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Research Article

Comparative mutagenesis and effect of EMS, Gamma rays and Electron beam on biological parameters of Kalbhat and black rice (*Oryza sativa* L.) non-basmati aromatic rice landraces

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Abstract
Kalbhat and Black rice are two non-basmati scented rice landraces famous for their taste, aroma, and nutritional properties. Mutation was attempted by using ethyl methanesulfonate, gamma radiation, and electron beam in Kalbhat and Black rice. In M_1 generation studies, the effect of mutagens on several biological variables was studied, like lethality, chromosomal abnormalities, pollen sterility, and plant survival. In M_2 generation studies, different chlorophyll mutants like Albina, Xantha, Chlorina, and Viridis were identified. The frequency of chlorophyll mutants was relatively higher in Black rice (9.54) as compared to Kalbhat (7.72). Mutagenic effectiveness and efficiency with respect to seedlings height, lethality, and pollen sterility were studied, and the maximum effectiveness and efficiency were observed in EMS treatment in both Black rice and Kalbhat.The lethality and pollen sterility was maximum in EMS treated Kalbhat, while mutagenic efficiency in terms of seedlings height and pollen sterility were maximum in the electron beam treated black rice. EMS was found to be more effective and efficient than gamma radiation and electron beam treatment in both the landraces.

Keywords: Black rice, Effectiveness, Kalbhat, landraces, Mutation frequency

INTRODUCTION

Rice is cultivated all over the world and is a staple food in the most populated parts of the world. Asia is known as the birthplace of rice crops. China and India produce more than 95% of global rice (Tiwari *et al.,* 2018). In Asia, rice is considered an economically and traditionally important cereal food crop. India ranks second after China for the production of rice in the world. (Chakaravarti *et al.,* 2013).

Blessed with a natural gift, the Indian subcontinent is home to the world-famous Basmati rice, which is highly valued in both domestic and foreign market for its exceptional flavor and long grain. Traditional rice landraces also have outstanding aroma and nutritional properties, like basmati rice. However, many unrecognized rice landraces, such as Kalbhat, Black rice, Ajra Ghansal, Kala Jirga, Govinbhog, Ambemohar, Kalanamak, Kalimuch, Chinur, Raibhog, Tamsal, etc., are interesting cost-effective replacements for expensive basmati rice. Low yield, tallness, lodging, and longer maturity period are common problems in non-basmati aromatic rice landraces.They show notable variations in morphological, agricultural, nutritional, and disease-resistance traits. (Mondol *et al.,* 2021).

Kalbhat is a non-basmati scented rice landrace from Pune district (Maharashtra), which is cultivated on a small scale in some pockets of Pune district only. Kalbhat possesses the highest amount of 2-Acetyl-1-pyrroline (2AP) content and has great demand, and fetches good prices in the market. Black rice is cultivated in the Northeast states of India like Manipur, and it is popular for its nutritional and medicinal values. Black rice is an excellent source of vitamins, minerals, and dietary fiber (Nitinkumar and Murali, 2020). According to Kushwaha (2016), purple rice is a rich source of antioxidants and vitamins (Vit. A, B, and E) and is free from gluten. Black rice, is known popularly as “Chakhao” (Manipuri), which means “delicious rice” (Nitinkumar and Murali, 2020). Many health advantages of the rice have been described, including protection of heart health (Pal, 2018), control of hypertension, detoxification of the body, improvement of lipid profile, improvement of vision, increased life span, and reduced atherosclerosis (Ling *et al.,* 2001), osteoporosis, allergy, cancer growth (Chen *et al.,* 2006), risk of asthma, and diabetes (Peng *et al*., 2021). Many researchers consider Black rice as a superfood due to its health benefits and nutritive value (Saha, 2016). There is a requirement to improve the agronomic traits of such traditional rice landraces without changing their original characters.

In self-pollinated crops, mutation breeding performs a crucial role. It is recognized for its ability to develop high variability in a shorter period (Gowthami *et al*., 2016). This approach has been employed to develop about 3362 mutant varieties worldwide (Shadakshari *et al.,* 2001). The present experiment was undertaken to improve Kalbhat and Black rice non-basmati aromatic rice landraces by using ethyl methanesulfonate, gamma rays, and electron beam.

MATERIALS AND METHODS

Dry, uniform, and healthy seeds of Kalbhat were procured from the Pune district of Maharashtra, and seeds of Black rice non-basmati aromatic rice landrace were obtained from Manipur. Ethyl methanesulfonate (0.8,1.0 and 1.2%), gamma rays (100,200 and 300Gy) and Electron beam (100, 200 and 300 Gy), were used for induction of mutation. To determine the exact concentration or doses of mutagens required to induce mutation in both non-basmati aromatic rice landraces, LD 50 was calculated based on seed germination percentage of various doses or concentrations. For each treatment, 500 dry, uniform, and healthy seeds were used. Before starting the EMS treatment seeds were soaked in distilled water for 10 h. Then, EMS treatment (0.8, 1.0, and 1.2%) was given to the soaked seeds for 6 h with constant shaking. The treated seeds were then washed in tap water for two hours. For radiation treatment 500 dry, healthy, and uniform seeds were exposed to 100, 200, and 300Gy doses of gamma radiation at (BARC) Bhabha Atomic Research Centre Trombay, Mumbai and Electron beam from Raja Ramana Centre for Advanced Studies, Indore, Madhya Pradesh. The treated and control seeds were sown in the field. Seedling height was measured 15 dyas after germination and three weeks after germination, the M_1 generation seedlings were transplanted, treatment-wise, in the field. The treated seeds were kept in the petri plates containing wet blotting paper, and after seven days, the germination percentage was calculated. For cytology studies, roots tips were fixed, treatment-wise, in 70% alcohol and used for the chromosomal studies. Root tips were squashed using 1% acetocarmine stain, and chromosomal abnormalities were observed. During seedlings’ development, the chlorophyll-deficient sectors like Albina, Xantha, Chlorina, and Viridis were observed. For pollen sterility studies, the pollen buds were fixed and stained with I_2KI stain to know the pollen sterility percentage (Vinithashri *et al.,* 2020 and Desai *et al.,* 2021). At the end of the M_1 generation, the plants were harvested individually, and survival percentage was determined. In M_2 generation, individually harvested plants were sown on a plant-to-row basis. Seedlings were transplanted in a randomized block design 21 days after germination and different chlorophyll mutants were observed. with. For the determination of mutagenic effectiveness, efficiency and mutation rate the formulae suggested by Konzak *et al* (1965) were used.

Mutagenic effectiveness

$$= \frac{\text{Mutagenic frequency}}{\text{Dose (Krad) or Concentration (C) X Time (t)}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutagenic frequency (M)}}{\text{Biological damage (L or I or S)}} \text{ X100}$$

Mutation rate Sum of value of efficiency or effectiveness of particular mutagens/number of treatments of particular mutagens

Where,

M - Mutation frequency.

Krad Kilorad.

L - Percent reduction in survival.

I - Percent reduction in height.

S - Percent pollen sterility.

RESULTS AND DISCUSSION

In both the landraces, germination percentage was observed to decrease with increasing concentrations of mutagen or radiation dose. A significant reduction in germination percentage was observed at the maximum concentration of EMS, gamma rays, and electron beam in Kalbhat and Black rice landraces (**Table 1 and Table 2**). Similar findings were reorted by Talebi *et al.* (2012), Chakravarti *et al.* (2017), Sharma *et al.* (2020), and Desai *et al.* (2021), in rice. Both landraces showed an increased lethality percentage with higher concentrations of mutagen or radiation doses**.** At higher concentrations of EMS, 1.2%, 10 and 11.34% lethality were observed in Kalbhat and Black rice, respectively. In gamma ray and electron beam treatments, maximum lethality was observed at 300 Gy . The chlorophyll-deficient sectors were increased with rising concentrations of ethyl methanesulfonate, gamma rays, and electron beam treated plants from Kalbhat and Black rice. Maximum chlorophyll deficient sectors were observed in higher

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Table 1. Effect of different mutagens on biological parameters in the M_1 generation of Kalbhat.

Treatment	Germination %	Lethality %	Chlorophyll deficient sectors %	Chromosomal abnormality %	Pollen sterility %	Plant survival %
Control	100	0.0	0.0	0.0	0.0	96.70
EMS0.8%	93.33	06.67	0.4	4.85	4.82	84.20
EMS1.0%	91.66	08.33	0.8	3.84	3.31	86.11
EMS1.2%	90	10.00	0.8	4.19	18.35	78.33
GR 100Gy	95	05.00	0.6	4.60	3.43	87.40
GR 200Gy	93.33	06.67	0.8	3.97	33.65	75.10
GR 300Gy	86.66	13.64	0.8	3.49	54.27	76.22
EB 100Gy	91.36	08.64	0.4	4.01	4.62	75.20
EB 200Gy	80.15	19.85	0.6	3.65	34.24	70.12
EB 300Gy	58.78	41.22	0.6	3.20	31.57	70.40

Table 2. Effect of different mutagens on biological parameters in the M_1 generation of Black Rice

Treatment	Germination %	Lethality %	Chlorophyll deficient sectors %	Chromosomal abnormality Unit %	Pollen sterility %	Plant survival %
Control	100	0.0	0.0	0.0	0.0	95.60
EMS0.8%	90.80	09.20	0.4	3.48	6.78	81.23
EMS1.0%	90.00	10.00	0.6	3.71	9.26	76.44
EMS1.2%	91.66	11.34	0.8	3.58	9.22	79.05
GR 100Gy	91.99	08.01	0.4	3.76	4.61	85.50
GR 200Gy	91.66	08.34	0.8	3.81	5.19	76.20
GR 300Gy	88.33	11.67	1.0	3.63	6.90	79.40
EB 100Gy	88.76	11.24	0.6	4.72	3.81	74.42
EB 200Gy	77.56	22.44	0.8	3.51	6.80	70.12
EB 300Gy	52.22	47.78	0.8	3.20	15.90	67.50

concentration of ethyl methanesulfonate, gamma rays and electron beam doses. Chromosomal abnormalities like laggard, bridge formation, sticky metaphase, and ring formation, were observed from different mutagenic treatments (**Fig 1 and Fig 2**). In Kalbhat, the maximum chromosomal abnormalities were observed in 0.8% EMS treatment (4.85%), while in Black rice, it was observed in 100Gy electron beam dose (4.72%) (**Table 1 and Table 2**). The pollen sterility was observed to increase with an increasing dose or concentration of mutagens from both the landraces. The maximum pollen sterility percentage was observed in the highest dose of gamma radiation and electron beam in Kalbhat and Black rice, respectively. Similar observations were reported by Gowthami *et al.* (2016), Vinithashree *et al.* (2020) and Desai *et al* (2021) in rice.

In the M_2 generation, chlorophyll mutants like Albina, Xantha, Chlorina and Viridis were observed in Kalbhat and Black rice. Among chlorophyll, mutant albina mutant occurred in highest frequency, while Viridis occurred in least frequency in both the landraces. Similar kinds of results in rice were reported by Singh *et al*. (1998), Shadakshari *et al.* (2001), and Desai *et al* (2021). In case of Kalbhat, a maximum of 235 chlorophyll mutants were observed under EMS treatment with a frequency of 2.75%, followed by electron beam (225) with a frequency of 2.75% and gamma rays (160) with a frequency of 2.22% . In Black rice, the highest number of chlorophyll mutants were observed from electron beam treatment (256) with 3.65% frequency, followed by EMS (239) mutants with 3.48% and gamma rays (209) mutants with 2.41% frequency. Black rice exhibited a relatively higher mutation frequency of 9.54% (704 chlorophyll mutants) as compared with Kalbhat of 7.72% (620 chlorophyll mutants) **Table 3 and Table 4.** Similar kinds of observations were reported by Cheema and Atta (2003), Imam *et al.* (2019) Desai *et al.* (2021) and Sao *et al*.(2021) in rice**.**

The frequency of mutation induced by the mutagen is called mutagenic effectiveness, while the mutagenic efficiency indicates the amount of mutation associated changes in biological variables like chromosomal abnormalities, lethality, pollen sterility, and germination percentage (Konzak *et al.,* 1965). Maximum mutagenic effectiveness was observed from EMS treatment from

Table 3. Frequency and spectrum of chlorophyll mutant in M_2 Generation of Kalbhat.

Treatment	Concentration	Total no. of seedlings screened	Total no. of chlorophyll mutant	Total mutation frequency	No. of individual mutants			
					Albino	Xantha	Chlorina	Viridis
Control	-	6221	-	-	-	-	-	-
EMS	0.8%	9180	61	0.66	22	16	18	5
	0.1%	8510	78	0.91	26	21	22	9
	1.2%	8108	96	1.18	34	24	30	8
	Total	25798	235	2.75	82	61	70	22
Gamma Rays	100Gy	10110	43	0.42	18	7	14	4
	200Gy	6416	54	0.84	23	10	18	3
	300Gy	6533	63	0.96	25	15	20	3
	Total	22059	160	2.22	66	32	52	10
Electron Beam	100Gy	12564	59	0.46	25	12	18	4
	200Gy	9812	72	0.73	27	15	21	9
	300Gy	5996	94	1.56	34	22	27	11
	Total	28372	225	2.75	86	49	66	24
Grand Total		76229	620	7.72	234	142	188	56

Table 4. Frequency and spectrum of chlorophyll mutant in M_2 generation of Black Rice.

Treatment	Concentration	Total no. of seedlings screened	Total no. of chlorophyll mutant	Total mutation frequency	No. of individual mutants			
					Albino	Xantha	Chlorina	Viridis
Control	-	8201	-	-	-	-	-	-
EMS	0.8%	8148	68	0.83	21	15	24	8
	1.0%	6726	82	1.21	24	20	26	12
	1.2%	6148	89	1.44	29	26	24	10
	Total	21022	239	3.48	74	61	74	30
Gamma Ray	100Gy	9544	57	0.59	19	15	18	5
	200Gy	8934	70	0.78	23	13	27	7
	300Gy	7817	82	1.04	26	18	27	11
	Total	26295	209	2.41	68	46	72	23
Electron Beam	100Gy	7934	69	0.86	26	11	22	10
	200Gy	7081	89	1.25	31	17	28	13
	300Gy	6329	98	1.54	37	20	29	12
	Total	21344	256	3.65	94	48	79	35
Grand Total		68661	704	9.54	236	155	225	88

both the landraces followed by electron beam and gamma rays (**Table 5 and Table 6**). In case of Kalbhat, maximum mutagenic efficiency with respect to seedling injury and lethality was noticed under 300Gy electron beam and 200 Gy gamma ray treatments, respectively. Whereas in black rice it was observed that highest seedling injury and lethality occured under 300 Gy electron beam and 1.2% EMS treatments, respectively.Similar results were reported by Siddiqi *et al.*(1968), Singh *et al.* (2001) in basmati rice **,** and Singh *et al.* (2015). In case of mutagenic efficiency, maximum mutagenic efficiency with respect to seedling injury, lethality, and pollen sterility was noticed under EMS treatment followed by gamma rays and electron beam treatment from Kalbhat. In Black rice, the highest mutagenic efficiency was observed under EMS treatment, followed by electron beam and gamma rays. The highest mutagenic efficiency with respect to seedling injury was observed under electron beam treatment followed by EMS and gamma rays in both the landraces. With respect to pollen sterility, highest efficiency was observed under EMS treatment, followed by electron beam and gamma ray treatment from Kalbhat

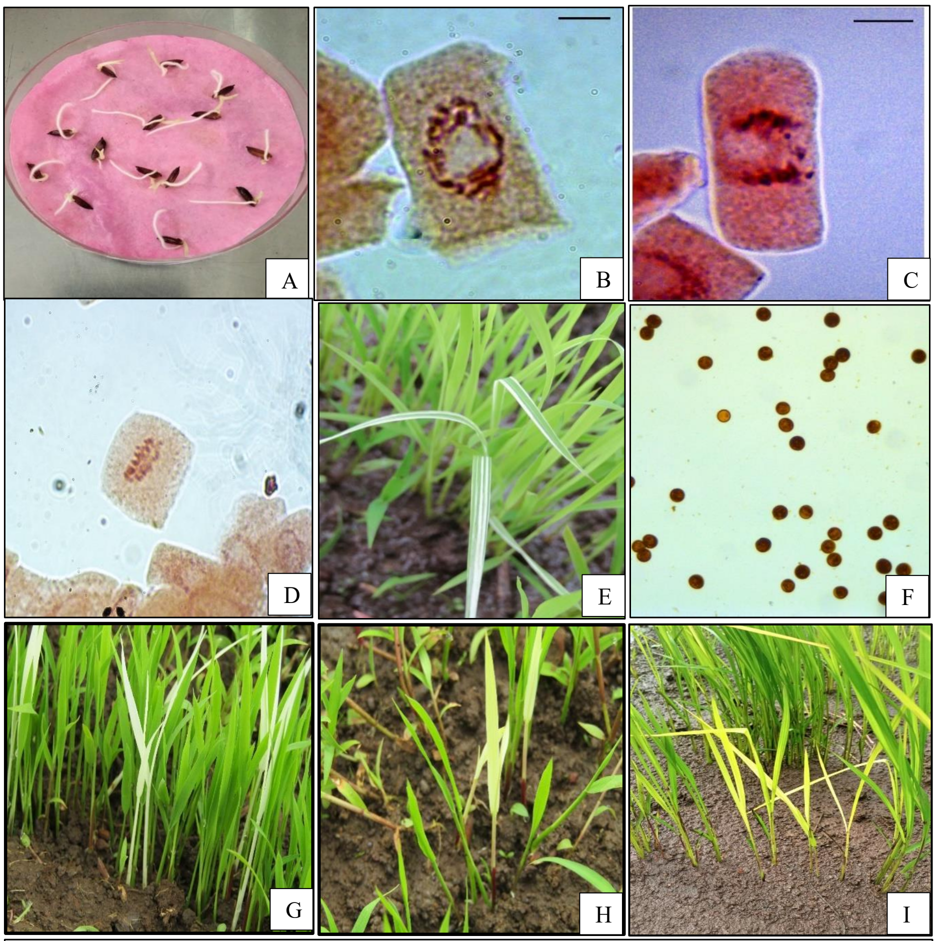

Fig. 1. **A- Seed Germination B- Ring Formation C- Laggard D- Bridge formation E- Chlorophyll deficient Sector F- Pollen sterility G- Albino H- Clorina I-Xantha.**

Table 5. Mutagenic effectiveness and efficiency of gamma rays, EMS, & electron beam treatments in Kalbhat from M_1 and M_2 generation.

Mutagen	Concentration / dose	Seedling height in (I)M1 (cm)	Lethality (L)%	Pollen sterility (S)%	Mutation frequency (MF)	Effectiveness Mf/Gy, cons x time	Efficiency (%)			
							Mx100 (I)	Mx100 (L)	Mx100 (S)	Total
	Control	32.67	-	-	-	-	-	-	-	-
EMS	0.8%	25.94	6.67	4.82	0.66	0.13	2.54	9.89	13.69	26.12
	1.0%	19.22	8.33	3.31	0.91	0.15	4.73	10.92	27.49	43.14
	1.2%	21.55	10.0	18.35	1.18	0.16	5.47	11.8	6.43	23.7
	Total				2.75	0.44	12.74	32.61	47.61	92.96
Gamma Rays	100Gy	23.33	5.00	3.43	0.42	0.004	1.80	8.4	12.24	22.44
	200Gy	17.33	6.67	33.65	0.84	0.004	4.84	12.59	2.49	19.92
	300Gy	17.50	13.64	54.27	0.96	0.003	5.48	7.03	1.76	14.27
	Total				2.22	0.011	12.12	28.02	16.49	56.63
Electron Beam	100Gy	21.38	8.64	4.62	0.46	0.004	2.15	5.32	9.95	15.42
	200Gy	20.33	19.85	34.24	0.73	0.003	3.59	3.67	2.13	9.39
	300Gy	21.33	41.22	31.57	1.56	0.005	7.31	3.78	4.94	16.03
	Total				2.75	0.012	13.05	12.77	17.02	40.84

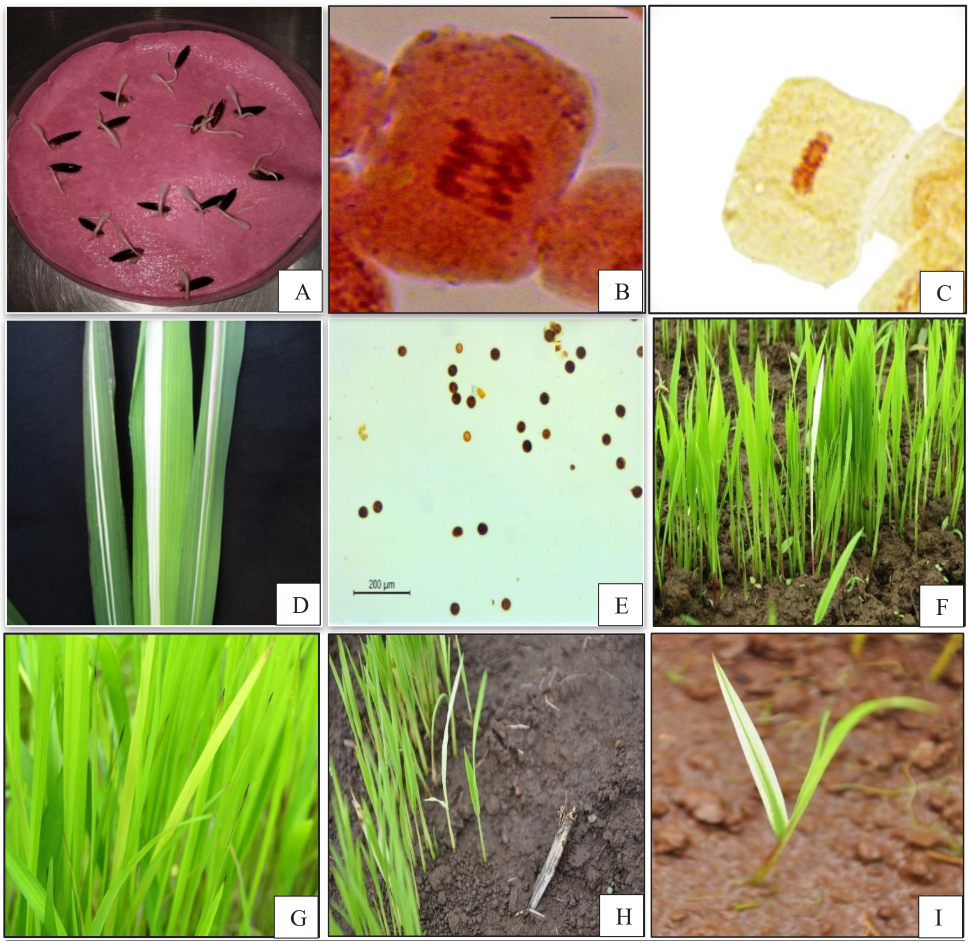

Fig. 2. A- Seed germination B-Bridge formation C- Sticky metaphase D- Chlorophyll deficient sectors, E- Pollen sterility F- Albina G-Xantha H- Chlorina and I- Viridis

Table 6. Mutagenic effectiveness and efficiency of Gamma rays, EMS, & electron beam treatments in Black rice from M_1 and M_2 generation.

Mutagen	Concentration / dose	Seedling height in(I) M1 (cm)	Lethality (L)%	Pollen sterility (S)%	Mutation frequency (MF)	Effectiveness Mf/Gy, cons x time	Efficiency (%)			
							Mx100 (I)	Mx100 (L)	Mx100 (S)	Total
	Control	33.10	-	-	-	-	-	-	-	-
EMS	0.8%	30.76	9.2	6.78	0.83	0.17	2.69	9.02	12.24	23.95
	1.0%	28.62	10	9.26	1.21	0.20	4.22	12.11	13.06	29.39
	1.2%	29.22	11.34	9.22	1.44	0.2	4.92	12.69	15.61	33.22
	Total				3.48	0.57	11.83	33.82	40.91	86.56
Gamma Rays	100Gy	25.05	8.01	4.61	0.59	0.005	2.35	7.36	12.79	22.50
	200Gy	27.32	8.34	5.19	0.78	0.003	2.85	9.35	15.02	27.22
	300Gy	21.37	11.67	6.9	1.04	0.003	4.86	8.91	15.07	28.84
	Total				2.41	0.011	10.06	25.62	42.88	78.56
Electron Beam	100Gy	28.04	11.24	3.81	0.86	0.008	3.06	7.65	22.57	33.28
	200Gy	26.5	22.44	6.8	1.25	0.006	4.71	5.57	18.38	28.66
	300Gy	22.3	47.78	15.90	1.54	0.005	6.90	3.22	9.68	19.8
	Total				3.65	0.019	14.67	16.44	50.63	81.74

Table 7. Mutation rate in terms effectiveness and efficiency in Kalbhat.

Mutagen	Mutation rate in terms of effectiveness %	Mutation rate in terms of efficiency			
		Reduction in seedling height %	Lethality %	Sterility %	Total
EMS	0.14	4.24	10.87	15.87	30.98
Gamma Rays	0.003	4.04	9.34	5.49	18.87
Electron Beam	0.004	4.35	4.25	5.67	14.27

Table 8. Mutation rate in terms of effectiveness and efficiency in Black Rice.

Mutagen	Mutation rate in terms of effectiveness %	Mutation rate in terms of efficiency			
		Reduction in seedling height %	Lethality %	Sterility %	Total
EMS	0.19	3.94	11.27	13.63	28.84
Gamma Rays	0.003	3.35	8.54	14.29	26.18
Electron Beam	0.006	4.89	5.48	16.86	27.23

landrace, whereas in case of Black rice highest efficiency was reported with electron beam treatment, followed by gamma rays and EMS treatments. In case of Kalbhat, maximum mutagenic efficiency with respect pollen sterility was noticed under 1.0 % EMS treatment,whereas in black rice, it was observed in 100 Gy electron beam treatment. Similar results were reported by Agrawal *et al*. 2000, Rajarajan *et al*. 2014, Gowthami *et al*. 2016, Imam *et al.* 2019, and Lalitha *et al*. 2020, in rice.

Mutation effectiveness was high in EMS treatment followed by electron beam and gamma rays treatment. Whereas mutation rate in terms of efficiency was highest under EMS treatment, follwed by gamma rays and electron beam in Kalbhat landrace, while in case of Black rice, it was highest in EMS treatment, followed by electron beam and gamma rays treatment. Similar kinds of results were reported from rice (L.) by (Rao, 1977, and Ramchander *et al*., 2014).

From the present investigation, it can be concluded that among two landraces , Black rice was more sensitive for all the mutagenic treatments compared with Kalbhat . This may be due to differential response of these landraces towards different mutagenic treatment. Among the three mutagenic treatments, EMS treatment was found to be the most effective and efficient as compared with electron beam and gamma rays for induction of desirable changes like reduced plany height, early flowering and high yield, in Kalbhat and Black rice landraces.

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