13.1	19.7	0.02	0.04	2197.6	4.02
4	150 Gy	66.7	11.9	16.3	0.01	0.03	1880.9	2.67
5	200 Gy	63.3	11.7	15.4	0.01	0.03	1718.6	2.53
6	250 Gy	43.3	9.1	7.9	0.01	0.03	736.96	1.73
7	300 Gy	20.0	0.9	1.8	0.00	0.02	53.20	0.40
8	400 Gy	16.7	0.4	1.4	0.00	0.01	31.10	0.18
9	500 Gy	0.0	0.0	0.0	0.00	0.00	0.00	0.00
10	600 Gy	0.0	0.0	0.0	0.00	0.00	0.00	0.00
11	700 Gy	0.0	0.0	0.0	0.00	0.00	0.00	0.00
	Mean	40.5	7.4	9.5	0.01	0.02	1186.70	2.20
	SD	34.3	7.2	9.2	0.01	0.02	1320.34	2.49

Abbreviations: PT- Paper Towel, MRL- Mean Root Length, MSL- Mean Shoot Length, MRDW- Mean Root Dry Weight, MSDW- Mean Shoot Dry Weight, VI- I- Vigour Index I, VI- II- Vigour Index II.

Figure 2. Per cent reduction in growth parameters of Electron Beam treated inbred line, PML 93



As a consequence of the decrease in mean root and shoot length compared to control, Vigor Index- I values also decreased to 3049.0, 2807.22, 2197.60, 1880.94, 1718.60, 736.96, 53.20, and 31.06 at the doses of 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, and 400 Gy, respectively from the control value of 3493.77. The reduction of 50 per cent VI-I was observed at the dose 200 Gy. A similar result was observed on VI-II, where the increased dose from 50 Gy to 700 Gy resulted in the decreased VI-II from 5.37 to 0.0 compared to control (6.42). The reduction of 50 per cent VI-II was observed at dose 150 Gy.

Breeders are practicing the mutation breeding approach to induce genetic variability in crops to have an exploitable genetic variation for further crop improvement (Holme *et al.*, 2019). Generally, in plant breeding, physical and

chemical mutagenesis are the two major methods used to induce mutations in crop plants (Leitao, 2011; Mba *et al.*, 2011). But, the selection of particular mutagen depends on their efficiency and the purpose for which it is being used. Gamma irradiation is one of the frequently used physical mutagens to induce mutation in plant and animal systems and it was earlier reported that the effectiveness of EB is more than that of gamma rays (Mondal *et al.*, 2017; Gowthami, 2021). However, it is very important to standardize the dose of mutagen before its specific application to the targeted system, as the indiscriminate dose may be harmful. Hence, the present study was carried out to fix the appropriate dose of the EB mutagen to generate sufficient variations in a maize inbred line, PML 93, with minimum impact on the genome to sustain the negative effect of mutagen (Álvarez-Holguín *et al.*, 2019).

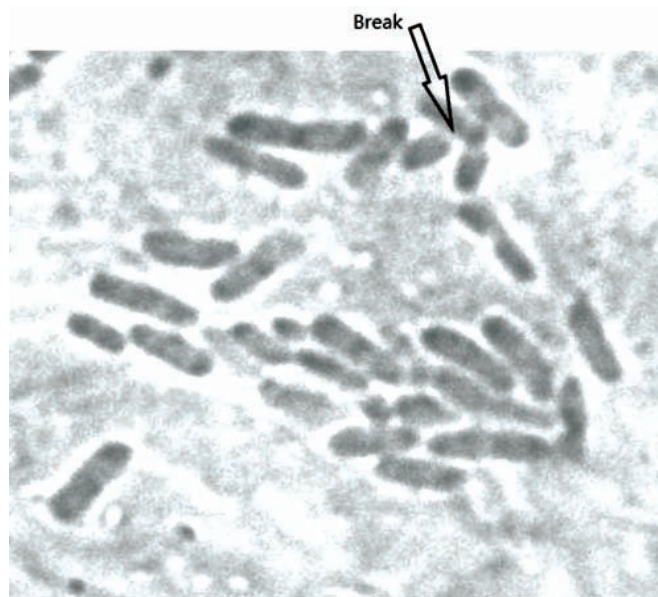


Figure 3. Chromosomal aberrations by the electron beam at 200 Gy in PML 93

In the ANOVA of different doses of EB, the mean sum of squares due to germination percentage, survival, seedling length (root and shoot), dry weight, wet weight, vigor index, I and II (Table 1) were highly significant indicating the induction of significant variation in growth parameter. It further implied that the tested EB doses can create genetic variation *vis-a-vis* its survivability in the target genotype (Kurtar *et al.*, 2017, Kumar *et al.*, 2018).

Further analysis of individual growth parameters for deciding GR 50 dose indicated that irradiation adversely affected both seed germination and the elongation of the root and shoot, similar to that noticed in other crops, during early seedling growth (Marcu *et al.*, 2013). Compared to the control treatment, germination, mean root length, and mean shoot length were reduced approximately to half at 250 Gy. The reduction of seedling growth after irradiation has been earlier explained based on auxin destruction, changes in ascorbic acid content and physiological injury, and biochemical disturbances in rice seedlings (Yusuf and Nair, 1974). The use of reduction in the germination percentage or root elongation and shoot elongation as an index to establish the relationship between the irradiation dose and early seed growth has its own limitations. Since, the process of seed germination and shoot elongation can be affected by various factors and their interactions, including temperature, water availability, oxygen, light, substrate, maturity of seed, and physiological age of seed (Smýkal *et al.*, 2014). Therefore, the vigor index is considered to be a more reliable parameter for estimating the 50 per cent growth reduction

dose in plants (Kumar *et al.*, 2018). Seedling vigor is a measure of the extent of damage that accumulates as viability declines, and the damage accumulates in seeds until the seeds are unable to germinate and eventually die (Copeland and McDonald, 2012; Zhao *et al.*, 2016). Vigor Index-I and II showed a reduction trend as the concentration of the EB was increased from 0 Gy to 400 Gy and further increase in mutagen dose from 500–700 Gy became lethal. Compared to the control treatment, GR 50 with respect to Vigor Index-I and II were recorded at doses 200 Gy and 150 Gy, respectively. This was depicted in the growth of the seedling also (Figure 1).

Therefore, taking into consideration of per cent germination, MRL and MSL, MRDL and MSDL, in PML 93, it can be concluded that GR 50 dose required for inducing useful mutations in this inbred line is 200 Gy. But, the 50 per cent reduction of V-I and V-II compared to the control appeared at 150 Gy (Figure 2). Hence, further karyotype analysis was conducted to decide the effective GR 50 dose. The root samples from seedlings treated with different doses as described in the material and methods section were studied for this purpose. The karyotype analysis revealed that chromosomal aberrations started at 200 Gy as minor breakage in the chromosomes and progressively heavier aberrations at doses higher than 200 Gy (Figure 3). Hence, dose 200 Gy was considered as GR 50 dose for maize genotype in general and the inbred line like PML 93.

Conclusion

Standardization of dose for obtaining effective mutation is the basic requirement of a mutation breeding program. In the present investigation, among the 10 doses of EB employed in the study, 200 Gy was revealed as a GR 50 dose in the inbred line, PML 93. This dose can be utilized to obtain potentially useful mutations in the inbred line, PML 93.

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References

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

- Álvarez-Holguín, A., Morales-Nieto, C. R., Avendaño-Arrazate, C. H., Corrales-Lerma, R., Villarreal-Guerrero, F., Santellano-Estrada, E. & Gómez-Simuta, Y. (2019). Mean lethal dose (LD50) and growth reduction (GR50) due to gamma radiation in Wilman lovegrass (*Eragrostis superba*). *Revista mexicana de ciencias pecuarias*, **10**: 227–238.
- Bado, S., Rafiri, M. A., El-Achouri, K., Sapey, E., Niele, S., Ghanim, A. M. A., Forster, B. P. & Laimer, M. (2016). In vitro methods for mutation induction in potato (*Solanum tuberosum* L.). *African Journal of Biotechnology*, **15**(39): 2132–2145.
- Copeland, L. O. & McDonald, M. F. (2012). *Principles of Seed Science and Technology*. Springer Science & Business Media.
- Gowthami, R., Vanniarajan, C., Souframanien, J., Veni, K. & Renganathan, V. G. (2021). Efficiency of electron beam over gamma rays to induce desirable grain-type mutation in rice (*Oryza sativa* L.). *International Journal of Radiation Biology*, **97**(5): 727–736.
- Holme, I. B., Gregersen, P. L. & Brinch-Pedersen, H. (2019). Induced genetic variation in crop plants by random or targeted mutagenesis: convergence and differences. *Frontiers in Plant Science*, **10**: 1468. DOI: 10.3389/fpls.2019.01468.
- Kumar, V., Vishwakarma, G., Chauhan, A., Shitre, A., Das, B. K., Nair, J. P. & Gupta, A. K. (2018). Use of proton beam as a novel tool for mutations in rice. *BARC Newsletter*, **366**: 5–9.
- Kurtar, E. S., Balkaya, A. & Kandemir, D. (2017). Determination of semi-lethal (LD50) doses for mutation breeding of winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.). *Fresenius Environmental Bulletin*, **26**(5): 3209–3216.
- Leitao, J. M. (2011). *Plant mutation breeding and biotechnology*. (In) *Chemical Mutagenesis*, Eds. Shu, Q. Y., Forster, B. P. & Nakagawa, H. (Wallingford: CABI), 135–158. DOI: 10.1079/9781780640853.0135
- Ma, L., Kong, F., Sun, K., Wang, T. & Guo, T. (2021). From classical radiation to modern radiation: past, present, and future of radiation in mutation breeding. *Frontiers in Public Health*, **9**: 768071. doi: 10.3389/fpubh.2021.768071
- Marcu, D., Damian, G., Cosma, C. & Cristea, V. (2013). Gamma radiation effects on seed germination, growth and pigment content, and ESR study of induced free radicals in maize (*Zea mays*). *Journal of Biological Physics*, **39**(4): 625–634.
- Mba, C., Afza, R. & Shu, Q. Y. (2011). *Plant mutation breeding and biotechnology*. (In) *Mutagenic radiations: X-rays, ionizing particles and ultraviolet*, Eds. Shu, Q. Y., Forster, B. P. & Nakagawa, H. (Wallingford: CABI), pp. 83–90. DOI: 10.1079/9781780640853.0083.
- Mittal, K. C. (2012). High power electron accelerators for radiation processing and its safety aspects. *Journal of Pure and Applied Physics*, **50**: 772–775.
- Mondal, S., Petwal, V. C., Badigannavar, A. M., Bhad, P. G., Verma, V. P., Goswami, S. G. & Dwivedi, J. (2017). Electron beam irradiation revealed genetic differences in radio-sensitivity and generated mutants in groundnut (*Arachis hypogaea* L.). *Applied Radiation and Isotopes*, **122**: 78–83.
- Saif-u-Malook, S. A., Qaisarani, M. K., Shabaz, H., Ahmed, M., Nawaz, M. & Qurban, A. (2015). Mutation breeding approach to breed drought-tolerant maize hybrids. *International Journal of Biosciences*, **6**(2): 427–436.
- Sao, R., Sahu, P. K., Sharma, D., Vishwakarma, G., Nair, J. P., Petwal, V. C. & Das, B. K. (2020). Comparative study of radio-sensitivity and relative biological effectiveness of gamma rays, X-rays, electron beam and proton beam in short grain aromatic rice. *Indian Journal of Genetics and Plant Breeding*, **80**(4): 384–394.
- Shrivastava, R., Mondal, S., Patel, N. B., Purkayastha, S. & Devi, Y. L. (2021). Standardization of GR50 dose of gamma rays for mutation breeding experiments in safflower (*Carthamus tinctorius* L.). *Indian Journal of Genetics and Plant Breeding*, **81**(3): 474–477.
- Smýkal, P., Vernoud, V., Blair, M. W., Soukup, A., & Thompson, R. D. (2014). The role of the testa during development and in the establishment of dormancy of the legume seed. *Frontiers in Plant Science*, **5**: 351.
- Viana, V. E., Pegararo, C., Busanello, C. & Oliveira, A. C. (2019). Mutagenesis in rice: The basis for breeding a new super plant. *Frontiers in Plant Science*, **10**: 326.
- Yusuf, K. K. & Nair, P. M. (1974). Effect of gamma irradiation in indole acetic acid synthesizing system and its significance in sprout inhibition of potatoes. *Radiation Botany*, **14**: 251–256.
- Zhao, X., Joo, J., Kim, D., Lee, J. & Kim, J. (2016). Estimation of the seedling vigor index of sunflowers treated with various heavy metals. *Journal of Bioremediation and Biodegradation*, **7**(3): 1–6.