Radiation effect on germination and seedling traits in rice (*Oryza sativa* L.)

R. Lalitha, P. Arunachalam, A. Mothilal, N. Senthil, G. Hemalatha, C. Vanniarajan, and J. Souframanien

ISSN: 0975-928X

Volume: 10

Number:3

EJPB (2019) 10(3):1038-1048

DOI: 10.5958/0975-928X.2019.00133.9

https://ejplantbreeding.org

Electronic Journal of Plant Breeding, 10 (3): 1038-1048 (Sep 2019) DOI: 10.5958/0975-928X.2019.00133.9
ISSN 0975-928X

Research Article

Radiation effect on germination and seedling traits in rice (*Oryza sativa* L.)

R. Lalitha[1], P. Arunachalam[2*], A. Mothilal[3], N. Senthil[4], G. Hemalatha[5], C. Vanniarajan[2], and J. Souframanien[6]

[1]Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore – 641 003, India
[2]Department of Plant Breeding and Genetics, Agricultural College and Research Institute, TNAU, Madurai -625 104, India
[3]Regional Research Station, TNAU, Vridhachalam – 606 001
[4]Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore – 641 003, India
[5]Community Science College and Research Institute, TNAU, Madurai -625 104, India
[6]Nuclear Agriculture & Biotechnology Division, BARC, Trombay, Mumbai - 400085.
[*]**E-Mail**: arunachalp@gmail.com

(Received: 07 Aug 2019; Revised: 27 Sep 2019; Accepted: 28 Sep 2019)

Abstract

The biological effect of new source of physical mutagen *viz.*, electron beam over gamma rays were studied with different dosages in the M_1 generation of rice during 2017-18. The seeds of rice variety Anna (R) 4 rice were treated with 100 to 400 Gy of electron beam and gamma rays. At higher dosage level of 400 Gy both in gamma rays and electron beam, the germination percentage was reduced to 28 and 24 per cent respectively. The LD_{50} value was fixed as 273.27 Gy for electron beam, which is much lower dose than gamma rays (376.57Gy). During germination, root length was more affected than shoot length in both radiations. The electron beam irradiation affected maximum reduction in pollen fertility (61.87%) and in spikelet fertility (72.75%) at 400 Gy, whereas at same dose lesser reduction was observed in gamma rays. The relationship between pollen and spikelet fertility showed positive significant. The above information obtained from this study is more useful to understand the effect of electron beam on rice over gamma rays and also help to decide the dose for future mutation breeding programme in rice.

Key words

Rice, mutation, electron beam, gamma rays, $LD_{50,}$ fertility

Introduction

Rice is the most important cereal crop which belongs to the family Poaceae. It feeds two third of the world population. Mutation is the primary source of all genetic variations existing in any organism, including plants. It provides a raw material for natural selection and is a driving force in evolution, particularly in crops. Asia leads the world in developing mutant plant varieties with an approximate amount of 1965 varieties while comparing the three Asian countries, India ranked top the list globally (Kharkwal, 2018). Electron beam is a new source of physical mutagen interacts with atoms by a variety of Coulomb force interactions compared to gamma rays because of its low penetrating power (< 2 inches). The absorbed dose rate of electron beam on biomaterials may reach even upto 10^{10} Gy.s^{-1}, which is much higher than that of gamma rays (usually under 60 Gy.s^{-1}) (Zhu *et al.* 2008). The present investigation is focused to determine the lethal dose, to understand the effect on seedling growth and fertility due to electron beam over conventionally used gamma rays in rice.

Materials and Methods

Freshly harvested Anna (R) 4 rice cultivar seeds were used for mutation studies. The seeds were obtained from Agricultural Research Station (ARS), Paramakudi, Tamil Nadu, India. Details of Anna (R) 4 rice variety used for irradiation in the present investigation has been summarized in Table 1.

Each 1000 well filled seeds of Anna (R) 4 rice were exposed to different doses of gamma rays and electron beam ranged from 100 to 400Gy with an interval of 50Gy. Gamma rays (^{60} Co) treatment of seeds was undertaken at Bhabha Atomic Research Centre, Mumbai. The seeds were treated with electron beam at 10 MeV at electron beam accelerator facility at Electron Beam Centre, Bhabha Atomic Research Centre, Kharghar, Navi Mumbai, India. Non-irradiated seeds of Anna (R) 4 rice cultivar were considered as control.

Irradiated seeds of Anna (R) 4 rice were allowed to germinate in roll towel method using fifty seeds for each three replication. To study the radiation extension on mean germination percentage, the treated seeds were considered as germinated one when they exhibited a radical extension of >2mm at seventh day. Based on the per cent germination of different dose, the LD_{50} value was assessed using probit analysis (Finney, 1971, 1978) for gamma rays and electron beam. The shoot and root

Electronic Journal of Plant Breeding, 10 (3): 1038-1048 (Sep 2019) DOI: 10.5958/0975-928X.2019.00133.9
ISSN 0975-928X

length reduction was recorded at fourteen days after placing the seeds in a germination paper to examine the effect of radiation sources in early stages of germination and growth.

The field experiment was conducted at Agricultural College and Research Institute, Madurai from October 2017 to January 2018. The remaining seeds of gamma ray and electron beam treated Anna (R) 4 rice with a dosage of 100Gy, 150Gy, 200Gy, 250Gy, 300Gy, 350Gy and 400Gy were raised in nursery. The seedlings were transplanted to main field at 25 days after sowing and the recommended agricultural practices for short duration rice were followed to raise a healthy crop.

The pollens were collected from unopened flowers of five randomly selected plants in each treatment including control. The collected flowers were placed in a petridish poured with water followed by dissect out the flowers and placed in a slide. The pollens were treated with 1% potassium iodide (KI) and fix it with cover slide and were examined under microscope with camera attachment. Well stained and round pollens grains were considered as fertile and shriveled and empty pollen grains were counted as sterile.

Pollen fertility percentage = Total number of well stained pollen grains/ Total number of well stained and unstained pollen grains x 100

The number of well filled grains and unfilled grains were counted from primary panicle in each treatment. The spikelet fertility percentage was derived from the formula given below.

Spikelet fertility percentage = Total number of well filled grains/Total number of well filled and unfilled (chaffy) grains x 100.

Based on linear regression analysis, the equation was obtained and it is used to determine the per cent growth reduction (GR) *viz.*, GR_{30}, GR_{50} and GR_{75} due to radiation effect on shoot and root length. Similarly, the effect of radiation on pollen fertility and spikelet fertility reductions were calculated.

Results and Discussions

The effect of gamma rays and electron beam irradiation of Anna (R) 4 rice showed gradual inhibition of germination with increased doses from 100 Gy to 400 Gy. The germination percentage of irradiated seeds ranged from 92 (100Gy) to 28 per cent (400Gy) for gamma rays and 80 (100Gy) to 24 per cent (400Gy) for electron beam. It indicates that there is significant influence of gamma rays and electron beam irradiation towards germination in Anna (R) 4 rice cultivar. The LD_{50} value was determined from germination percentage, which is 376.57 Gy for gamma rays and 273.27 Gy for electron beam treated Anna (R) 4 rice. Higher reduction of germination was noticed in electron beam compared to gamma rays (Fig.1 and Table 2). Though electron beam and gamma rays have same linear energy transfer value, electron beam is exposed as short and intense pulses as compared to gamma rays. Hence, electron beam produce more biological effect in lower doses. Similar results were reported by Gowthami *et al*. (2016) for electron beam in rice. Preuss and Britt (2003) opined that the cell cycle arrest in G_2/M phase during somatic cell division might be a cause of germination inhibition with increased dosage of gamma radiation in Arabidopsis. When cells are exposed to higher doses of gamma radiation in *Dolichos lablab,* a significant reduction of active mitotic index and increment of total abnormality percentage such as stickiness, precocious movement, laggard and disorientation were observed by Kumar and Swati (2017).

In M_1 generation, gamma rays and electron beam influences on early stage of germination was determined by shoot and root length growth at 14^{th} day of germination. The per cent reduction of shoot length due to irradiation of gamma rays and electron beam varied from 9.08 (100 Gy) to 53.80 (400 Gy) and 13.89 (100 Gy) to 53.71 (400 Gy) respectively. Similarly, the per cent reduction in root length ranged from 17.61 (100 Gy) to 73.32 (400 Gy) for gamma rays and 34.47 (100 Gy) to 71.21 (400 Gy) for electron beam (Table 2). It revealed that increase in radiation doses resulted in gradual reduction of shoot and root length in both gamma ray and electron beam. Among the traits, root length was more sensitive for gamma rays and electron beam treated Anna (R) 4 rice when compared to shoot length. These findings were in accordance with Sasikala and Kalaiarasi (2010) for higher root length reduction with increase in dose compared to shoot length for gamma rays and (Gowthami *et al.* (2017), for both gamma ray and electron beam in rice. Reduced mitotic activity in meristematic tissues and reduction of moisture content in seeds after irradiation with higher dose of gamma irradiation may be responsible for shoot and root length reduction (Khalil *et al*. 1986). Girija *et al*. 2013 reported cytological changes in root tip cell of *Vigna unguiculata* like anaphasic bridges, laggards and stickiness which arised due to increase in dose of gamma ray treatment in seeds from 100 to 300 Gy. However, Raines *et al.* (2016) observed cytokinin response factors (CRFs) important for modulating shoot and root growth in *Arabidopsis thaliana*. Further, they observed certain disruption of multiple CRF_S which in turn leads to the formation of smaller root meristems, reduced primary and lateral roots, etiolated seedlings and a shorter hypocotyls.

The pollen fertility and spikelet fertility percent was found to be inversely proportional to the dose of mutagen in all the treatments. The per cent reduction for pollen fertility varied from 8.52 (100 Gy) to 54.06 (400 Gy) in gamma rays and 20.08 (100Gy) to 61.87 (400Gy) in electron beam. For spikelet fertility per cent reduction ranged from 2.18 (100 Gy) to 32.07 (400Gy) in gamma rays and 2.76 (100Gy) to 72.75 (400Gy) in electron beam (Table 3 and Fig. 2). Similar results were reported by Akilan *et al*. (2019) for pollen fertility and spikelet fertility and Chemma and Atta (2003) for spikelet fertility in gamma rays. Compared to gamma rays, electron beam had a higher level of fertility reduction in rice and also supported by Gowthami *et al*. (2017) in rice. The decrease in fertility of rice after irradiation is considered due to the chromosomal abnormalities (Matsuo and Onazawa, 1961). Anther development and viable pollen formation is a prerequisite for spikelet fertility (Liu *et al.* 2013). Phytohormones like auxin, jasmonic acid and gibberellic acid also play a crucial role in spikelet fertility. The anther and pollen formation regulated by GAMYB (GA **my**elo**b**lastosis) gene in rice and barley participate in GA signaling (Millar and Gubler 2005). The GA deficient and *gamyb* mutants exhibited common defects in programmed cell death (PCD) of tapetal cells and formation of exine and ubisch bodies in rice (Aya *et al.,* 2009).

The relation between the pollen sterility and spikelet sterility was estimated by correlation coefficient (r). The results revealed that irrespective of source of irradiation the pollen fertility and spikelet fertility shows strong positive correlation in gamma rays (0.95) and electron beam (0.91) mutant population (Table 4.)

The growth reduction by 50 per cent (GR_{50}) was studied in shoot and root length of Anna(R) 4 rice derived from linear regression analysis. Significant growth reduction by 50 per cent in shoot length was observed at 403.26 Gy in gamma rays and 359.71 Gy in electron beam. Similarly GR_{50} for root length was 246.31 Gy for gamma rays and 245.10 Gy for electron beam (Table 5 and Fig 3.). The spikelet fertility reduction by 50 per cent for gamma rays and electron beam mutants was estimated as 806.45Gy and 413.22Gy respectively, and for pollen fertility reduction was 387.60 in gamma rays and 335.57 in electron beam. The findings revealed that root length and pollen fertility are sensitive to irradiation.

This M_1 generation study revealed that electron beam had ability to cause intense reduction in germination at lower dose (LD_{50} = 273.27 Gy) compared to gamma rays (LD_{50} = 376.57 Gy). In early stages of germination the root growth was found to more sensitive to irradiation than shoot growth. The pollen fertility reduction was dose dependent, which was high in electron beam as compared to gamma rays in rice. The pollen fertility also significantly impact on spikelet fertility as it shows positive correlation. Therefore, electron beam at minimum dosage level of 350Gy and less can be better used for irradiation of rice seeds in future rice improvement programmes to reduce injury and sterility for also to obtain the viable mutants.

Acknowledgements

We extend our sincere thanks to Government of India, Board of Research in Nuclear Sciences, Mumbai for providing a research grant [35/14/13/2017-BRNS] for this study, Agricultural College and Research Institute, Madurai for extending laboratory and field facilities. Further first author thanks BRNS for providing Junior Research Fellowship.

References

Akilan, M. Anand, G. Vanniarajan, C. Subramanian, E. and Anandhi, K. (2019). Study on the Impact of mutagenic treatment on pollen and spikelet fertility and its relationship in rice (*Oryza sativa* L.). *Electronic journal of Plant Breeding*, **10**(2): 525 – 534.

Aya, K. Ugeuchi- Tanaka, M. Kondo, M. Hamada, K. Yano, K. Nishimura, M. and Matsuoka, M. (2009). Gibberellin modulates anther development in rice via the transcriptional regulation of GAMYB. *The Plant cell*, **21** (5): 1453 – 1472.

Cheema, A. A. and Atta, B. M. (2003). Radiosensitivity studies in basmati rice. *Pak. J. Bot*, **35**(2): 197 -207.

Finney, D.J. (1971). Probit analysis. Cambridge University Press.

Finney, D.J. (1978). Statistical method in biological assay. Charles Griffin & Co.

Girija, M. Gnanamurthy, S. and Dhanavel, D. (2013). Cytogenetics effect of gamma rays on root meristem cells of *Vigna unguiculata* (L.). *European Journal of Experimental Biology*, **3**(2), 38-41.

Gowthami, R. Vanniarajan, C. Souframanien, J. and Arumugam Pillai, M. (2016). Effect of gamma rays and electron beam on various quantitative traits of rice (*Oryza sativa* L.) in M_1 Generation. Advances in life *sciences*, **5**(5), 1876-1882.

Gowthami R., Vanniarajan C., Souframanien J., and Arumugam Pillai M. (2017). Comparison of radiosensitivity of two rice (*Oryza sativa* L.) varieties to gamma rays and electron beam in M_1 generation. *Electronic Journal of Plant Breeding*, **8**(3), 732-741.

Khalil, S. J. Rehman, S. Afridi, K. and Jan, M. T. (1986). Damage induced by gamma irradiation in morphological and chemical characteristics of barley. *Sarhad Journal of Agricultural*, 2: 45-54.

Kharkwal, C. B. (2018). Mutation breeding for crop improvement. Geography andYou.

Kumar, G and Swati, K. (2017). Germination and cytological aspects of *Dolichos lablab* L. exposed to gamma irradiation . *Chromosome Botany*, **12**(3), 63-71.

Liu, W., Zhang, D., Tang, M., Li, D., Zhu, Y., Zhu, L., Chen C (2013). THIS1 is a putative lipase that regulates tillering, plant height, and spikelet fertility in rice. *Journal of Experimental Botany*, **64**(14), 4389–4402.

Matsuo, T. and Onozawa. (1961). Mutations induced in rice by ionizing radiations and chemicals. In: Effects of Ionizing Radiations on Seeds, IAEA,Vienna. Pp. 495 – 501.

Millar, A.A., & Gubler, F. (2005). The Arabidopsis GAMYB-like genes, MYB33 and MYB65, are microRNA-regulated genes that redundantly facilitate anther development. *The Plant Cell*, **17**(3), 705–721.

Preuss, S. B. and Britt A.B. (2003). A DNA-Damage-Induced Cell Cycle Checkpoint in Arabidopsis. *Journal of Genetics*, **164,** 323–334.

Raines, T. Carly Shanks, Chia-Yi Cheng, Duncan McPherson, Cristiana T. Argueso, Hyo J. Kim, Jose M. Franco-Zorrilla, Irene Lopez-Vidriero, Roberto Solano, Radomira Vankova, G. Eric Schaller, Joseph J. Kieber. (2016). The cytokinin response factors modulate root and shoot growth and promote leaf senescence in Arabidopsis. *The Plant Journal*, **85**, 134–147.

Sasikala, R. and Kalaiyarasi, R. (2010). Sensitivity of rice varieties to gamma irradiation. *Electronic Journal of Plant Breeding*, **1**(4), 885-889.

Zhu H., Xu J., Li S., Sun X., Yao, S., and Wang S. (2008). Effect of high-energy-pulse-electron beam radiation on biomolecules. *Science in China Series B-Chemistry*, **51**, 86-91.

Electronic Journal of Plant Breeding, 10 (3): 1038-1048 (Sep 2019) DOI: 10.5958/0975-928X.2019.00133.9
ISSN 0975-928X

Table 1. Particulars of Anna (R) 4 rice variety

Year of release	:	2009
Pedigree	:	Pantdhan 10 X IET 9911
Duration (in days)	:	100 -105
Average Yield (kg/ha)	:	3700
Thousand grain weight(g)	:	25.7
L/B ratio	:	3.45
Growth habit	:	Semi-dwarf, erect and non-lodging
Rice quality and colour	:	Long slender white rice
Special features	:	Drought tolerance

Table 2. *In vitro* studies on radiation effect of germination, shoot length and root length in Anna(R) 4 rice

Irradiation doses (Gy)	Germination(%) on 7th day	Shoot length(cm) on 14th day	Root length (cm) on 14th day)
Gamma rays			
Control	98	11.45 (0.00)	20.05(0.00)
100 Gy	92	10.41(9.08)	16.52(17.61)
150 Gy	90	9.20(19.65)	14.10(29.68)
200 Gy	86	9.19(19.74)	11.61(42.10)
250 Gy	78	8.93(22.01)	9.61(52.07)
300 Gy	84	6.70(41.49)	6.32(68.48)
350Gy	48	6.22(45.68)	5.43(72.92)
400 Gy	28	5.29(53.80)	5.35(73.32)
Treatment mean	72.29	7.99	9.85
Electron beam			
100 Gy	80	9.86(13.89)	13.14(34.47)
150 Gy	70	9.56(16.51)	13.69(31.70)
200 Gy	60	9.27(19.04)	10.58(47.24)
250 Gy	56	7.12(37.82)	8.27(58.75)
300 Gy	50	6.12(46.55)	7.10(64.59)
350Gy	46	6.03(52.66)	6.65(66.80)
400 Gy	24	5.30(53.71)	5.77(71.21)
Treatment mean	55.14	7.61	9.31
Overall mean	**66.00**	**8.04**	**10.28**
SEd	2.58	0.12	0.24
CD(P=0.05)	5.06	0.24	0.47

*Values in parentheses indicates per cent reduction over control [Anna (R) 4]

 DOI: 10.5958/0975-928X.2019.00133.9
ISSN 0975-928X

Table 3. Effect on pollen and spikelet fertility in gamma rays and electron beam in rice

	Gamma rays			Electron beam		
Irradiation (Gy)	**Pollen fertility (%)**	**Reduction over control (%)**	**Sterility (%)**	**Pollen fertility (%)**	**Reduction over control (%)**	**Sterility (%)**
Control	98.60	0.00	1.40	98.60	0.00	1.40
100	90.20	8.52	9.80	81.20	17.65	18.80
150	86.43	12.34	13.57	78.80	20.08	21.20
200	73.33	25.63	26.67	68.40	30.63	31.60
250	69.30	29.72	30.70	64.80	34.28	35.20
300	57.60	41.58	42.40	58.04	41.44	41.96
350	53.00	46.25	47.00	44.30	55.07	55.70
400	45.30	54.06	54.70	37.60	61.87	62.40

	Gamma rays			Electron beam		
Irradiation (Gy)	**Spikelet fertility (%)**	**Reduction over control (%)**	**Sterility (%)**	**Spikelet fertility (%)**	**Reduction over control (%)**	**Sterility (%)**
Control	90.40	0.00	9.60	90.40	0.00	9.60
100	88.43	2.18	11.57	87.91	2.76	12.09
150	87.50	3.21	12.50	83.58	7.54	16.42
200	86.19	4.66	13.81	74.21	17.91	25.79
250	76.44	15.44	23.56	66.57	26.36	33.43
300	71.51	20.90	28.49	64.62	28.52	35.38
350	71.59	20.81	28.41	58.09	35.74	41.91
400	61.41	32.07	38.59	24.63	72.75	75.37

Table 4. Correlation between pollen and spikelet sterility induced by irradiation in rice

Gamma rays	Pollen sterility	Spikelet sterility
Pollen sterility	1	
Spikelet sterility	0.95	1

Electron beam	Pollen sterility	Spikelet sterility
Pollen sterility	1	
Spikelet sterility	0.91	1

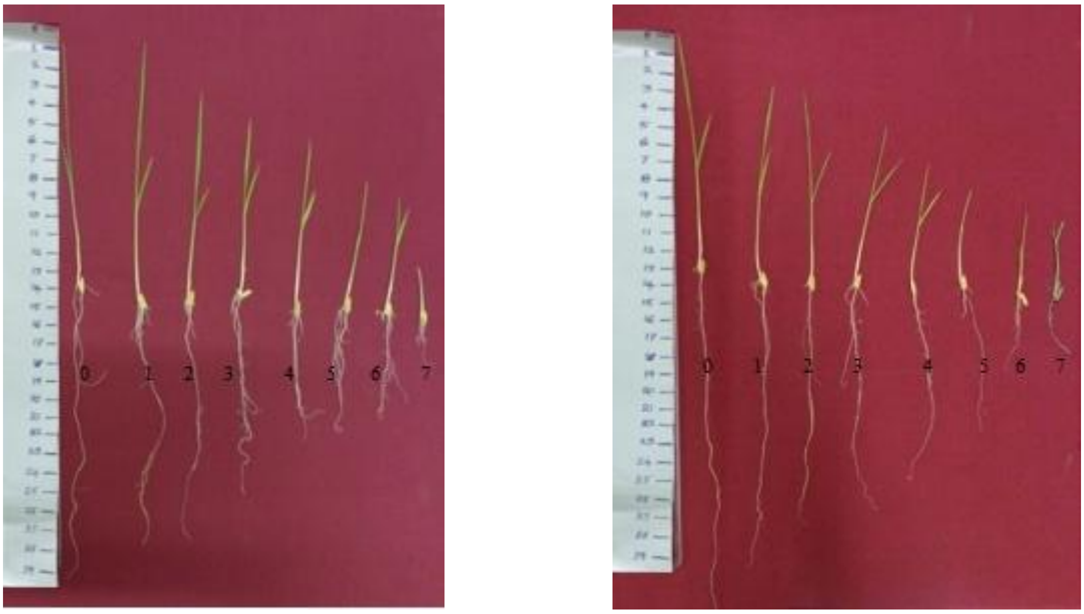
Electronic Journal of Plant Breeding, 10 (3): 1038-1048 (Sep 2019) DOI: 10.5958/0975-928X.2019.00133.9
ISSN 0975-928X

Table 5. The effect of radiation in Anna (R) 4 rice on seedling and fertility characters

Traits	Sources	Linear equation	Growth and fertility reduction (Gy)		
			30 %	**50 %**	**75 %**
Shoot length (14^{th} day)	Gamma rays	Y=0.124x (R^2 = 0.935)	241.94	403.26	604.84
	Electron beam	Y=0.139 x (R^2 = 0.948)	215.83	359.71	539.57
Root length (14^{th} day)	Gamma rays	Y=0.203 x (R^2 = 0.974)	147.78	246.31	369.46
	Electron beam	Y= 0.204 (R^2 = 0.890)	147.06	245.10	343.18
Pollen fertility (%)	Gamma rays	Y=0.129 x (R^2=0.965)	232.56	387.60	581.40
	Electron beam	Y = 0.149 x (R^2 = 0.982)	201.34	335.57	503.36
Spikelet fertility (%)	Gamma rays	Y=0.062 x (R^2 = 0.817)	483.87	806.45	1209.68
	Electron beam	Y = 0.121 x (R^2 = 0.754)	247.34	413.22	619.83

Electronic Journal of Plant Breeding, 10 (3): 1038-1048 (Sep 2019) DOI: 10.5958/0975-928X.2019.00133.9
ISSN 0975-928X

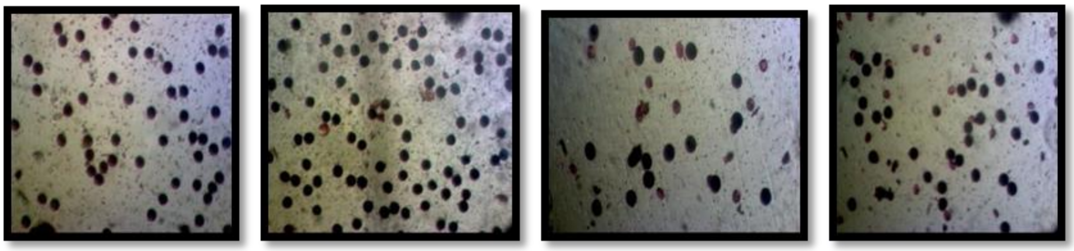
Gamma rays Electron beam



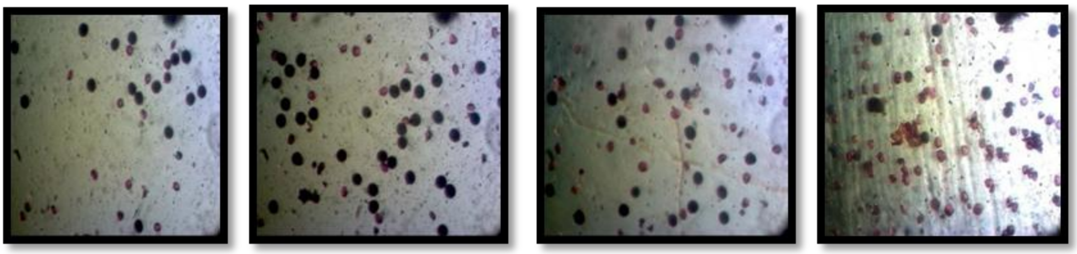
Dose: 0 –Control, 1- 100Gy, 2 -150Gy, 3- 200Gy, 4- 250Gy, 5-300Gy, 6-350Gy, 7-400Gy

Fig. 1. Shoot and root length variation at 14th day in roll towel method for gamma rays and electron beam treated Anna(R) 4 rice

Gamma rays

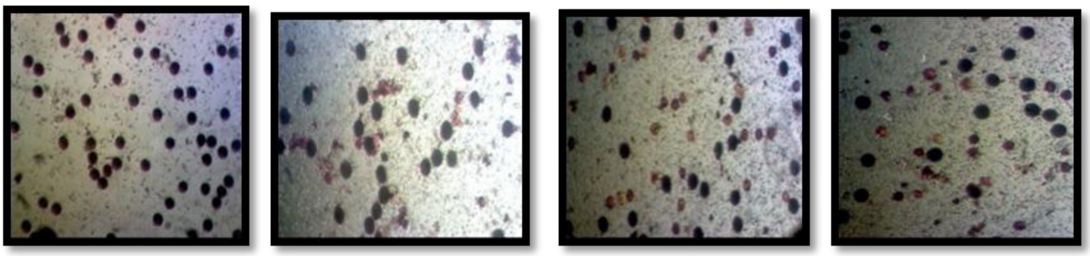


C-Anna (R) 4 100Gy 150Gy 200Gy

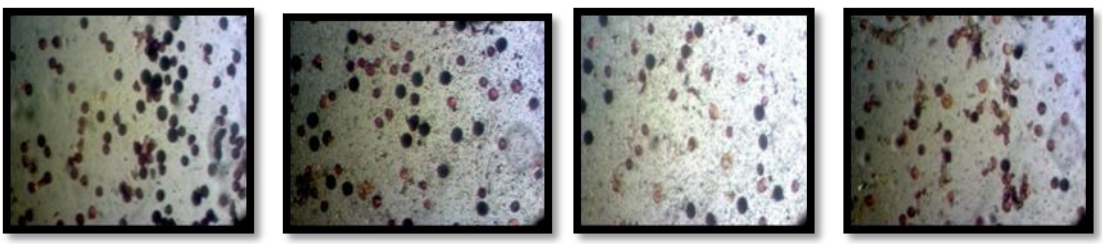


250Gy 300Gy 350Gy 400Gy

Electron beam



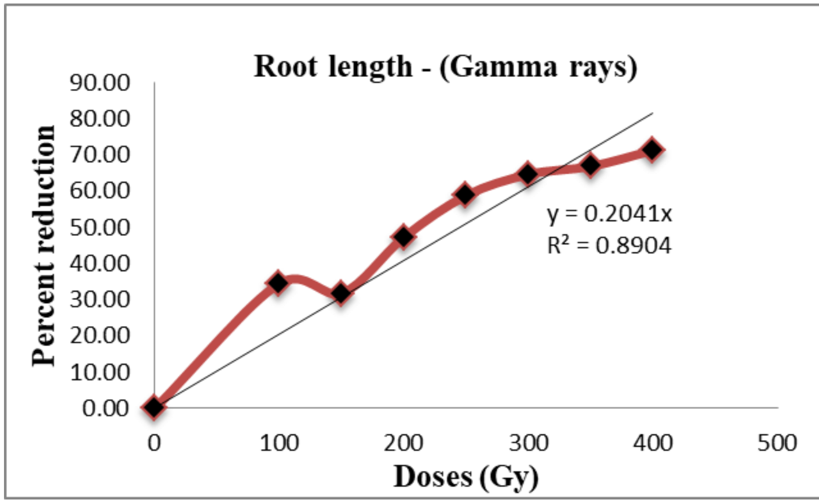
C-Anna (R) 4 100Gy 150Gy 200Gy

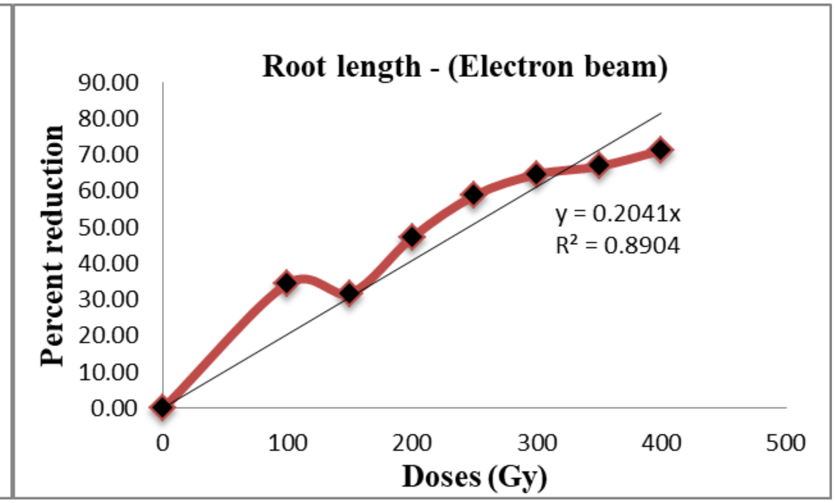


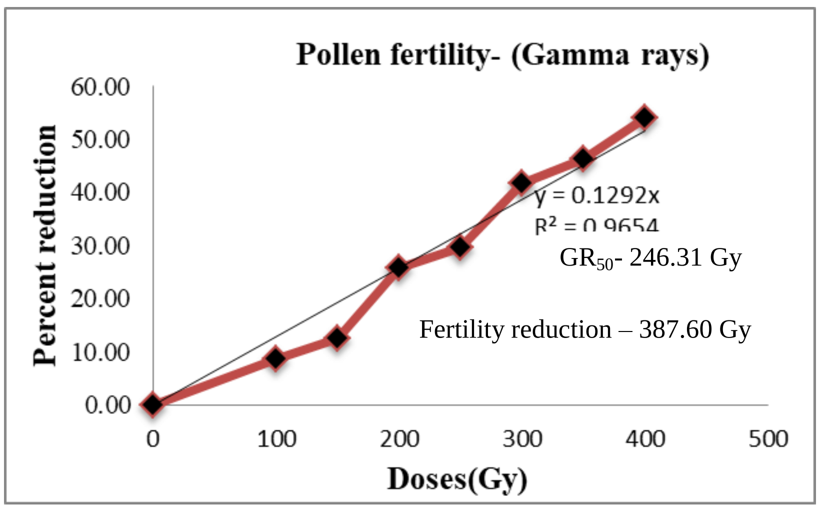
250Gy 300Gy 350Gy 400Gy

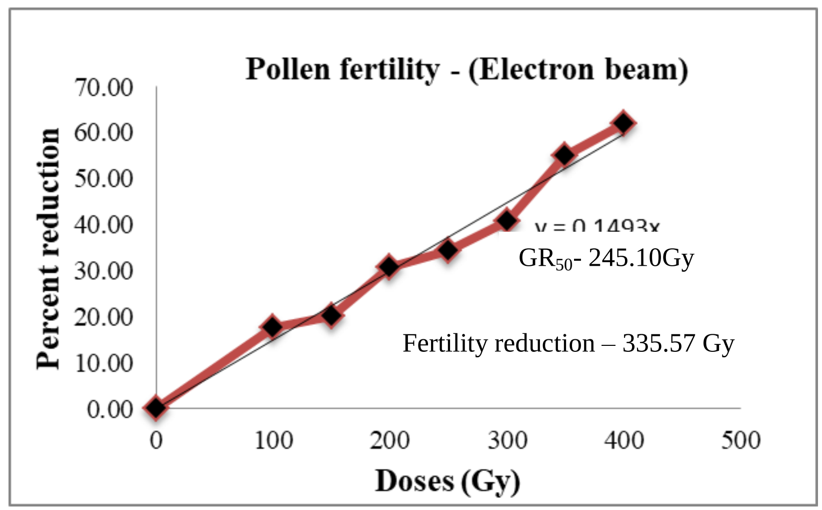
Fig. 2. Effect of gamma rays and electron beam on Pollen fertility in M_1 generation of Anna (R) 4 rice

Electronic Journal of Plant Breeding, 10 (3): 1038-1048 (Sep 2019) DOI: 10.5958/0975-928X.2019.00133.9
ISSN 0975-928X









Electronic Journal of Plant Breeding, 10 (3): 1038-1048 (Sep 2019) DOI: 10.5958/0975-928X.2019.00133.9
ISSN 0975-928X

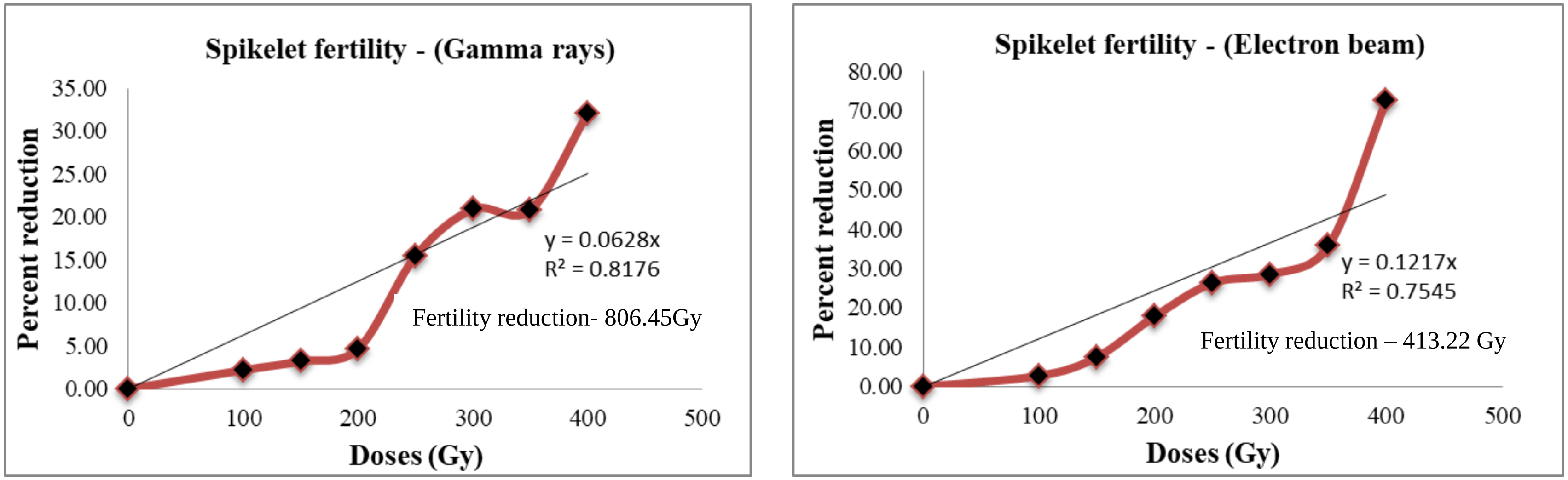


Fig. 3. The effect of radiation in Anna(R) 4 rice on growth and yield attributing characters based on GR_{50}

INDIAN SOCIETY OF PLANT BREEDERS

ESTD 1995

https://ejplantbreeding.org