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ORIGINAL ARTICLE

Radiation sensitivity on Pollen and Spikelet Fertility associated with Grain yield in non-basmati aromatic "Badshabhog" rice

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ABSTRACT
Dry, uniform healthy seeds of Badshabhog rice was exposed by gamma radiation from 200Gy to 400Gy with interval of 50Gy for find out the immediate radiation sensitivity on pollen grains and spikelet fertility. All treatments with control were grown primarily in the nursery and 25 days old seedlings were transplanted to the main field with three replications. At the time of flowering, 1% potassium iodide stain was used to stain pollens and examined under microscope to check the fertility percentage over control and different kinds of pollens were observed. At the time of maturity stage, the main panicles were harvested from randomly selected plants and spikelet fertility percentage and grain yield was calculated. The results revealed that pollen as well as spikelet fertility percentage over control associated with grain yield/plant was decreased with increasing gamma irradiation in Badshabhog rice. Optimal radiation dose at 50% reduction were estimated for pollen fertility, spikelet fertility and grain yield at 260.89Gy, 390.05Gy and 329.78Gy irradiation respectively. In M_1 generation, less biological damages due to irradiation plays an important role to produced viable and desirable mutants in M_2 and subsequent generation.
Keywords: *Mutation, Gamma ray, Aromatic rice, Pollen and spikelet fertility.*

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INTRODUCTION
Rice (*Oryza sativa* L.) is a self-pollinated autogenous crop and have 24 diploid (2n) chromosome number [1]. Among the rice species aromatic rice is a small and special group of rice which is known for their fragrance and best in quality. An important pleasant flavour aroma component 2-acetyle-l-pyrroline, has been reported in several aromatic varieties, it gives pleasant aromatic fragrance from cooked or uncooked aromatic rice or in field at the time of flowering [28, 30]. Due to aroma, aromatic rice is more popular or demandable in Middle-East, European counties and United States. But mostly trade of aromatic rice is from India, Pakistan and Thailand. Some aromatic local land races such as Badshabhog, Gobindabhog, Gopalbhog, Kaminibhog, Radgunipagol, Seetabhog, Tulaipanja etc. are very popular in eastern India. Although these cultivars have excellent grain quality and appealing aroma. Among all these cultivars, Badshabhog is more acceptable by the farmers due to they have a greater number of filled grains per panicle with high number of effective tillers [5]. But they have some undesirable traits such as higher plant height along with less study stem which promote lodging susceptibility and long harvesting duration (150-178 days) also encourage blast attract caused poor yield [6]. Therefore, Badshabhog cultivar required to improvement undesirable traits without affecting their fragrance and grain quality.
In a short period, to create new variability and changing plant types in aromatic rice by means of mutation breeding through mutagenesis one of the sound technique considered by the plant breeders. Mutation breeding is utilised in many autogenous crops improvement programmes because it breaks undesirable linkage between genes and develops desirable genotypes with recombination. Induced mutagenesis has played a crucial role in developing rice mutant having short height, less duration for maturity and disease tolerance with high yielding abilities [26]. Gamma rays are electromagnetic shorter

wave length type physical mutagen widely employed in induced mutagenesis[15, 31]. Therefore, they are more deep penetrating into the tissues compare than other types of physical mutagens [16]. It can be useful for the alteration of physiological as well as morphological traits. It has been effective in rearrangements of mitotic division or increasing pollen abnormalities. High doses radiation causes higher pollen and spikelet sterility in M_1 plants [8, 9]. This may be due to various chromosomal aberrations and meiotic abnormalities including chromosome univalent, multivalent, stickiness and laggards, bridges etc. [3, 29]. But some doses range of gamma ray in some cases change plant types and produce viable mutants that can be useful further as an improved variety or parents in breeding programs. Therefore, pollen fertility and spikelet fertility are important parameters which have been used to determine mutation effect in plants [2]. Hence, the present experiment was undertaken to study the immediate radiation sensitivity on pollen and spikelet fertility associated with grain yield in “Badshabhog” rice.

MATERIAL AND METHODS

Plant material and mutagen

Dry, uniform, healthy seeds of rice cultivar namely ‘Badshabhog’ each weighing 100g were taken in six packets for the experiment. Five packets were placed inside the gamma chamber to get the seeds irradiated with five different doses *viz*., 200Gy, 250Gy, 300Gy, 350Gy and 400Gy with 17 Gy/min dose rate at BARC, Trombay, Mumbai. The Cobalt ^{60} was used as a source of gamma rays. The remaining unexposed seed packet was maintained as control.

Experimental design

The experiment was conducted at Agriculture farm in Viava-bharati University. The all irradiated seeds combination along with control seeds were sown on well fertile raised nursery bed. Twenty-five days old seedlings were transplanted to main field at 15cm plant to plant and 20cm row to row spacing with three replications. Recommended fertiliser N P K ratio and cultural practices applied for better crop growth.

Observations and data collections

Once the plants started flowering, firstly fifteen hills from each treatment were selected randomly per replication wise including control, then from their one main panicle were selected for each selected hill and anthers were collected from each selected unopened spikelet (just before anthers dehiscence) by considering top, middle and bottom portions. Collected anthers were crushed and pollen were fixed with cover slip on glass slide and examined under microscope to check the pollen fertility by using 1% potassium iodide (KI) stain (Iodine test) [4]. The pollen fertility percent was determined by using formula Ts ×100/ T where, Ts and T indicates total number of round shape well stained pollens and total number of pollens (unstained and stained), respectively. Round shape well stained, half stained, unstained pollens were counted as fertile, partial sterile, complete sterile, respectively. Whereas, shrivelled and triangle shape unstained pollens were categorised under shapeless and triangle sterile, respectively [12, 23].

The plants attained maturity stage, firstly ten plants for each treatment were selected randomly per replication wise including control, then from their primary or main panicle was selected from each selected plant. Total number of well filled grains and total number of grains were counted from selected primary panicle. The spikelet fertility percentage was calculated for each treatment. After harvesting, grain yield per plant for each treatment was calculated from ten randomly selected plant.

At last percentage over control for pollen fertility (%) and spikelet fertility (%) and grain yield per plant for each treatment were calculated by using formula Mt ×100 /Mc where, Mt and Mc denote mean of irradiated treatment and mean of control treatment, respectively [27].

The data was statistically analysed by linear regression equation, $y = mx + c$ (where y is response variable, x is the irradiated dose) using Microsoft excel 2016.

RESULTS AND DISCUSSIONS

Radiation sensitivity on pollen and spikelet fertility associated with grain yield

Figure 1 represented radio sensitivity of gamma rays on pollen fertility, spikelet fertility and grain yield per plant in Badshabhog rice. Figure 2 and Figure 3 represented microscopic view of pollen grain and view of panicle morphology due to gamma rays respectively. The results exhibited that percentage over control for all three parameters were decreased with increasing dose of gamma irradiation. The maximum percentage over control for pollen fertility (66.53%), spikelet fertility (82.97%) and grain yield (74.59%) were recorded at 200Gy irradiation. Whereas, pollen fertility (33.76%), spikelet fertility (50.34%) and grain yield (40.84%) at 400Gy irradiation were recorded minimum. These similar types finding were reported by [2, 12, 13, 14, 17, 19]. Through regression analysis, dose at 50% reduction refers to as optimal dose (OD) was recorded at 260.89Gy, 390.05Gy and 329.78Gy for pollen fertility, spikelet fertility and grain yield per plant, respectively. [19] was reported 50% reduction for pollen fertility at

387.60Gy in Anna (R) 4 variety and [2] for spikelet fertility at 300.57Gy in rice variety BPT 2231. Pollen fertility is a genetically controlled trait and highly negatively associated with percent of meiotic aberrations [2]. In the present investigation, pollen grains were very sensitive at higher dose of irradiation due to causes chromosomal aberrations [25], point mutations and inactivation of certain genes [10] during anther and pollen grain development. According to [20,22], the pollen fertility reduction may be caused by various anomalies in chromosome and physiological changes.[10]reported that gamma rays exhibit negative impact on spikelet fertility which is not heritable as it is due physiological damage. Spikelet fertility positively correlated with pollen grains fertility or survivability and sometimes environmental fluctuations [19]. Phytohormones such as gibberellic acid, auxin and jasmonic acid is needs to develop fertile spikelet and GAMYB (GA myeloblastosis) gene is regulate the formation of anther and pollen in rice [21]. In present experiment different doses of gamma radiation was affected the pollen grain and spikelet fertility which is caused reduction in grain yield. Similar results were agreeing with [2].

Radiation sensitivity on pollen shape categories

In the present experiment, the pollen grains sterility categorised into four broad forms based on shapes such as round complete sterile, round partial sterile, triangle shaped sterile and shapeless sterile as shown in Table 1 and Figure 4. In control hills of Badshabhog rice, partial sterile form was observed maximum (5.48%) with higher relative percent (51.98%) followed by completely sterile type (4.01%)accompanied relative percent (38.06 %), triangle shaped (0.61%) with relative percent (5.79%)and shapeless sterile forms (0.44%) with relative percent (4.17 %). The relative percentage of different forms in irradiated populations of Badshabhog rice, the completely sterile form of pollen sterility among the total sterility ranged from 51.61 % (200 Gy) to 56.24 % (400 Gy), partially sterile from 4.47 % (400 Gy) to 21.62 (200 Gy), triangle shaped sterile 4.02% (250 Gy) to 5.20% (300 Gy) and shapeless sterile form with 22.08 % (200 Gy) to 35.15 % (400 Gy) sterility was occurred.It was caused by the irregular disjunction of chromosomes at anaphase or chromosome breakage through formation of anti-metabolic compound in the cell [11, 18]. As a result, abnormal or malformed pollen grains were found. The maximum sterility was found at 400 Gy (69.79 %) with high percentage of round completely sterile (39.25 %) followed by shapeless sterile pollen (24.53 %). The control or non-irradiated hills was exhibited only 10.54 % sterility with low percentage of all forms of sterility also. The present results on different forms of pollen shape were revealed that, relative percentage was linearly increased with increasing mutagen dose for completely sterile form followed by shapeless sterile and other remaining forms. The similar findings also were reported by [2, 12, 17] the rapidly pollen shapes disturb by increased mutagens dose *via*., poor growth, chlorophyll deficiencies, low starch content, aborted metabolic activity, chromosomal abnormalities and alteration of cell divisions. Cytogenetical studies was carried out by [24] have revealed that physical mutagen causes chromosomal rearrangements due to cryptic deletions and specific gene mutations.

Table 1: Radiation sensitivity on pollen shape.

Mutagen (dose)	Fertility %	Sterility %								
		Total	Different groups of sterility based on pollen shape							
			Completely		Partially		Tri angle Shaped		Shapeless Sterile	
			Mean	Relative %	Mean	Relative %	Mean	Relative %	Mean	Relative %
Control	89.46	10.54	4.01	38.06	5.48	51.98	0.61	5.79	0.44	4.17
200Gy	59.52	40.48	20.89	51.61	8.75	21.62	1.90	4.69	8.94	22.08
250Gy	36.30	63.70	35.56	55.82	4.59	7.21	2.56	4.02	20.99	32.96
300Gy	31.95	68.05	36.57	53.74	4.12	6.05	3.54	5.20	23.82	35.00
350Gy	31.48	68.52	37.12	54.17	5.21	7.60	3.26	4.76	22.93	33.47
400Gy	30.21	69.79	39.25	56.24	3.12	4.47	2.89	4.14	24.53	35.15

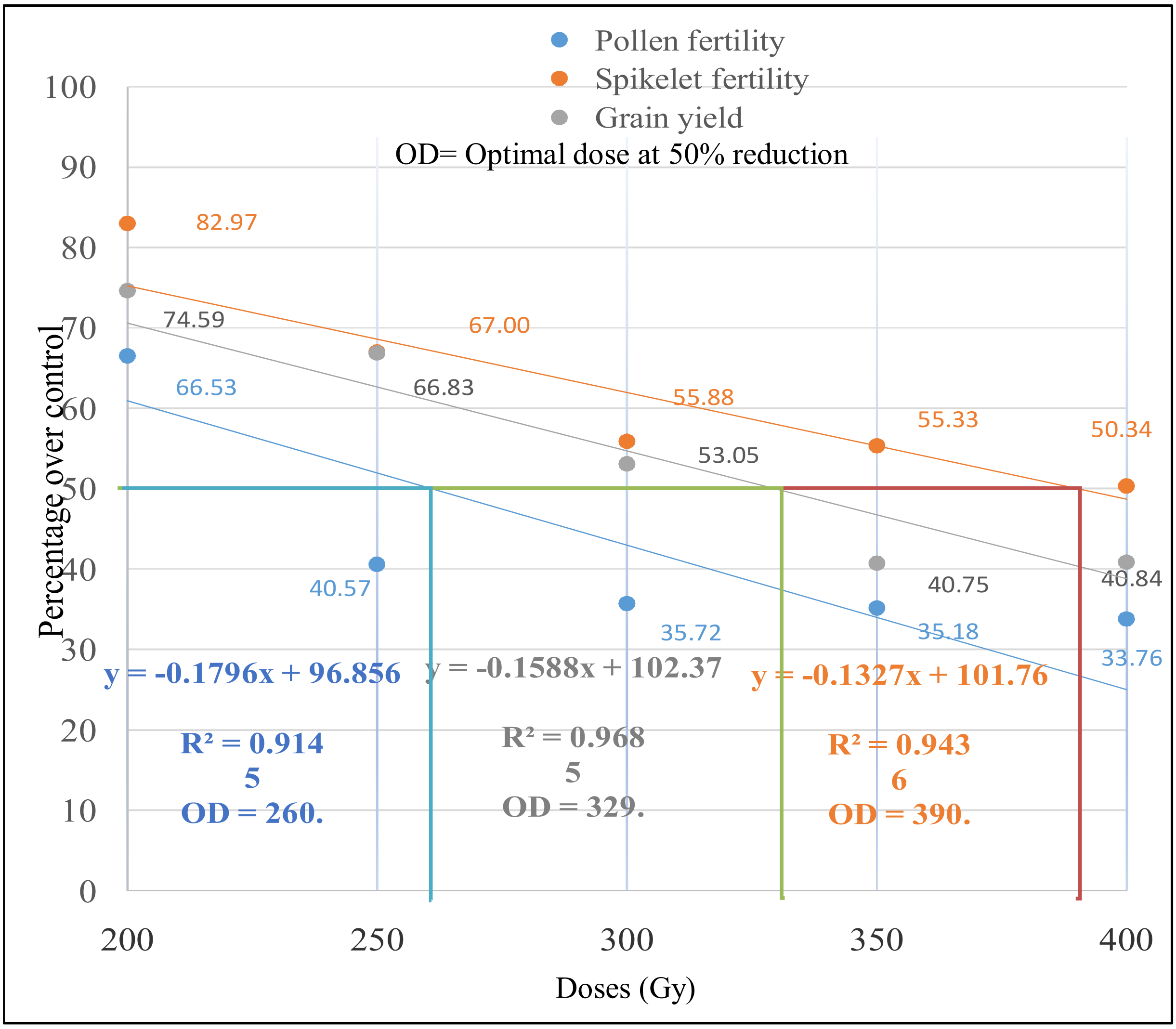
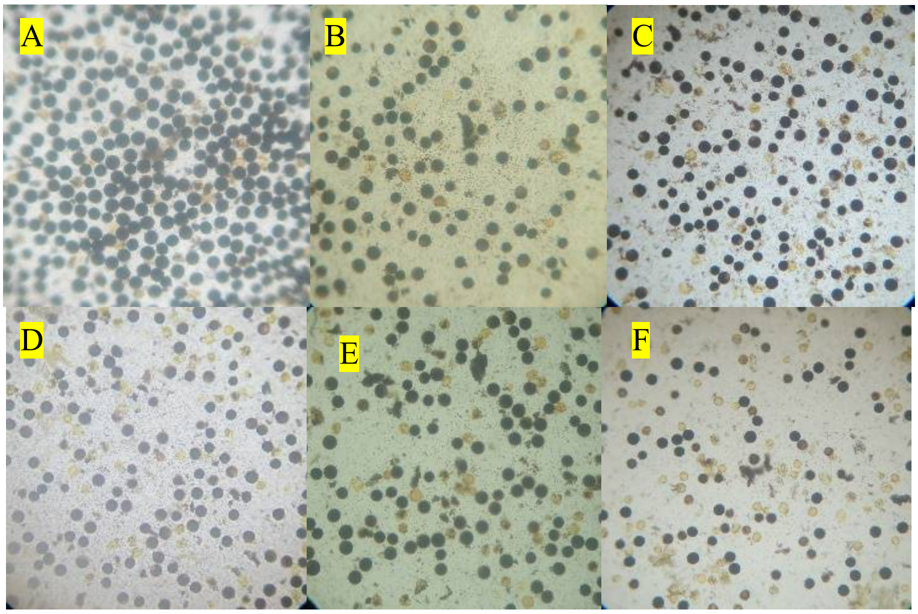


Figure 1: Radiation sensitivity on pollen fertility, spikelet fertility and grain yield.



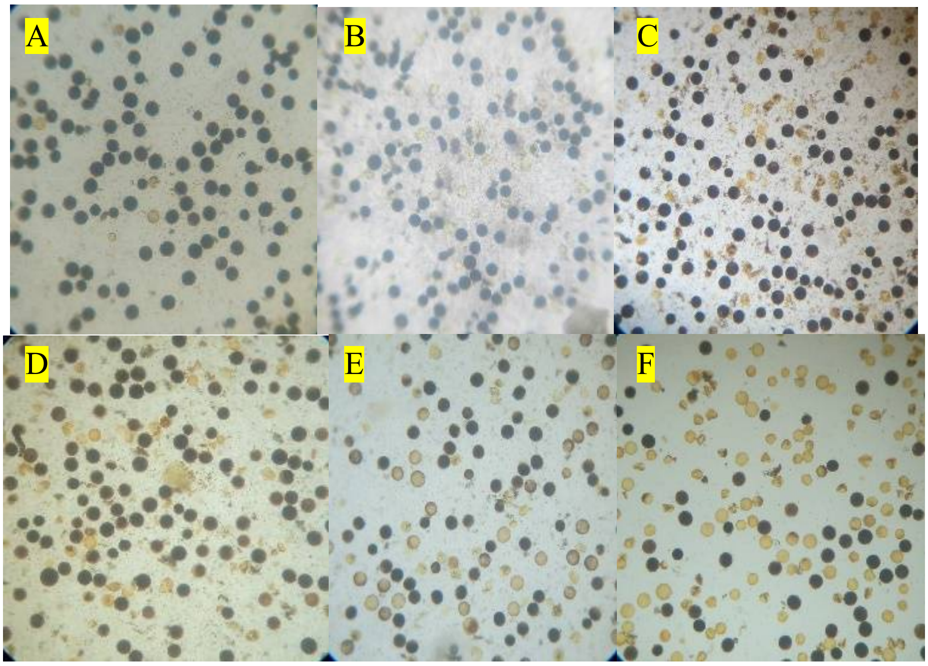
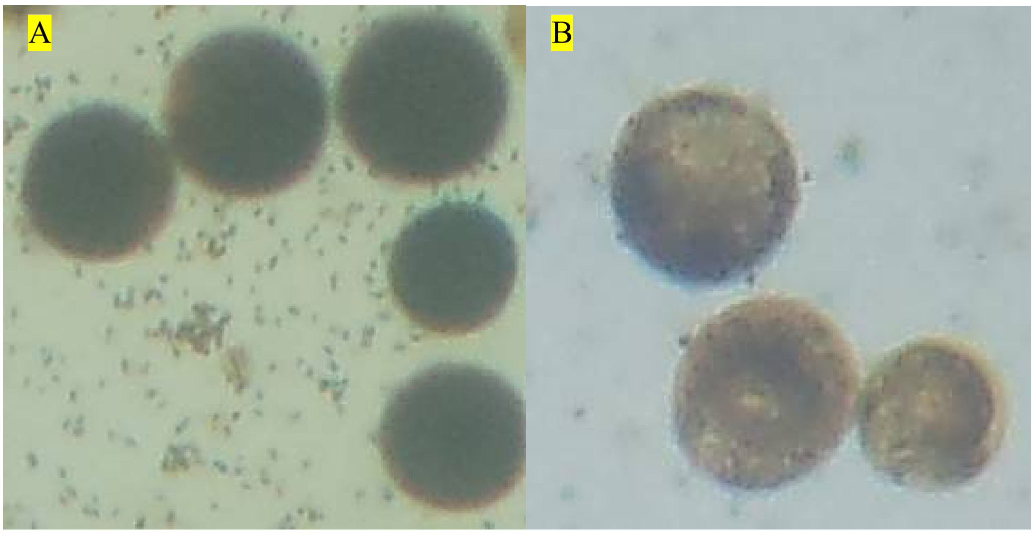


Figure 2: Radiation sensitivity on pollen fertility. A) Control B) 200Gy C) 250Gy D)300Gy E) 350Gy F) 400Gy

Figure 3: Radiation sensitivity on spikelet fertility



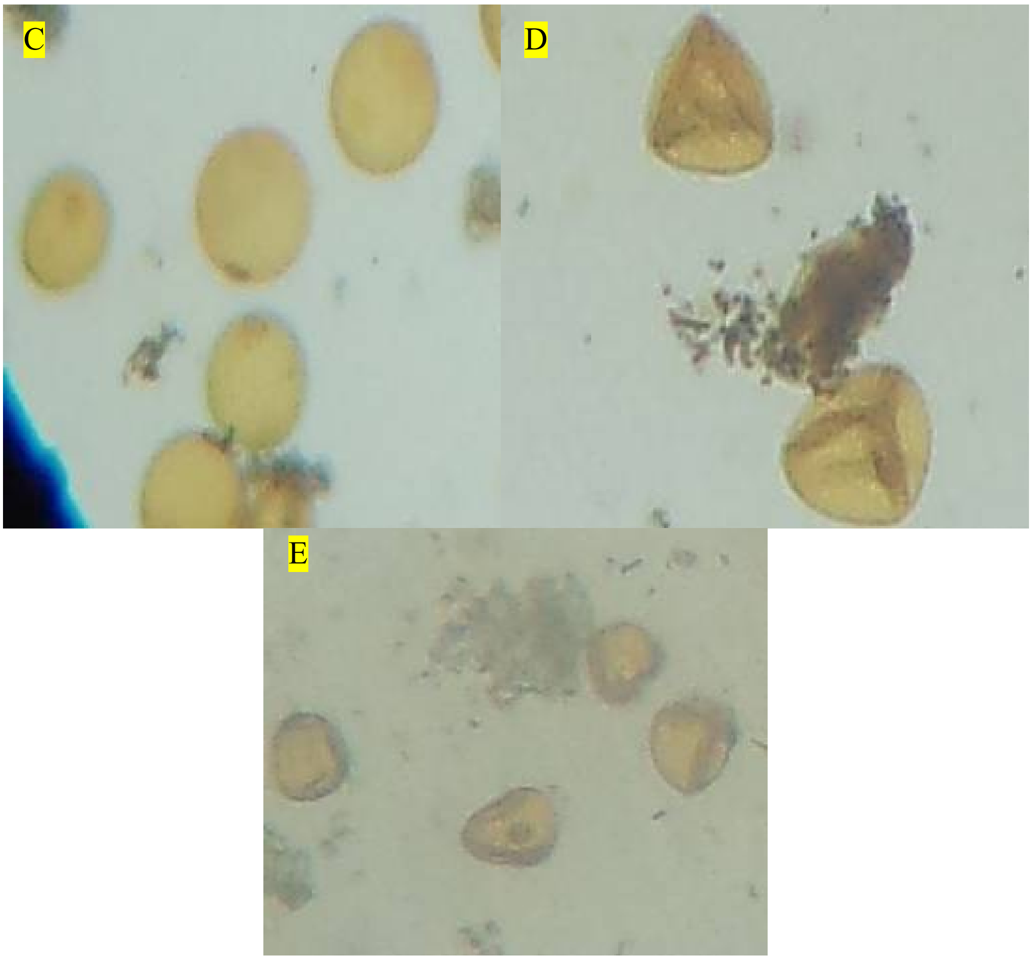


Figure 4: Radiation sensitivity on pollen shape. A) Completely fertile B) Partially sterile C) Completely sterile D) Tri angle shaped sterile E) Shapeless sterile

CONCLUSIONS

The pollen grain shapes were found to be very sensitive under increasing doses of gamma radiation. 50 percent pollen and spikelet population were found sterile at 260.89Gy and 390Gy irradiation respectively in Badshabhog cultivar. Spikelet fertility are also found affected at higher dose of gamma ray due to alteration of pollen fertility. The number of spikelet per panicle reduction due to present high pollen sterility and directly affected the yield. Finally, these finding helps to understand the optimal doses of gamma ray for production of viable sound mutations in Badshabhog rice with less cytological damages.

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DECLARATION OF INTEREST

Authors of article, declare that there is no conflict of interest.

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