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ASSESSING THE MUTAGENIC IMPACT OF GAMMA RADIATION AND EMS ON SEED GERMINATION AND POLLEN STERILITY IN MOTH BEAN (*VIGNA ACONITIFOLIA* (JACQ.) MARECHAL)

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ABSTRACT

Traditional plant breeding methods have demonstrated limited efficacy in generating genetic variability for the genetic improvement of moth bean. In contrast, mutation breeding has proven to be a highly effective strategy for inducing variability, particularly in self-pollinated crops. In the present study, the moth bean variety RMO-435 was subjected to gamma irradiation (200, 400, 600, and 800 Gy), ethyl methane sulphonates (EMS) treatments (0.15%, 0.3%, 0.45%, and 0.6%), and their respective combinations. A total of 26 treatment combinations of physical and chemical mutagens, along with a control (seeds soaked in distilled water for 4 hours) and an absolute control (untreated dry seeds), were evaluated for germination, lethality, and pollen fertility under both field and laboratory conditions. The findings revealed that RMO-435 exhibited heightened sensitivity to both physical and chemical mutagens in the M_1 generation. The lethal dose 50 (LD_{50}) for gamma radiation was estimated to be between 400 Gy and 600 Gy, whereas for EMS, it was suggested that an LD_{50} threshold lies slightly below 0.6%. Pollen sterility at a 600 Gy dose of gamma radiation reached 55.06%, while for EMS, sterility was approximately 48.03% at 0.45%. Furthermore, a dose-dependent increase in lethality was observed with rising concentrations of both mutagens. The study confirms the effectiveness of gamma radiation and EMS in inducing genetic variability in moth bean. However, gamma irradiation was found to exert a more pronounced effect, significantly impacting seed germination and pollen fertility compared to chemical mutagenesis. These findings provide valuable insights for future breeding programs aimed at enhancing the genetic potential of moth bean.

Keywords: EMS, Gamma radiation, Pollen fertility, Moth bean, Mutation breeding.

Introduction

Mothbean, *Vigna aconitifolia* (Jacq.) Marechal, is an important short-duration pulse crop well-adapted to arid and semi-arid regions. It is primarily cultivated in India, where it serves as a vital source of dietary protein, particularly in drought-prone areas. This crop is highly resilient to extreme environmental conditions, making it an essential component of sustainable agriculture and food security in regions with limited water availability (Meena *et al.,* 2020). Moth bean is a rich source of high-quality protein, carbohydrates, and micronutrients, contributing significantly to the nutritional requirements of populations in arid regions (Verma *et al.,* 2019; Bhadkaria, 2022). It also plays a crucial role in improving soil fertility through biological nitrogen fixation, thereby enhancing soil health and sustainability (Patel *et al.,* 2016). Despite its adaptability and nutritional significance, moth bean exhibits limited genetic variability due to its predominantly self-pollinated nature, which restricts conventional breeding efforts (Sharma *et al.,* 2018). The narrow genetic base poses a challenge in developing high-yielding, stress-tolerant, and nutritionally superior varieties.

To overcome this limitation, mutation breeding has emerged as an effective approach for generating genetic variability and improving key agronomic traits. Induced mutations have been successfully utilized to enhance yield, drought tolerance, and other desirable characteristics in moth bean (Yadav *et al.,* 2021). Both physical (gamma radiation) and chemical (EMS) mutagens have been employed to develop improved mutants with superior agronomic and nutritional traits. Understanding the genetic control of important traits is crucial for designing efficient breeding strategies aimed at moth bean improvement (Singh *et al.* 2020).

Mutation breeding provides an accelerated approach to crop improvement by inducing beneficial genetic variations that may not be readily available in natural populations. The development of high-yielding, climate-resilient moth bean varieties through mutation breeding holds great potential for ensuring food and nutritional security, particularly in regions affected by climate change and soil degradation.

Materials and Methods

Field and laboratory studies were conducted at College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner during kharif 2022. Moth bean variety RMO-435 was exposed to varying doses of gamma radiation (200, 400, 600 and 800 Gy), ethyl methane sulphonate (0.15, 0.3, 0.45 and 0.6%) and their combinations. Healthy dry seeds were irradiated with gamma radiation at Bhabha Atomic Research Center, Trombay (Mumbai) and for chemical mutagen treatment, firstly seeds were uniformly soaked in distilled water for 4 hrs at room temperature and after that treated with freshly prepared 0.15, 0.3, 0.45 and 0.6% ethyl methane sulphonate (EMS) in phosphate buffer solution for 6 hrs at room temperature. Total 26 treatment combinations with control (seed soaked in distilled water for 4 hrs) and absolute control (normal dry seed) were laid out on July 19, 2022 using Randomized Block Design with three replications accommodating 5 m long 9 rows at 30 cm spacing. The data was recorded on germination at seven days after sowing (ISTA 1993) and after flowering, pollen sterility with the help of microscope by staining the pollens with 1% aceto-carmine stain was recorded. The fully stained pollen grains were considered fertile (Fig. 1), while unstained, abnormal shaped and improperly filled pollen grains were considered sterile (Fig. 2). Pollen sterility (%) was measured as the ratio of sterile pollen grains to the total number of pollens observed under microscopic field and Lethality percentage was analyzed using standard formulae (Lethality = 100 – Germination percentage).

Results and Discussion

Germination

Analysis of variance indicated highly significant variation among treatments for germination (Table 1). Gamma radiation alone and its combined application with EMS adversely affected seed germination and effects were found more severe at higher doses of mutagens. A treatment combination of gamma radiation 800 Gy along with 0.6% EMS exhibited minimum seed germination (Table 2). A gradual decline in seed germination was also observed at gradual increase in doses of gamma and concentration of EMS however treatment differences were not found statistically significant. Based on seed germination percentage, the lethal dose 50 (LD_{50}) for gamma radiation was estimated to be between 400 Gy and 600 Gy, whereas for EMS, it was suggested that an LD_{50} threshold lies slightly below 0.6%. Figure 2 reveals that both field and laboratory studies exhibited a similar pattern of decline in germination percentage with increasing doses of gamma radiation and EMS. Although absolute values varied slightly between the two conditions, the overall trend remained consistent higher mutagen doses led to reduced germination. This parallel response confirms the reliability of laboratory screening for predicting field performance in mutation studies of moth bean. Narayan *et al.* (2015) also recorded germination percent ranged from 12.50 (800 Gy) to 78.40 (200 Gy) in moth bean. Other similar results have been reported by Kumar (2014), Vairam and Ibrahim (2014), Hemavathy (2015), Mori *et al.* (2016), Rukesh *et al.* (2017), Sofia *et al.* (2019), Bolbhat and Korade (2020), Bonde *et al.* (2020), Vikhe and Nehul (2020), Kumar *et al.* (2023) and Thounaojam *et al*. (2024).

Pollen sterility

Analysis of variance indicated highly significant differences among treatments for pollen sterility. Gamma radiation alone and its combined application with EMS adversely affected pollen fertility and effects were found more severe at higher doses of mutagens. In both varieties, a treatment combination of 800 Gy gamma radiation and 0.6% EMS resulted in maximum pollen sterility (Table 2, Fig. 3). At greater EMS concentrations, pollen sterility began to increase gradually. Based on pollen sterility percentage, gamma radiation @ 600 Gy and @800 Gy alone and its combined application with EMS was found near to LD_{50} in moth bean variety RMO-435. Similar results were also recorded by Kulthe (2019), Swain et al. (2019), Khan and Goyal (2009), Tah (2006) and Khan (1981). Pollen fertility declines as gamma ray exposures raise, indicating a positive liner connection, which could be related to an increase in the frequency of meiotic chromosomal aberrations in PMC or microspores. Induced sterility can be caused by gene mutations, hidden deficits and cytoplasmic causes (Malinoveskii *et al.* 1973).

Lethality

Analysis of variance indicated highly significant differences among treatments for lethality. The degree of lethality was found to be associated with the dose of gamma radiation. Higher doses of gamma radiation (400, 600 and 800 Gy) alone and their combined application with EMS were found lethal to moth bean and maximum lethality was exhibited with combined application of gamma radiation 800 Gy along with 0.6% EMS (Table 2), however, lower dose of gamma radiation (200 Gy) and solitary application of EMS were not found lethal to moth bean. Konzak (1965) reported that higher rate of gamma radiation is responsible for more lethality that might be due to biological damage caused by radiation. Physical and biochemical damage, chromosomal variation and induced sterility, in addition to mutation effects, are all factors that influence the effectiveness of a mutagenic agent (Hemnani, 2017). Similar results were also reported by Jain *et al.* (2013), %). Narayan *et al.* (2015), Hemnani (2017) and Rukesh *et al.* (2017).

Conclusion

The narrow genetic base is a major impediment to breeding progress in moth bean. The application of gamma radiation and EMS may be helpful in regeneration and restoration of genetic variability in self-pollinated crops. Moth bean variety RMO-435 responded to mutagen treatments but expressed in a different way under various treatment combinations of gamma radiation and ethyl methane sulphonate. Combined application of physical and chemical mutagens was more hazardous to seed germination and pollen fertility as compared to solitary treatments. Therefore, mutation breeding has enormous potential and opportunities in creating genetic variability in moth bean and provides scope of selection of desirable genotypes.

Table 1 : Analysis of variance (mean square) for different characters in M_1 generation of mothbean variety RMO-435 under field conditions

Source of variation	d.f.	Characters		
		Germination percentage (%)	Pollen Sterility	Lethality percent (%)
Replication	2	8.17	21.45	8.17
Treatments	25	1836.09**	2265.83**	1836.09**
Error	50	12.65	11.91	12.65

**Significant at p=0.01%

Table 2 : Effect of physical and chemical mutagens on different characters in M_1 generation of moth bean variety RMO-435 under field conditions

S. No.	Treatments/Dose	Germination Percentage (%)	Pollen sterility (%)	Lethality (%)
1	200 Gy	76.33	23.32	23.67
2	400 Gy	61.00	40.27	39.00
3	600 Gy	43.67	55.06	56.33
4	800 Gy	16.00	83.59	84.00
5	0.15 % EMS	84.67	17.88	15.33
6	0.3 % EMS	73.33	21.75	26.67
7	0.45 % EMS	60.33	48.03	39.67
8	0.6 % EMS	46.67	64.65	53.33
9	200 Gy + 0.15% EMS	72.67	37.90	27.33
10	200 Gy + 0. 3% EMS	64.00	35.81	36.00
11	200 Gy + 0.45% EMS	52.67	54.13	47.33
12	200 Gy + 0.6 % EMS	41.00	70.11	59.00
13	400 Gy + 0.15% EMS	55.67	46.32	44.33
14	400 Gy + 0. 3 % EMS	45.67	56.51	54.33
15	400 Gy + 0.45% EMS	41.33	69.15	58.67
16	400 Gy + 0.6 % EMS	35.00	78.56	65.00
17	600 Gy + 0.15% EMS	40.67	58.77	59.33
18	600 Gy + 0. 3% EMS	35.67	65.73	64.33
19	600 Gy + 0.45% EMS	31.00	75.01	69.00
20	600 Gy + 0.6 % EMS	23.67	83.01	76.33
21	800 Gy + 0.15% EMS	15.00	87.47	85.00
22	800 Gy + 0. 3 % EMS	13.67	90.86	86.33
23	800 Gy + 0.45% EMS	9.67	95.57	90.33
24	800 Gy + 0.6% EMS	5.33	98.54	94.67
25	Control (Absolute)	84.33	3.72	15.67
26	Control (Water soaked)	92.67	2.89	7.33
	Mean	46.99	56.33	53.01
	Range	5.33-92.67	2.89-98.54	7.33-94.67
	CD (p=0.05%)	5.83	5.66	5.83
	Sem ±	2.05	1.99	2.05
	Sed ±	2.90	2.82	2.90
	CV (%)	7.57	6.13	6.71

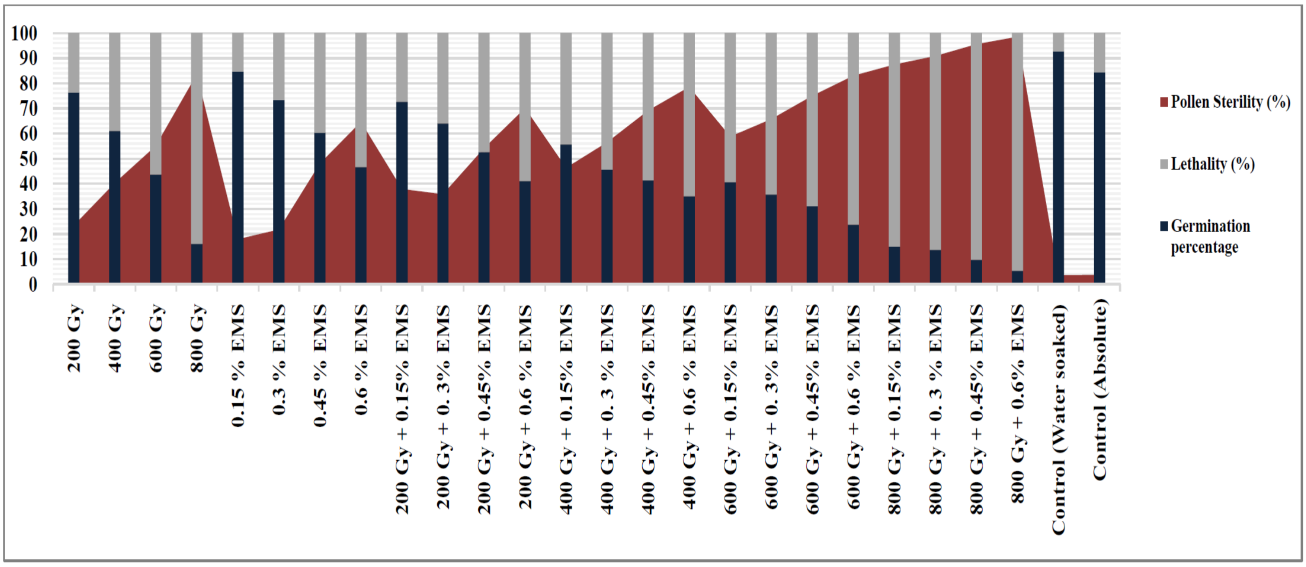

Fig. 1 : Comparative effect of different mutagenic treatments on germination percentage, lethality percentage and pollen sterility percentage in mothbean variety RMO-435 during field trial of M_1 generation

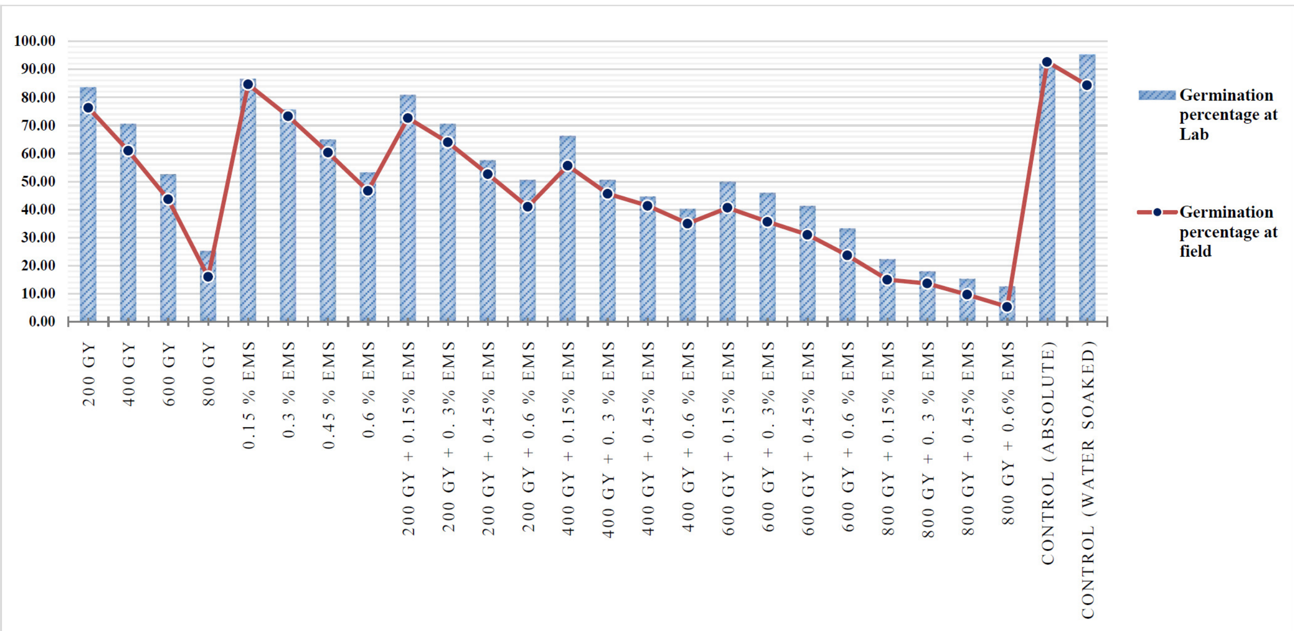

Fig. 2 : Comparison of different mutagenic treatments on germination percentage under laboratory versus field conditions in mothbean variety RMO-435 during M_1 generation

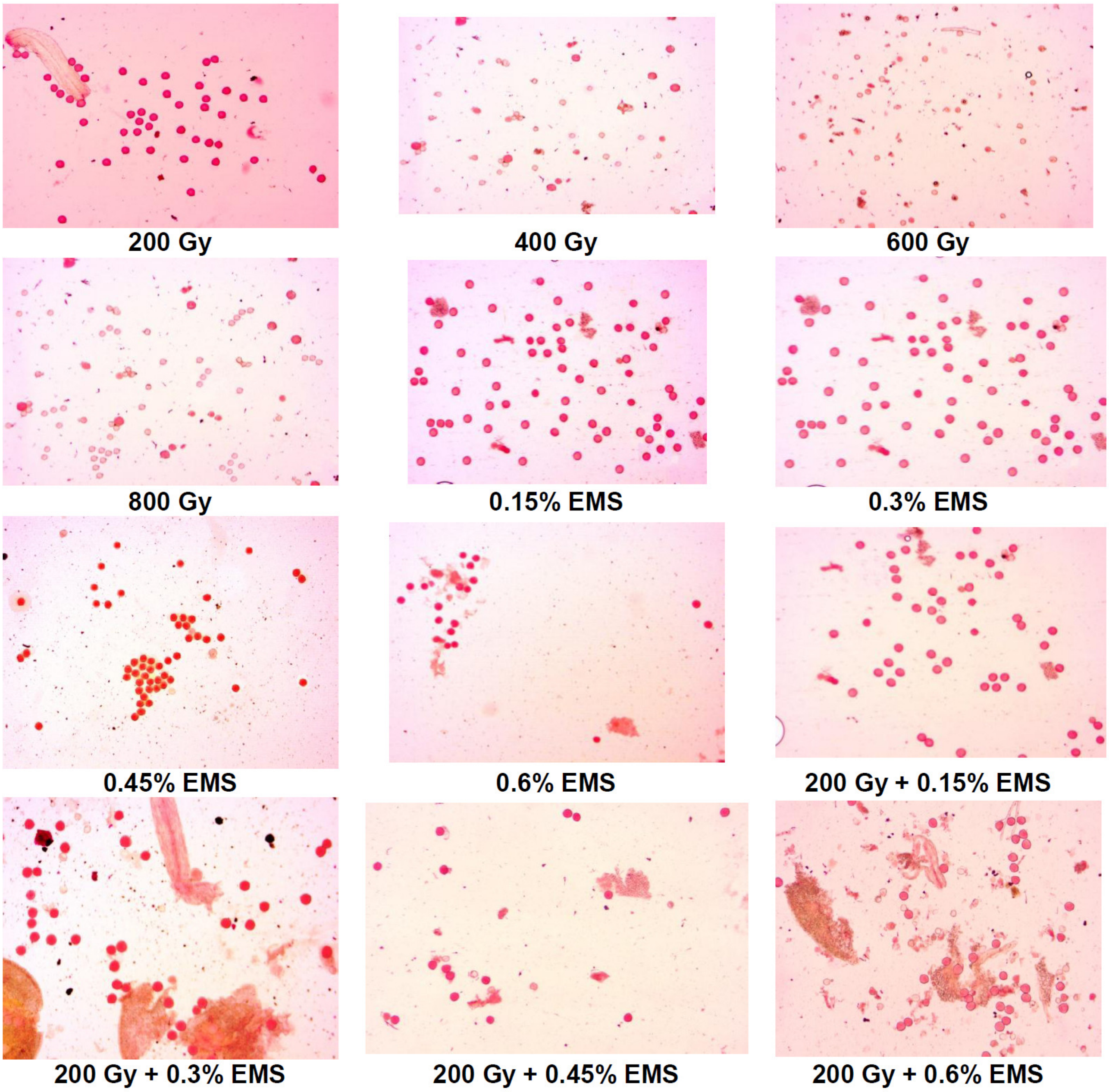

Fig. 3a: Differences in pollen fertility percentage at different doses of physical and chemical mutagens in mothbean variety RMO-435

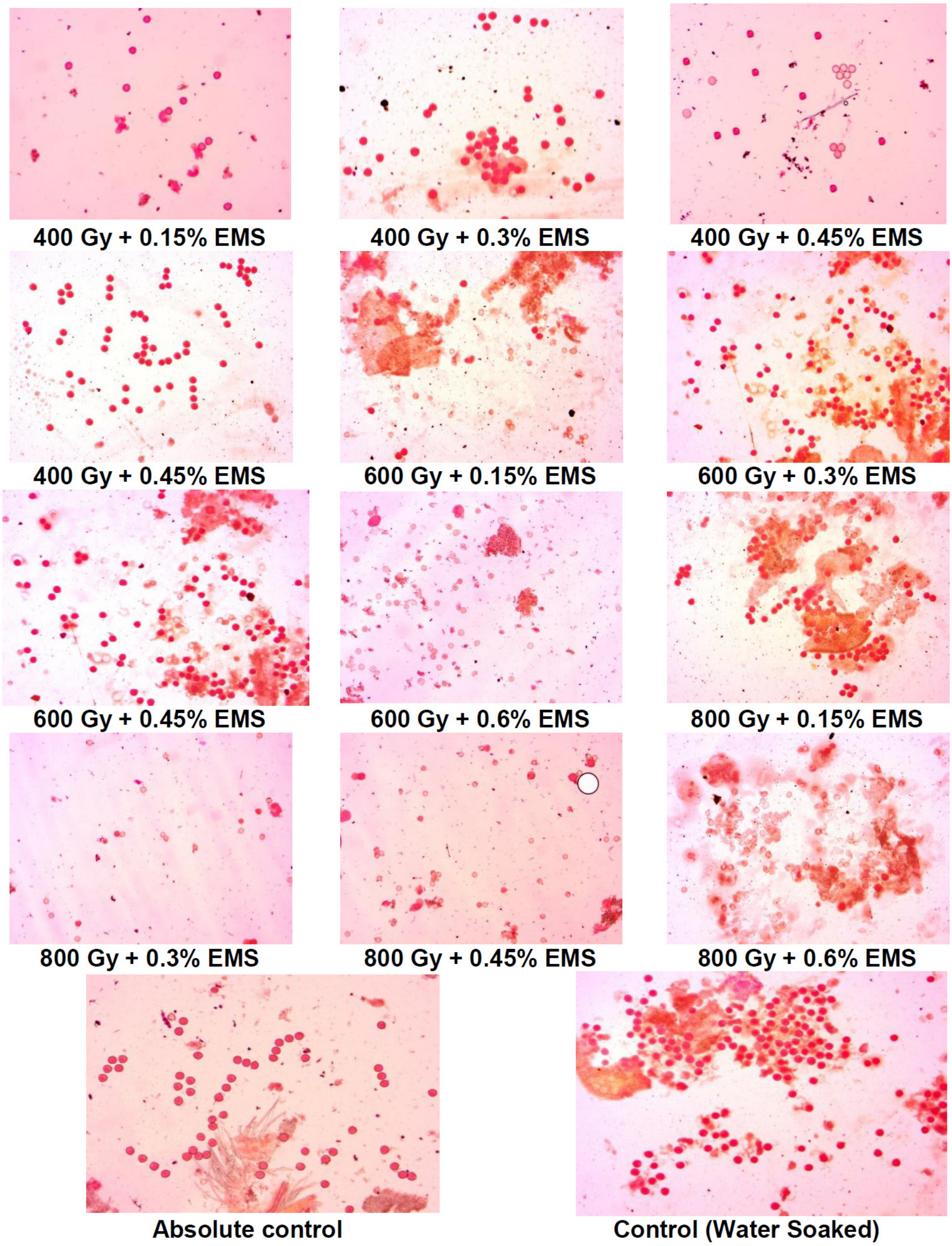

Fig. 3b: Differences in pollen fertility percentage at different doses of physical and chemical mutagens in mothbean variety RMO-435

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