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INDUCTION OF MUTATION THROUGH GAMMA IRRADIATION IN NON-BASMATI AROMATIC 'BADSHABHOG' RICE (*Oryza sativa* L.)

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ABSTRACT

Induction of several types chlorophyll and viable morphological mutations were studied in M_2 generation of non-basmati aromatic Badshabhog rice in all five different doses (200, 250, 300, 350 and 400 Gy) of gamma rays. Chlorophyll frequency was increased with increasing dose of gamma rays. Among different chlorophyll mutations albino was the most frequent type. Several morphological mutants such as short-stature, early maturity, increased tillering, dark green broad leaf, narrow leaf, short exerted panicle, complete spikelet sterility, double-triple spikelet, double kernel within spikelet and lodging resistant type were appeared with variable frequencies by different doses of gamma rays in 2,07,960 individual plantlets from M_2 population. Among them short-stature mutant was predominate type. A nonlinear relationship was noticed between dose of gamma rays and frequency of viable mutations. The mutagenic efficiencies were increased with increase in irradiation dose but no relationship between the doses of mutagen and mutagenic effectiveness was established. Less damages in biological traits such as lethality, injury and sterility were observed at 200 to 300 Gy dose of gamma rays. Desirable mutants were found at 200 and 300 Gy.

Keywords: Badshabhog rice, chlorophyll, gamma-irradiation, mutants

INTRODUCTION

Rice (*Oryza sativa* L.) is a major food commodity throughout the world and people all over world prefers rice as per their culture and habitat. Aromatic or scented rice is an important sub-group of rice which became popular in Asia and gained wide acceptance in Europe and United States due to its fragrance, flavour and other grain quality parameters. An aromatic compound, 2-acetyle-l-pyrroline, is 10 to 15 times more in aromatic rice than non-aromatic rice (Weber *et al.*, 2000) and it is responsible for aromatic odour in several Asian aromatic rice varieties (Wakte *et al.*, 2017). The aroma trait is controlled by a single recessive gene located on chromosome 8 (Ahn *et al.*, 1992; Roy *et al.*, 2020).

Basmati and non-basmati rice are categorized on the basis of grain type and the amount of aroma present in grains. Basmati rice having long slender grain type with moderate to strong aroma (Mahindru, 1995) are grown in north-western part of India and Pakistan (Singh *et al.*, 2018). However, their adaptation for cultivation is unsatisfactory in Eastern India due to the breakdown of its original aroma, grains quality, difficulty in milling because of very poor head rice recovery and high susceptibility to insects-pests (Chakraborty and Kole, 2014).

The small and medium grain type non-basmati aromatic rice adapts to specific eco-geographic conditions and are distributed in different parts of India (Singh *et al.*, 2000). A number of non-basmati scanted rice cultivars such as 'Badshabhog', 'Gobindabhog', 'Bahadurbhog', 'Radhunipagol', 'Seetabhog', 'Blackjoha', 'Tulaipanja', etc. are available in the eastern India and popular due to their pleasant aroma, grain quality and excellent cooking quality features (Chakraborty and Kole, 2014; Sharma *et al.,* 2017). Among them, cv. "Badshabhog" is very popular short-medium grain type non-basmati scented rice and is cultivated by the marginal farmers for their local consumptions and rituals in some part of eastern India. The area under its cultivation is limited due to expansion of area under high yielding non aromatic varieties (Singh *et al.*, 2000). 'Badshabhog' has some drawbacks such as lodging susceptibility due to tall height (>165 cm.) along with weak culm, long duration (>155 days), photoperiod sensitive and blast susceptibility in fluctuating weather caused low yield (Chakravarti *et al.*, 2012). So, improvement in yield potential in 'Badshabhog' rice by rectifying the undesirable traits without disrupting its important quality parameters including aroma is the prime objective.

Mutation breeding is one option to improve a cultivar without hampering its inbuilt qualitative traits. Induced mutagenesis, particularly using gamma (γ) irradiation, plays an important role in generating genetic variability in crops including cereals, pulses and oilseeds for desirable traits of economic importance and these variants were used further in cross breeding programme of several seed propagated crops (Oladosu *et al.,* 2016; Rampure *et al.,* 2017). Induced mutation has successfully been employed in rice breeding worldwide (Oladosu *et al.*, 2016). As of now 3329 mutant varieties of several crops, including 513 mutant varieties in rice have been registered in FAO/IAEA mutant variety database by using γ-rays (IAEA, 2020).

Mutagenic effectiveness (mutation per dose of mutagen) and mutagenic efficiency (mutation in connection to undesirable changes/damages such as lethality, injury and sterility etc.) plays a pivotal role in mutation breeding programme to produce a high frequency and a spectrum of desirable mutation which is free from undesirable ones (Ramesh *et al.,* 2019). The present experiment was carried out to study the frequency and spectrum of morphological mutations along with mutagenic effectiveness and efficiency of γ-rays in non-basmati aromatic "Badshabhog" rice.

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MATERIALS AND METHODS

Plant materials and mutagen treatment

The seeds of rice cultivar 'Badshabhog' (whitish creamy, short-medium cylinder seeds, maturity 150-160 days) was collected from Rice Research Station Chinsurah, Hooghly, West Bengal (India) for irradiation. Genetically pure, disease free and healthy seeds with 12-14% moisture each weighing 100 g were taken in six packets. Five such packets were placed inside a γ-irradiation chamber to get the seeds exposed to five different doses of γ-rays *viz.*, 200, 250, 300, 350 and 400 Gy [1 Gy (Gray) = 0.1 KR]. The γ-irradiation was done at BARC, Trombay, Mumbai (India) where $Cobalt^{60}$ was used as source of γ rays for appropriate time for each dose based on the half-life of the source. The sixth packet was kept unexposed seed and maintained as control.

Experimental design and data collection

The experiments were carried out in the Department of Genetics & Plant Breeding and Agriculture Farm of Palli Shikha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan, West Bengal (India). Fifty seeds of each treatment along with unexposed seeds (control) of 'Badshabhog' were grown separately on vertical stand blotting paper (*in vitro*) and the remaining seeds of each treatment were sown in nursery bed (*in vivo*) at a time with three replications during kharif season, 2016. Reduction percent over control in seedling survival (referred to as % lethality) and seedling height (referred to as % injury) were calculated under *in vitro* as well as *in vivo* conditions at 14^{th} and 25^{th} DAS, respectively, by using the following formula of Leng *et al.* (2017):

$$\text{Reduction in seedling survival} = \frac{Mc - Mt}{Mc} \times 100$$

where Mc and Mt denote the mean of control treatment and mean of irradiated treatment, respectively. After 25-30 days all the survived M_1 seedlings of each dose along with control were transplanted to the main field by adopting inter-plant spacing of 15 cm with single seedling hill^{-1} and 20 cm inter-row spacing in a completely randomized block design with three replications. Spikelet fertility reduction percent over control (refers to as sterility) for each treatment was calculated from main panicle of ten randomly selected plants by using the above formula. Since most mutations are recessive in nature, therefore selection was not done in M_1 generation.

The main panicle of each plant treatment^{-1} was selfed and harvested individually as suggested by Ukai and Nakagawa (2012) and M_2 population was raised separately along with control on the nursery bed during kharif season, 2017. Spectrum and frequency of chlorophyll mutation (both viable and non-viable) was determined at seedling stage between 7 to 12 days. The chlorophyll mutants were classified according to Gustafsson (1940). Then, 25-30 days old all M_2 seedlings (at least 60 seedlings plot^{-1}) for each treatment were transplanted separately to the main field following plant to row progeny method by adopting 15 cm × 20 cm spacing. The spectrum and frequency of different types viable macro-mutations based on altered plant height, maturity duration, tillers hill^{-1}, leaf morphology, panicle morphology and lodging resistant without disrupting original aroma were observed. Dose-wise total mutation frequency (Mf) was determined based on the frequency of chlorophyll mutations and viable macro-mutations.

Mutagenic effectiveness and efficiency were estimated based on the formulae adopted by Konzak *et al.* (1965). Data on total mutation frequencies (Mf) and mutagen dose (Gy) were used to determine the mutagenic effectiveness by using the formula Mf× 100 / Gy. Data on total mutation frequencies and biological damages (lethality, injury and sterility) in M_1 generation were used to calculate the mutagenic efficiency by using the formulae (Mf/L), (Mf/I) and (Mf/Ss) where Mf, L, I and Ss indicated the total mutation frequency in M_2 generation, lethality of survived seedlings, injures in seedling height and spikelet sterility in M_1 generation, respectively. Statistical analysis was done by using IBM SPSS Statistics 23 software and Microsoft excel 2016.

RESULTS AND DISCUSSION

Spectrum and frequency of chlorophyll mutation

Chlorophyll mutation helps to assess the genetic effects and sensitivity in any crops due to mutation in earlier growth stage of plant. Study of chlorophyll mutations support in understanding the potency of mutagen. Table 1 and Fig. 1 present different types of chlorophyll-deficient mutants and their frequency. It was observed that frequency of albino (8.95%) at 350 Gy was the most frequent chlorophyll mutant followed by Xantha (1.94%) at 250 Gy. Other chlorophyll mutant like albino green, striata, yellow viridis and chlorine were observed in low frequency (%). Earlier workers also reported a higher frequencies of albino mutant in rice (Lalitha *et al.*, 2020; Vinithashri *et al*., 2020). Present revealed the absence of chlorina type mutant at 250 Gy onwards. Rajarajan *et al.* (2014) reported that chlorina mutant was observed only at 200 Gy. Dose wise chlorophyll mutation frequency (%) was increased with increasing dose of gamma rays *viz*., 200 Gy (6.20%) < 250 Gy (9.17%) < 300 Gy (9.92%) < 350 Gy (10.03%) < 400 Gy (12.78%). The results of dose dependent increase in chlorophyll mutation frequency were in agreement with Tamilzharasi *et al.,* (2019) and Lalitha *et al.,* (2020). The absence of chlorophyll mutants in the M_1 generation and their appearance in M_2 generation indicates the recessive nature of the chlorophyll mutations. Appearance of mutation in one or other doses is evidently due to the genes for chlorophyll formation and also sensitivity of genes governing chlorophyll (Gaul, 1964). Chlorophyll mutation arose due to the disbalance of genes encoding enzymes which is responsible for weakening of chlorophyll biosynthesis activity, degrading

Table 1: Frequency and spectrum of chlorophyll mutation in M_2 generation of rice cv. 'Badshabhog'

Doses (Gy)	No. of M_2 progenies studied	No. of progenies segregating	No. of M_2 seedlings in segregating progenies	Frequency and spectrum of chlorophyll mutation													
				Albino		Xantha		Albino green		Striata		Yellow viridis		Chlorina		Dose wise (Total)	
				X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	Mutant seedlings	Frequency (%)
200	1093	358	54870	2176	3.97	747	1.36	173	0.32	151	0.28	118	0.22	35	0.06	3400	6.20
250	961	348	38598	2336	6.05	747	1.94	168	0.44	133	0.34	157	0.41	0	0.00	3541	9.17
300	887	252	25606	2107	8.23	212	0.83	24	0.09	90	0.35	107	0.42	0	0.00	2540	9.92
350	590	204	19913	1782	8.95	157	0.79	0	0.00	45	0.23	13	0.07	0	0.00	1997	10.03
400	377	133	12180	1024	8.41	201	1.65	173	1.42	32	0.26	126	1.03	0	0.00	1556	12.78
Mutant wise total*	3908	1295	151167	9425 (72.3)	6.23	2064 (15.8)	1.37	538 (4.1)	0.36	451 (3.5)	0.30	521 (4.0)	0.34	35 (0.3)	0.02	13034	8.62

Gy, Gray; X, Number of mutants; Y, Frequency %;
*Values given in parentheses relate to percent over total number of chlorophyll mutations

Fig. 1: Chlorophyll mutants in 'Badshabhog' rice; A) Albino; B) Xentha; C) Albino green; D) Striata; E) Yellow viridis; F) Chlorina

the chlorophyll content (Reddy and Annadurai, 1991), lack of carotenoids promotes bleaching (Bevins *et al.*, 1992) and alteration in certain regions of chromosomes (Lalitha *et al.*, 2020). Chlorophyll development likely is governed by polygenes which could be located near to the centromeric and proximal regions of chromosomes and mutation in these genes may induce chlorophyll mutation (Swaminathan, 1965).

Spectrum and frequency of viable mutation

A wide spectrum (10 types) of viable morphological mutations *i.e.* short-stature, early maturity, increased tillering, dark green broad leaf, narrow leaf, short exerted panicle, complete spikelet sterility, double-triple spikelet, double kernel within spikelet and lodging resistant type were observed in M_2 generation of γ-treated 207,960 population of 'Badshabhog' (Table 2; Fig. 2). Among these morphological mutation, mutant wise frequency (%) was found to be higher for short-stature type (19.25%) followed by lodging resistant (18.45%), increased tillering (15.51%) and short exerted

Table 2: Frequency and spectrum of induced viable mutations in M_2 generation in rice cv. 'Badshabhog'

Doses (Gy)	No. of M_2 progenies transplanted	No. of M_2 progenies segregating	M_2 plants studied		Frequency and spectrum of viable macro mutations with inbuilt aroma										
					Short stature plants	Early maturity	Increased tillering	Dark green broad leaf	Narrow leaf	Short exerted panicle	Complete spikelet sterility	Double & triple spikelet	Double kernel within spikelet	Lodging resistant	Dose wise total
200	1014	25	60840	X	38	0	26	5	9	25	2	0	0	25	130
				Y	0.06	0.00	0.04	0.01	0.01	0.04	0.00	0.00	0.00	0.04	0.21
250	897	10	53820	X	0	0	0	0	8	0	3	0	0	5	16
				Y	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.03
300	792	31	47520	X	19	21	11	0	5	15	7	0	25	28	131
				Y	0.04	0.04	0.02	0.00	0.01	0.03	0.01	0.00	0.05	0.06	0.28
350	493	9	29580	X	0	0	9	7	0	0	6	5	0	0	27
				Y	0.00	0.00	0.03	0.02	0.00	0.00	0.02	0.02	0.00	0.00	0.09
400	270	15	16200	X	15	0	12	0	10	10	9	3	0	11	70
				Y	0.09	0.00	0.07	0.00	0.06	0.06	0.06	0.02	0.00	0.07	0.43
Mutant wise total*	3466	90	207960	X	72 (19.2)	21 (5.6)	58 (15.5)	12 (3.2)	32 (8.6)	50 (13.4)	27 (7.2)	8 (2.14)	25 (6.68)	69 (18.45)	374
				Y	0.04	0.01	0.03	0.01	0.02	0.02	0.01	0.004	0.012	0.033	0.18

Gy, Gray; X, Number of mutants; Y, Frequency %; *, Values given in parentheses relate to percent over total number of viable macro mutations.

panicle (13.37%) while lower frequency (%) was observed for double -triple spikelet (2.14%) and dark green broad leaf (3.21%). Such morphological mutants have also reported by Chakravarti *et al.* (2012) and Manikandan and Vanniarajan (2017). Dose wise frequency (%) for viable mutation was highest (0.28%) at 300 Gy and lowest (0.03%) at 250 Gy irradiation. A nonlinear relationship was founded between dose of γ-rays and frequency of viable mutations. Similar findings have been reported by Sikder *et al.* (2013) in tomato genotypes. A total number of 72 short-stature (height less than 135 cm as compared to 165 cm in control) and 58 increased tillering (tiller more than 15 as compared to 8-10 tillers plant^{-1} in normal) mutants were isolated from 207,960 M_2 plants. Maximum number of short-stature mutant (38) was observed at 200 Gy. Semi-dwarf mutants with increased tillering in rice have been reported by Chakraborty and Kole (2009); Chakravarti *et al.* (2012) and Manikandan and Vanniarajan (2017). Different types of morphological mutation/altered phenotypes appeared through induced mutation due to the chromosomal rearrangements (Amri-Tiliouine *et al.*, 2018), disbalance auxin synthesis (Goyal and Khan, 2010), disruption of mineral metabolism and accumulation of amino acids (Lalitha *et al.*, 2020). The more frequent induced mutations might be attributed to the fact that the genes for these characters were relatively more responsive to γ-rays (Chakravarti *et al.*, 2012). Nilan (1967) reported that different treatment of mutagens might also be responsible to change the relative proportion of different mutation types.

Biological damages

The impact and tolerance level of cv. 'Badshabhog' to different γ-irradiation doses were manifested in M_1 generation in terms of lethality, injury and spikelet sterility (Table 3). Each treatment of γ-irradiation was exhibited inhibitory effect as reduction in seedlings survival and height and spikelet sterility in terms of lethality (%), injury (%) and sterility (%) with increase in dose from 200 to 400 Gy. The lethality (%) was minimum (10.53 and 11.85%) at 200 Gy and higher (61.05 and 66.01%) at 400 Gy under both *in vitro* and *in vivo* conditions, respectively. This appears due to the inhibitory effect of γ-rays on cytochrome oxidase content and accordingly reducing the respiration (Swaminathan *et al.*, 1962), drastic distortion of actively dividing phase in meristematic tissues of seed (Ramya *et al.*, 2014) and alteration in seed morphology (Gowthami *et al.*, 2017). The reduction in seedling height was 54.71 and 48.64% at 400 Gy under *in vitro* and *in vivo* conditions, respectively. Such injuries have been due to adverse effect of γ-irradiation on physiological systems (Gaul, 1977), growth hormone (Gunckel and Sparrow, 1967) and reduced mitotic activity index in meristematic

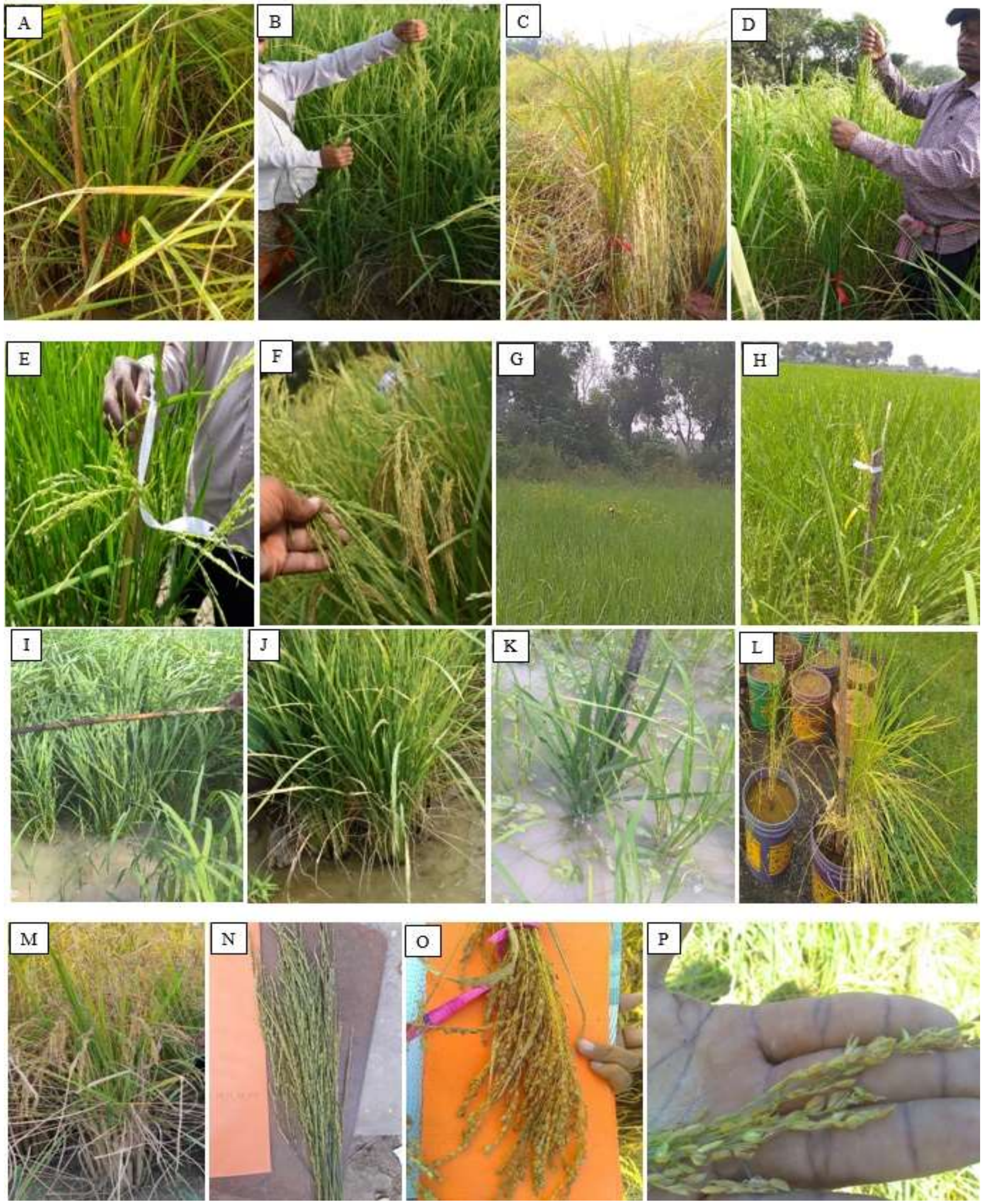

Fig. 2: Viable morphological mutants in 'Badshabhog' rice. A-D) Short-stature with logging resistance; E-H) Early maturity; I-J) Increased tillering; K) Dark green broad leaf; L) Narrow leaf; M) Short exerted panicle; N) Complete spikelet sterility; O) Double and triple spikelet; P) Double kernel within spikelet

tissues which resulted in reduction of seedlings height with increasing irradiation dose (Khalil *et al.,* 1986; Oney-Birol and Balkan, 2019). Spikelet sterility increased steadily with increase in mutagen dose. The spikelet sterility was minimum (17.04%) at 200 Gy and maximum (49.67%) at 400 Gy

Table 3: Effectiveness and efficiency of gamma irradiation in M_2 generation of rice cv. 'Badshabhog'

Doses (Gy)	Mf	L_{14}	L_{25}	I_{14}	I_{25}	Ss	ME	MeL_{14}	MeL_{25}	MeI_{14}	MeI_{25}	MeSs
200	6.41	10.53	11.85	13.05	7.85	17.04	3.21	0.61	0.54	0.49	0.82	0.38
250	9.20	17.89	21.98	21.08	10.85	33.00	3.68	0.51	0.42	0.44	0.85	0.28
300	10.20	27.37	28.65	29.99	21.37	44.13	3.40	0.37	0.36	0.34	0.48	0.23
350	10.12	48.42	50.78	38.39	28.75	44.68	2.89	0.21	0.20	0.26	0.35	0.23
400	13.21	61.05	66.01	54.71	48.64	49.67	3.30	0.22	0.20	0.24	0.27	0.27
Mutation rate							3.30	0.38	0.34	0.35	0.55	0.28

Gy = Gray; Mf = Total mutation frequency dose wise (chlorophyll frequency % + viable macro-mutation frequency %); ME = Mutation effectiveness %; L_{14} and $L_{25,}$ = Lethality (%) in M_1 generation at 14^{th} and 25^{th} DAS under *in vitro* and *in vivo* conditions, respectively; I_{14} and I_{25}, = Seedling injury (%) at 14^{th} and 25^{th} DAS under *in vitro* and *in vivo* conditions, respectively; Ss = Spikelet sterility (%) in M_1 generation; MeL_{14}, MeL_{25}, MeI_{14}, MeI_{25} and MeSs = Mutation efficiency based on L_{14}, L_{25}, I_{14}, I_{25} and Ss, respectively.

irradiation. The findings are supported by Lalitha *et al.* (2019) and Gowthami *et al.* (2017). Radiation induced sterility may be due to the chromosomal aberrations, meiotic abnormalities and genetic and physiological change in pollen mother cells (Ramya *et al.*, 2014). Anther cells and viable pollen formation is directly responsible for spikelet fertility (Liu *et al.*, 2013). Phytohormones like auxin, gibberellic acid and jasmonic acid also play a vital role in spikelet fertility because their deficiency exhibited common defects in tapetal cells and formation of ubisch bodies in rice (Aya *et al.*, 2009).

Effectiveness and efficiency

Knowledge on the mutation efficiency and effectiveness is very essential step to identify the useful kind of mutagens and their doses/concentrations in mutation breeding programme for obtaining high frequency of desirable mutations. In M_2 generation of cv. 'Badshabhog', dose-wise total mutation frequency (%) was used to find out the effectiveness and efficiency of γ-irradiation (Table 3). The effectiveness was maximum at 250 Gy (3.68%) with 3.30% mutation rate. Effectiveness was independent of the increase in dose of γ-rays and no linear trend was observed. These are in agreement with Lalitha *et al.* (2020) for γ-mutagen and Vinithashri *et al.* (2020) for sodium azide, wherein they reported no linear relationship between the doses of mutagen and the mutation frequency.

Production of desirable mutants depends on mutagenic efficiency as against associated undesirable biological damages such as lethality, seedling injury, spikelet sterility, pollen abnormalities due to chromosomal aberration, etc. (Sikder *et al.*, 2013). The results revealed that the efficiency was dose-dependent linear relationship with increasing γ-irradiation, except mutagenic efficiency based on spikelet sterility where the relation was independent of dose. The efficiency of γ-rays was highest at 200 Gy (followed by 250 and 300 Gy) for lethality under *in vitro* (0.61) as well as *in vivo* (0.54), seedling injury under *in vitro* (0.49) and spikelet sterility (0.38) with 0.38, 0.34, 0.35 and 0.28 mutation rate, respectively; except seedling injury under *in vivo* where maximum (0.85) efficiency was observed at 250 Gy with highest (0.55) mutation rate as compared to others. Similar findings have been reported for maximum efficiency of γ-rays at 250 Gy irradiation in aromatic rice cv. 'T- 23' by Chand *et al.* (2007); 200 Gy in cv. 'ADT(R) 47' by Rajarajan *et al.* (2014) and 250 Gy in cv. 'Anna (R) 4' by Lalitha *et al.* (2020). Jain and Khandelwal (2009) and Sikder *et al.* (2013) have reported that higher efficiency of a mutagen shows relatively minimum biological damage with respect to mutations induced.

***Conclusion*:** In present investigation 200 to 300 Gy dose of γ-rays was found more efficient in rice cv. 'Badshabhog' because of < 50% damages in biological traits such as lethality, injury and sterility. More spectrum of mutants such as short-stature, early maturity with high tillering ability, etc. was found at 200 and 300 Gy irradiation. Desirable changes appeared through efficient mutagenesis with minimum undesirable effects. Minimal deleterious effects with high mutation rate is desirable in any mutation breeding programme.

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