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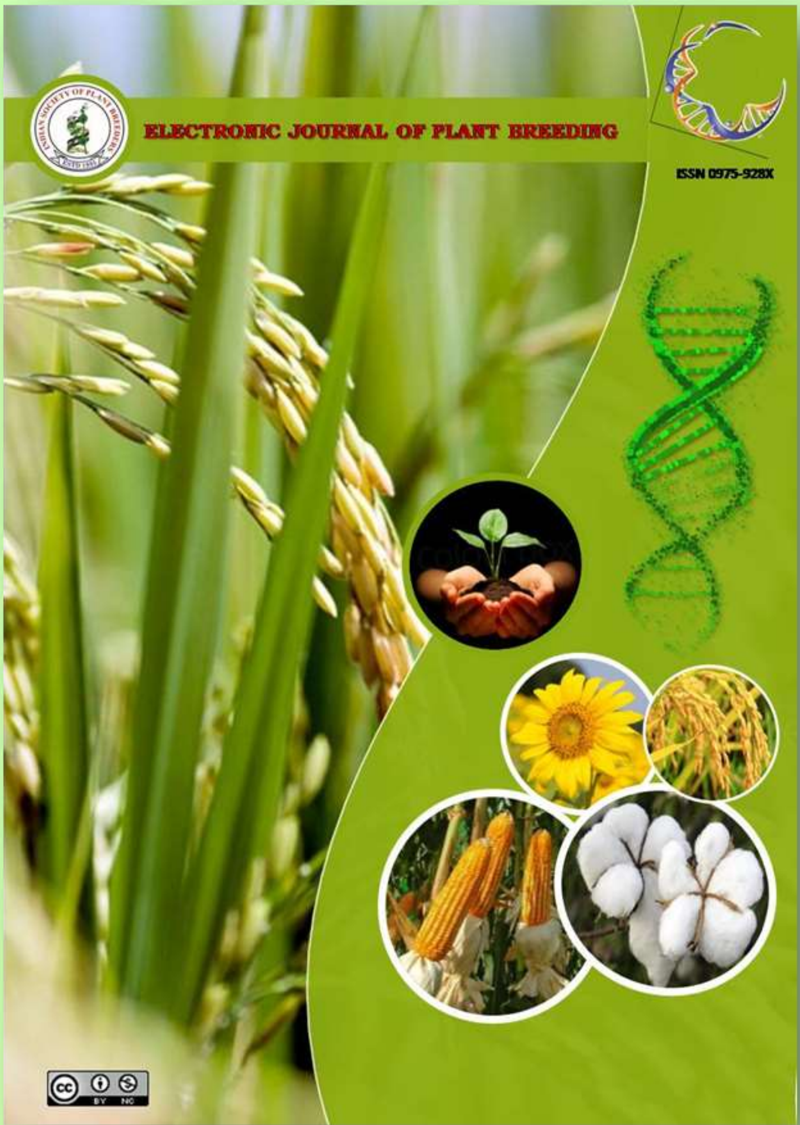

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Research Article

Study on the impact of mutagenic treatment on pollen and spikelet fertility and its relationship in rice (*Oryza sativa* L.)

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Abstract
The present study was conducted to study the impact of different mutagens on pollen and spikelet fertility. Pollen fertility and spikelet fertility per cent was found to be inversely proportional to increasing dose of the mutagen in all the treatments *viz.*, gamma rays, EMS and its combination treatment. The range of per cent reduction in pollen fertility was observed from 16.67 per cent (100 Gy) to 26.62 per cent (300 Gy) in gamma rays treatment of the seeds. Reduction in pollen fertility due to EMS treatment and its combination treatment ranged from 13.57 per cent reduction (10 mM) to 24.16 per cent reduction (30 mM) and 20.62 per cent reduction (100 Gy + 30 mM) to 31.43 per cent reduction (300Gy + 30 mM) respectively. Spikelet fertility also showed similar relationship in all the treatments. The correlation coefficient between pollen and spikelet fertility was found to be positive and significant. The optimum dose of mutagen was also determined by using pollen and spikelet fertility per cent. The optimum dose was found to be 300 Gy, and 173 Gy + 30 mM for gamma rays and combination treatment respectively. Hence, this proves to be an easy method for determining optimum dose of mutagen.

Keywords
Rice, mutation, pollen sterility, spikelet sterility

Introduction

Rice is one of the most important food crops across the globe. It has been in cultivation for over seven thousand years and it supports more than fifty per cent of the world population (Izawa and Shimamoto, 1996). It also has a significant role in social rites, rituals and festivals in many countries (Gowthami *et al.*, 2016). Induced mutations have been an important tool for crop improvement. Generally, crossing followed by selection of superior recombinants is used for crop improvement (Rutger *et al.*, 1976). But, mutation breeding is an efficient tool when only one or two simple modifications are needed in a cultivar (Micke, 1979). Both physical and chemical mutagens are employed for this purpose. Induced mutations have helped in improvement of rice cultivars by development of varieties with semi dwarf stature, early maturity, high tillering ability, disease resistance, low amylose and high yielding traits (Soomro *et al.*, 2006).

Mutated plants generally show considerable amount of pollen and spikelet sterility. This may be explained by induction of various chromosomal aberrations in treated plants. The different types of aberrations include stickiness, univalents, multivalents, precocious separation, stray bivalents, micronuclei, cytomixis, laggards, bridges, non-disjunction etc. (Dixit *et al.*, 2013; Wani and Bhat, 2017). Meiotic abnormalities occurring in the treated plants generally increases with increasing dose of the mutagen (Jayabalan and Rao, 1987). Pollen fertility and spikelet fertility have been important parameters for assessing mutation effect in plants. Hence, in the present investigation, the effect of gamma rays and Ethyl Methane Sulfonate (EMS) and their combination on pollen and spikelet sterility of the rice variety Akshaya (BPT 2231) has been analysed.

Materials and Methods

Around 650 well-filled seeds of the variety BPT 2231 per dose were taken for mutagen treatment. The seeds were packed in butter paper covers at a moisture content of 12 per cent and subjected to gamma rays treatment at Indira Gandhi Centre for Atomic Research, Kalpakkam. Seeds were exposed to gamma radiation of Cobalt 60 by placing it in a vertical drawer inside a lead flask. The duration of exposure depend on the dose rate of the source at the time of treatment. Seeds were treated with five different doses from 100 Gy to 500 Gy with 100 Gy interval. Two similar lots were treated in this manner for combination treatment with EMS. The treated seeds were sown in a nursery bed for germination on the next day of treatment. Untreated seeds were used as control.

Around 650 well filled healthy seeds per lot were used for chemical mutagenesis with Ethyl Methane Sulfonate. The seeds were pre-soaked for eight hours in distilled water. The seeds then treated with 10, 20 and 30 mM concentration of EMS with intermittent shaking for every 30 minutes for six hours. After treatment, the seeds were washed in running tap water to remove the residual chemical. The treated seeds were sown immediately in a raised bed nursery. Untreated seeds were used as control.

The second lot of gamma rays treated seeds were treated with 30 mM EMS. 100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy gamma irradiated seeds were treated with 30 mM EMS. Treatment methodologies remained the same as above. Untreated seeds were used as control. The M_1 generation field experiment was laid out in RBD design with two replications during *kharif*, 2018 at Agricultural College and Research Institute, Madurai. The twenty seven days old seedlings were transplanted in the main field with 20 cm × 15 cm spacing for evaluation. Recommended package of practices were followed for maintaining the healthy crop.

Unopened flowers from five randomly selected plants in each treatment were collected for the study. Pollen grains were dusted on a glass slide and stained using 1 per cent potassium iodide (1 g of iodine dissolved in 2 g of potassium iodide in 100ml of distilled water) and mounted using a cover slip. The glass slide was observed under a stereo microscope and the image was captured. Round and well stained pollen grains were classified as fertile, while unstained, half stained, empty, shrivelled pollen grains were classified as sterile and those having both the types were classified as partially sterile (Raj and Virmani, 1988) (Fig. 1). The pollen fertility was calculated by using the formula,

Pollen fertility per cent =

$$\frac{\text{Number of round well stained pollens}}{\text{Total number of pollens observed}} \times 100$$

Panicles from ten randomly selected plants in each treatment were collected. Total number of grains per panicle and number of well filled grains are counted from the selected panicle. The spikelet fertility per cent was calculated by using the formula,

Spikelet fertility per cent =

$$\frac{\text{Number of well filled grains}}{\text{Total number of grains per panicle}} \times 100$$

Results and Discussion

The extent of the effect of mutagen treatment can be studied using pollen and spikelet fertility. Pollen fertility per cent was found to be inversely proportional to increasing dose of the mutagen in all the treatments (Table 1; Table 2). Sterility per cent was converted into sterile index using the formula, Sterile index = arcsine$\sqrt{\text{Sterility percentage}}$. The range of per cent reduction in pollen fertility was observed from 16.67 per cent (100 Gy) to 26.62 per cent (300 Gy) in gamma rays treatment of the seeds (Fig. 2). Singh *et al.* (1998) used two different varieties for gamma irradiation and they reported that pollen and spikelet sterility increased with increasing dose of mutagen in both the varieties. Similar findings was reported by Jayabalan and Rao (1987) in tomato. They concluded that pollen fertility was inversely proportional to dose of the mutagen treated. Cytological, genetic and molecular studies have revealed that ionising radiations causes chromosomal rearrangements (Coe Jr *et al.*, 1988; Evans, 1962; Mcclintock, 1944; Sparrow, 1961; Stadler, 1928). Chromosomal abnormalities lead to development of sterile pollen grains (Muthusamy and Jayabalan, 2002; Rana and Swaminathan, 1964; Sinha and Godward, 1972). Chemical treatment with EMS also produced considerable pollen sterility. Reduction in pollen fertility due to EMS treatment ranged from 13.57 per cent reduction (10 mM) to 24.17 per cent reduction (30 mM). Dixit *et al.* (2013) reported that pollen sterility increased with increasing dose of EMS. Cytogenetic analysis in their study also has shown that pollen sterility is highly correlated with per cent of meiotic aberrations. Combination treatment of gamma rays and EMS produced more sterility compared to single treatment. Pollen fertility reduction ranged from 20.63 per cent reduction (100 Gy + 30 mM) to 31.43 per cent reduction (300Gy + 30 mM). These results were on par with Siddiq and Swaminathan (1968) and they reported that chromosome aberrations were higher in gamma rays treatment than EMS treatment. Sharma and Kumar (2004) concluded that sterility caused by irradiation treatment is due to cryptic deletions and specific gene mutations, while sterility produced by EMS treatment is due to chromosomal aberrations. Wani (2009) reported that combination treatment with Gamma rays and EMS did not show any pattern of efficient mutation in chick pea, but, 300 Gy + 0.2 per cent EMS was most efficient in producing lethality, sterility and injury, while, 200

Gy + 0.2 per cent EMS was most efficient in producing meiotic aberrations.

Similar trend was observed in case of spikelet fertility trait also (Table 3; Table 4). The range of per cent reduction in spikelet fertility was from 6.2 (100 Gy) to 39.88 (300 Gy) in gamma rays treatment. In case of EMS and its combination treatment, the range was from 0.37 per cent (10 mM) to 5.81 per cent (30 mM) and from 44.40 per cent (100 Gy + 30 mM) to 58.33 per cent (300 Gy + 30 mM) respectively. Cheema and Atta (2003) also reported that spikelet fertility was inversely related to dose of the treatment. The sterility produced by irradiation is believed to be partly transferred to subsequent generations (Anonymous, 1977) and the major part is not heritable as it is due to physiological damage.

The correlation coefficient, r is used to measure the strength of relationship between the two variables, i.e., pollen fertility and spikelet fertility. Correlation coefficient was calculated using data analysis in MS Excel. The relationship between pollen and spikelet sterility was studied based on all the doses and treatments (Table 5). In the present study, r was found to be equal to 0.75 indicating that, irrespective of the treatment, spikelet sterility increased when pollen sterility was more. Both the characters showed positive and significant relationship between each other. The results were on par with Sawada (1971) who also reported highly significant correlation for pollen and spikelet sterility.

Correlation coefficient for pollen and spikelet sterility among the different treatments were also found to be positive and highly significant (Table 6). The r value for pollen and spikelet sterility for gamma rays, EMS and combination treatment were 0.86, 0.77 and 0.99 respectively.

Graphs were constructed using sterile index of pollen against sterile index of spikelets. The pollen sterility per cent against the 50 per cent spikelet sterility was calculated by using the trendline equations derived from the graphs which are given below. ‘y’ in the equation denotes spikelet sterility per cent and ‘x’ denotes pollen sterility per cent. ‘x’ value is calculated by solving the equation when ‘y’ value is 50.

Equation 1 (Gamma treatment)	$y=1.411x-1.7915$
Equation 2 (EMS treatment)	$y=0.1821x+16.294$
Equation 3 (Combination treatment)	$y=1.7591x-2.3511$

Pollen sterility was found to differ between different treatment doses. The pollen sterility percent was found to be 36.71 per cent when the spikelet sterility was found to be 50 per cent in gamma rays treatment (Fig. 3). Similarly, the pollen sterility per cent for EMS treatment and its combination treatment with gamma rays was 185.10 per cent (Fig. 4) and 29.76 per cent (Fig. 5) respectively when its spikelet sterility was 50 per cent (Table 7). The results of EMS treatment has to be validated because only mild sterility occurred in this treatment. The concentration of EMS has to be increased to produce more sterility for a valid result. This shows that based on different treatments used, its impact on embryo sac fertility is different, producing considerable differences in pollen and spikelet fertility.

The dose of mutagen which produces 50 per cent spikelet sterility was also be estimated. It can be regarded as the optimum dose of that particular mutagen. The methodology is similar to the method of estimation of optimum pollen sterility. Around 300 Gy was found to be optimum dose for gamma rays treatment (Fig. 6). In case of EMS treatment, even the highest dose of 30 mM produced only mild sterility. Hence, the concentration of the mutagen has to be increased several times for producing 50 per cent sterility to find the optimum dose (Fig. 7). Combination treatment of gamma rays with EMS produced 50 per cent sterility and the optimum dose was found to be around 173 Gy + 30 mM (Fig. 8). The calculated optimum doses for all the treatments are given in Table 8. Hence, it is concluded that, even lower dose of combination treatment (200 Gy + 30 mM) could produce same amount of sterility that is produced by higher dose of gamma rays (300 Gy).

Pollen fertility and spikelet fertility per cent were found to be inversely related to the dose of mutagen in all the treatments studied. The 50 per cent spikelet sterility was arrived at 36.71 per cent and 29.76 per cent of pollen sterility respectively for gamma rays and its combination with EMS. The correlation coefficient was also found to be positive and significant between pollen and spikelet fertility. Hence, increasing the dose to higher level reflects in higher pollen sterility and results in spikelet fertility beyond 50 per cent level in M_1 generation in rice. As like sterility and fertility status, wide range of variability were observed for the characters *viz.*, days to 50 % flowering, plant height, number of tillers per plant, number of grains per panicle, 100 grain weight and single plant yield in M_1 generation. It will be subjected to selection for identification of better genotype in latter generation.

Determination of effective and efficient mutagens is a preliminary step for any mutation breeding programme. Assessment of pollen and spikelet fertility proves to be a good and rather easy method for this purpose. Optimum dose of mutagen which produces maximum frequency of mutation is required to produce a large mutant population. Large plant population is essential for isolation of desirable mutants. Treatment of plants with optimum dose of mutagen produces plants with high frequency of mutation with reduced lethality, thus not limiting the total plant population.

Acknowledgement
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Table 1. Reduction in pollen fertility due to gamma rays treatment

Treatment	% Fertility	Transformed mean	% Sterility	Transformed mean	% Over control	% reduction over control
Control	95.05	77.15	4.95	12.86	100	0
100 Gy	81.17	64.28	18.83	25.72	83.33	16.67
200 Gy	75.86	60.57	24.14	29.43	78.52	21.48
300 Gy	69.71	56.61	30.29	33.39	73.38	26.62

Table 2. Reduction in pollen fertility due to EMS and combination treatments

Treatment	% Fertility	Transformed mean	% Sterility	Transformed mean	% Over control	% reduction over control
Control	95.05	77.15	4.95	12.86	100	0
10 mM	84.32	66.67	15.68	23.33	86.43	13.57
20 mM	78.62	62.46	21.38	27.54	80.96	19.04
30 mM	72.7	58.50	27.3	31.50	75.83	24.17
100+30	76.84	61.23	23.16	28.77	79.37	20.63
200+30	69.64	56.57	30.36	33.44	73.32	26.68
300+30	63.61	52.90	36.39	37.10	68.57	31.43

Table 3. Reduction in spikelet fertility due to gamma rays treatment

Treatment	% Fertility	Transformed mean	% Sterility	Transformed mean	% Over control	% reduction over control
Control	89.09	70.71	10.91	19.29	100	0
100 Gy	83.88	66.33	16.12	23.67	93.80	6.20
200 Gy	49.2	44.54	50.8	45.46	62.99	37.01
300 Gy	45.67	42.52	54.33	47.49	60.12	39.88

Table 4. Reduction in spikelet fertility due to EMS and combination treatments

Treatment	% Fertility	Transformed mean	% Sterility	Transformed mean	% Over control	% reduction over control
Control	89.09	70.71	10.91	19.29	100	0
10 mM	88.8	70.45	11.2	19.55	99.63	0.37
20 mM	87.99	69.73	12.01	20.28	98.60	1.40
30 mM	84.23	66.60	15.77	23.40	94.19	5.81
100 + 30	40.15	39.32	59.85	50.68	55.60	44.40
200 + 30	29.03	32.60	70.97	57.40	46.10	53.90
300 + 30	24.2	29.47	75.8	60.53	41.67	58.33

Table 5. Correlation between pollen sterility and spikelet sterility irrespective of the treatment

	Pollen Sterility	Spikelet Sterility
Pollen Sterility	1	
Spikelet Sterility	0.75	1

Table 6. Correlation between pollen and spikelet sterility by different treatments

Gamma rays	Pollen Sterility	Spikelet Sterility
Pollen Sterility	1	
Spikelet Sterility	0.86	1

EMS	Pollen Sterility	Spikelet Sterility
Pollen Sterility	1	
Spikelet Sterility	0.77	1

Combination	Pollen Sterility	Spikelet Sterility
Pollen Sterility	1	
Spikelet Sterility	0.99	1

Table 7. 50 per cent spikelet sterility *vs* per cent pollen sterility

Gamma ray treatment	Spikelet Sterility	Pollen Sterility
Control	19.29	12.86
100 Gy	23.67	25.72
200 Gy	45.46	29.43
300 Gy	47.49	33.39
y=1.411x-1.7915	**50**	**36.71**
EMS treatment	**Spikelet Sterility**	**Pollen Sterility**
Control	19.29	12.86
10 mM	19.55	23.33
20 mM	20.28	27.54
30 mM	23.40	31.50
y=0.1821x+16.294	**50**	**185.10**
Combination treatment	**Spikelet Sterility**	**Pollen Sterility**
Control	19.29	12.86
100 Gy + 30 mM	50.68	28.77
200 Gy + 30 mM	57.40	33.44
300 Gy + 30 mM	60.53	37.10
y=1.7591x-2.3511	**50**	**29.76**

Table 8. 50 per cent spikelet sterility *vs* treatment dosage

Gamma ray treatment	Spikelet sterility	EMS treatment	Spikelet sterility	Combination	Spikelet sterility
0	19.29	0	19.29	0	19.29
100	23.67	10	19.55	100 Gy + 30 mM	50.68
200	45.46	20	20.28	200 Gy + 30 mM	57.40
300	47.49	30	23.40	300 Gy + 30 mM	60.53
300.57	**50**	**239.89**	**50**	**173.11**	**50**
(y = 0.1064x + 18.019)		(y = 0.1306x + 18.67)		(y = 0.1305x + 27.408)	

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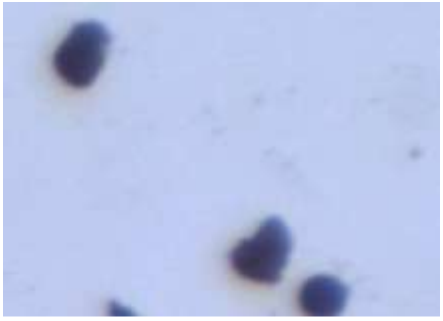
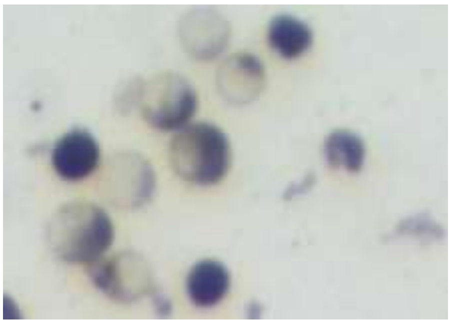
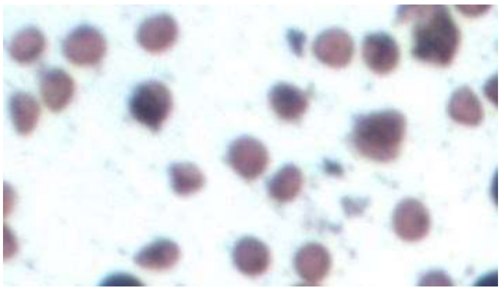


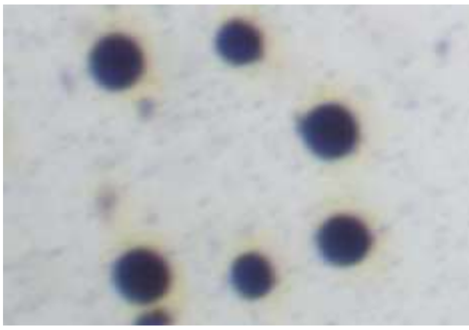
Shriveled pollen



Partially filled pollen



Completely sterile



Completely fertile

Fig. 1. Morphology of acetocarmine stained pollen grains

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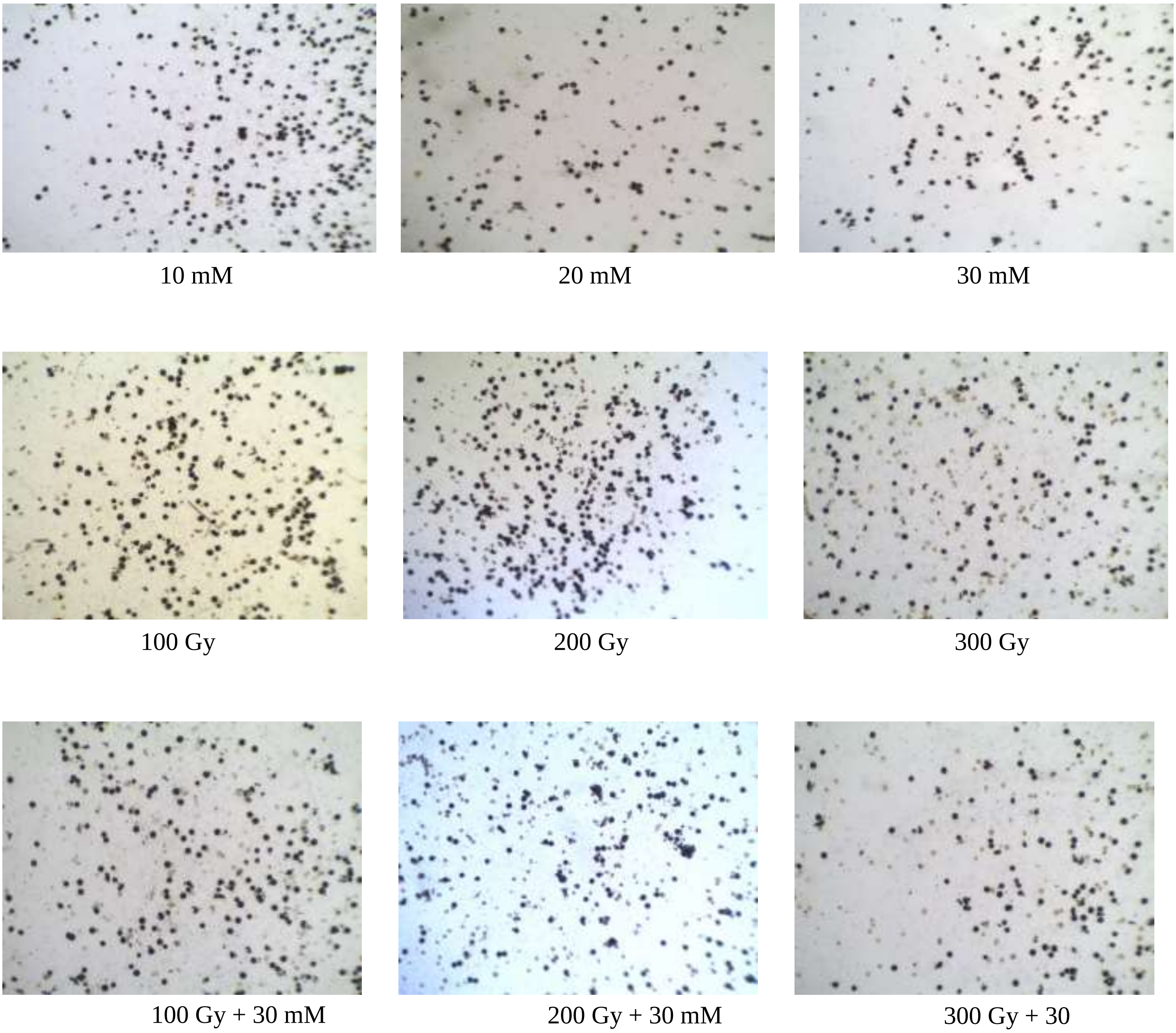

Fig. 2. Effect of gamma rays, EMS and its combination on pollen fertility

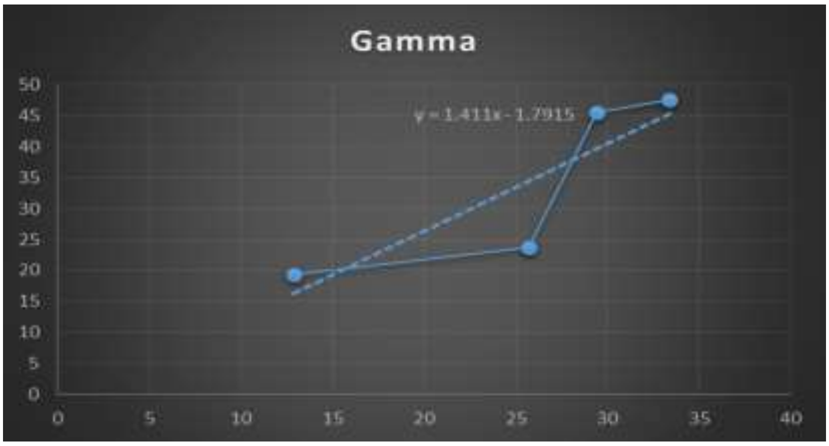

Fig. 3. Pollen sterility *vs* spikelet sterility in gamma ray treatment

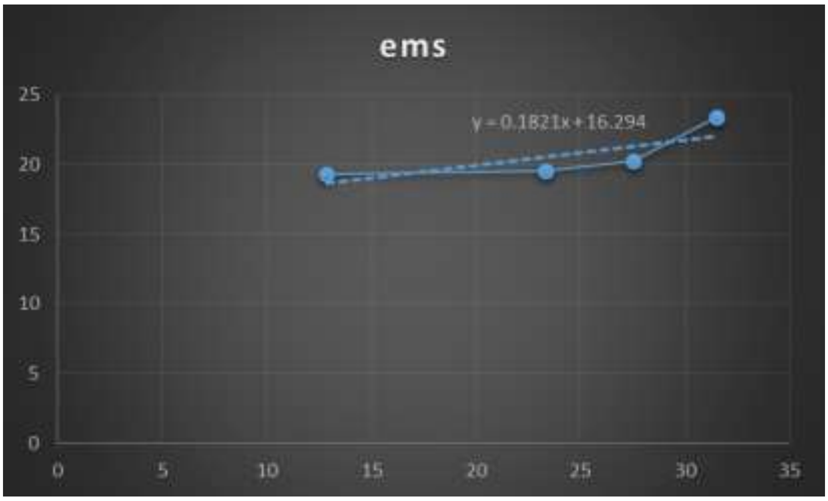

Fig. 4. Pollen sterility *vs* spikelet sterility in EMS treatment

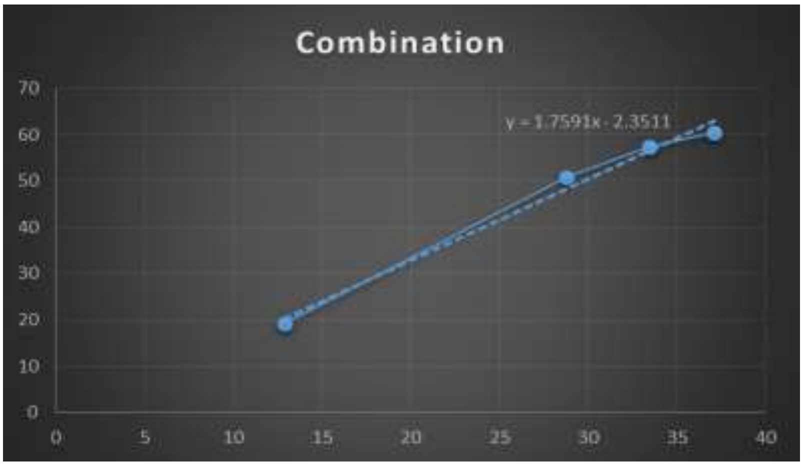

Fig. 5. Pollen sterility *vs* Spikelet sterility in combination treatment

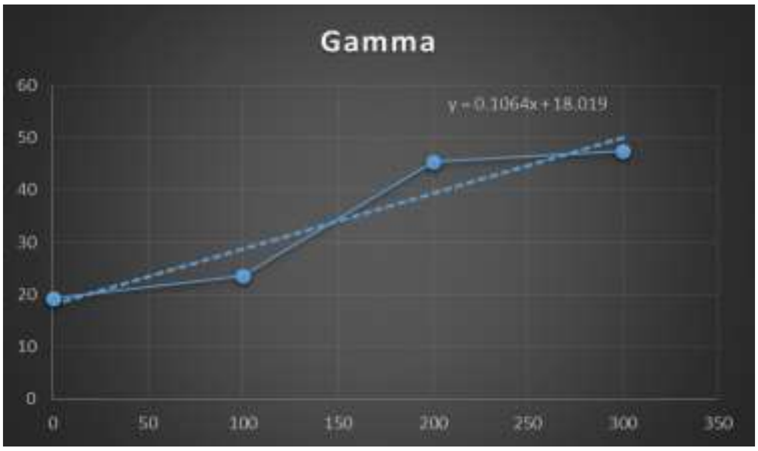

Fig. 6. Dose of mutagen *vs* spikelet sterility in gamma rays

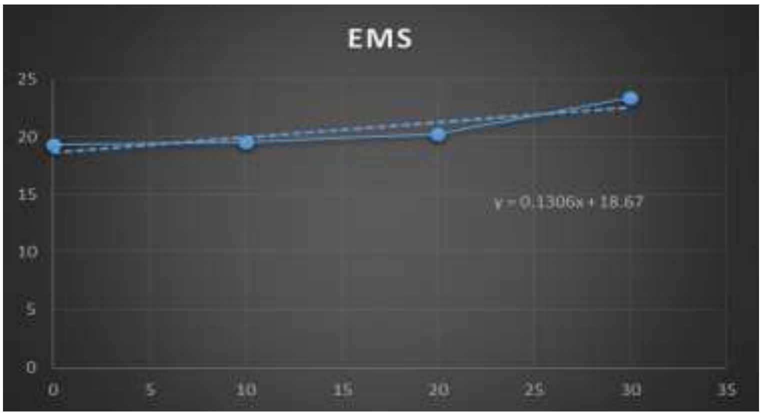

Fig. 7. Dose of mutagen *vs* spikelet sterility in EMS

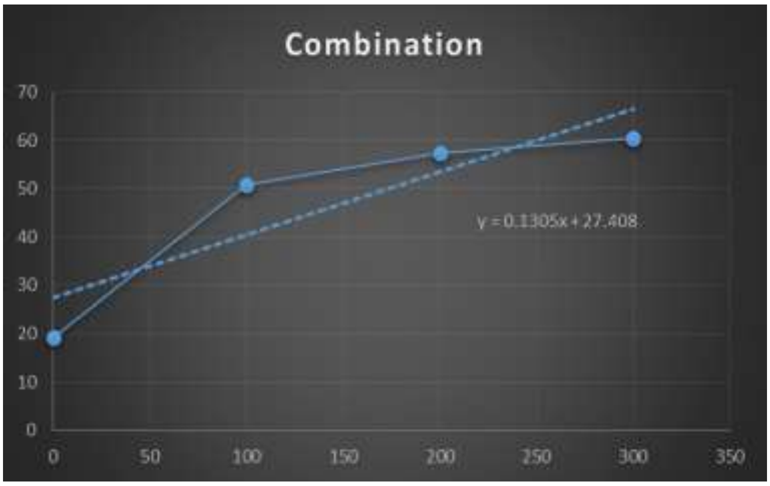

Fig. 8 . Dose of mutagen *vs* spikelet sterility in combination treatment

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