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Effect of Seed Treatment of Gamma Radiation and EMS on Germination and Pollen Fertility in Mungbean *Vigna radiata* (L.) Wilczek

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ABSTRACT

Traditional plant breeding is not very effective in creating genetic variability for mungbean improvement program. Mutation breeding is considered very effective in creating genetic variability for improvement of self-pollinated crops. In present study, two mungbean varieties Ganga-1 and GM- 4 were treated with gamma radiation (200, 400 and 600 Gy), ethyl methane sulphonate (0.1 and 0.2 %) and their combinations. Total 26 treatment combinations of physical and chemical mutagens along with control (seed soaked in distilled water for 4 hrs) and absolute control (normal dry seed) were studied for germination, lethality and pollen fertility under field and laboratory conditions. The results exhibited that in M_1 generation variety Ganga-1 was found more sensitive to both physical and chemical mutagens as compared to GM-4. Gamma radiation with 400 Gy was found near to LD_{50} in variety Ganga-1, however, it was 600 Gy in case of variety GM-4. At 600 Gy dose, pollen fertility was observed to be close to 50% (52.99%) in Ganga-1, however, it was 62.48% in GM-4 at the same dose. The rate of lethality was also found to be gradually increased with higher concentration of chemical mutagens. The use of physical and chemical mutagens has been found effective in creating variability in mungbean. Gamma radiation was found to be more effective and adversely affected the seed germination and pollen fertility of mungbean as compared to chemical mutagen.

Keywords EMS, Gamma radiation, Pollen fertility, Mungbean, Mutation breeding.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) it is one of most important short-duration pulse crop. In Asian diet, it is an excellent source of high-quality protein (Das *et al.* 2021). Mungbean cultivation has been going on in India since 2200 BC (Idress *et al.* 2006). It's native to India and Central Asia. This crop has the potential to significantly contribute to bridging the protein gap for the world's over 500 million under nourished people (Vairam *et al.* 2017). Dietary proteins provide amino acids that are used in the

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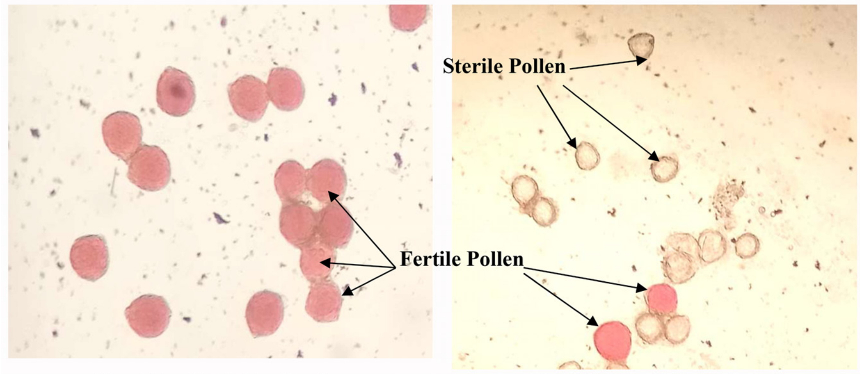

Fig. 1. Fertile pollen grains. **Fig. 2.** Sterile pollen grains.

synthesis of body proteins and other biologically important nitrogenous compounds (Dewanjee and Sarkar 2018). These are important for sustainable agriculture and enriching soil organic matter through biological nitrogen fixation, in addition to being a rich source of protein. Mungbean has very limited genetic variability because it is a self-fertilized crop (Kumar 2014). The lack of genetic variability for the major characters becomes a limiting factor for its improvement (Kumar *et al.* 2021). Knowledge of genes controlling important agronomic and quality traits is critical for plant breeders to develop proper strategies for efficient breeding programs. A mutation breeding program is a relatively faster method of crop improvement. Induced mutations have been shown to increase yield as well as other quantitative traits in plants (Sarkar and Kundagrami 2018). A wide range of physical and chemical mutagens have been used to induce useful mutants in a number of crops.

Table 1. Analysis of variance (mean square) for different characters in M_1 generation of mungbean under field conditions. **Significant at p=0.01.

Source of variation		Replication	Treatment	Error
df		2	12	24
Germination percent	Ganga 1	64.92	1239.53**	31.01
	GM 4	0.41	959.56**	30.05
Pollen fertility	Ganga 1	4.13	764.97**	47.46
	GM 4	13.38	543.27**	20.06
Lethality percent	Ganga 1	64.92	1239.53**	31.01
	GM 4	0.41	959.56**	30.05

MATERIALS AND METHODS

Field and laboratory studies were conducted at College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner during *kharif* 2021. Mungbean varieties namely Ganga-1 and GM-4 were exposed to varying doses of gamma radiation (200, 400 and 600 Gy), ethyl methane sulphonate (0.1 and 0.2%) and their combinations. Healthy dry seeds of both varieties 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 0.1 and 0.2% ethyl methane sulphonate (EMS) in phosphate buffer solution for 6 hrs at room temperature. Total 26 treatment combinations (13 treatments of each variety) with control (seed soaked in distilled water for 4 hrs) and absolute control (normal dry seed) were laidout on July 19, 2021 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 fertility 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 fertility (%) was measured as the ratio of sterile pollen grains to the total number of pollens observed under microscopic field and

Table 2. Effect of physical and chemical mutagens on germination percentage, pollen fertility and lethality percent in mungbean varieties.

Treatments	Germination percent		Pollen fertility		Lethality percent	
	Ganga 1	GM 4	Ganga 1	GM 4	Ganga 1	GM 4
Gamma radiation with 200 Gy	78.33	84.67	74.66	81.59	21.67	15.33
Gamma radiation with 400 Gy	51.67	61.67	61.69	76.77	48.33	38.33
Gamma radiation with 600 Gy	45.33	51.33	52.99	62.48	54.67	48.67
Seed soaking in 0.1% EMS	93.33	86.67	83.76	87.27	6.67	13.33
Seed soaking in 0.2% EMS	81.67	83.67	79.59	83.09	18.33	16.33
200 Gy + 0.1% EMS	75.33	81.33	72.76	79.39	24.67	18.67
200 Gy + 0.2% EMS	69.33	72.00	70.69	77.82	30.67	28.00
400 Gy + 0.1% EMS	50.33	60.67	58.35	71.47	49.67	39.33
400 Gy + 0.2% EMS	45.33	52.00	55.81	67.59	54.67	48.00
600 Gy + 0.1% EMS	42.33	45.67	50.76	59.55	57.67	54.33
600 Gy + 0.2% EMS	38.00	41.67	49.46	53.15	62.00	58.33
Control (Water soaking)	91.67	92.00	95.39	96.66	8.33	8.00
Control (Absolute)	86.33	88.00	94.96	97.41	13.67	12.00
Range	38.00-93.33	41.67-92.00	49.46-95.39	53.15-96.66	6.67-62.00	8.00-58.33
Mean	65.31	69.33	69.30	76.48	34.69	30.67
CD (p=0.05)	9.439	9.293	11.68	7.593	9.439	9.293
SEm±	3.215	3.165	3.978	2.586	3.215	3.165
SEd±	4.547	4.476	5.626	3.657	4.547	4.476
CV (%)	8.526	7.906	9.943	5.857	16.051	17.875

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 600 Gy along with 0.2% EMS exhibited minimum seed germination in both varieties (Table 2). A gradual decline in seed germination was also observed at higher concentration of EMS however treatment differences were not found statistically significant.

Variety ganga 1 was found more sensitive to mutagens for germinability as compared to GM- 4. Based on seed germination percentage, gamma radiation dose of 400 Gy was found near to LD_{50} (51.67%) in Ganga-1, whereas, it was 600 Gy in GM-4 (51.33%). Similar results were observed by Bonde *et al*. (2020), Sofia *et al.* (2020), Rukesh *et al.* (2017), Kumar (2014) and Vairam and Ibrahim (2014) in mungbean.

Pollen fertility : Analysis of variance indicated highly significant differences among treatments for pollen fertility. 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 600 Gy gamma radiation and 0.2% EMS resulted in minimal pollen fertility (Table 2, Fig. 3). At greater EMS concentrations, pollen fertility began to decline gradually. In comparison to GM-4, variety Ganga-1 was shown to be more sensitive to mutagens for pollen fertility. Based on pollen fertility percentage, gamma radiation @ 600 Gy alone and its combined application with EMS was found near to LD_{50} in both varieties. 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

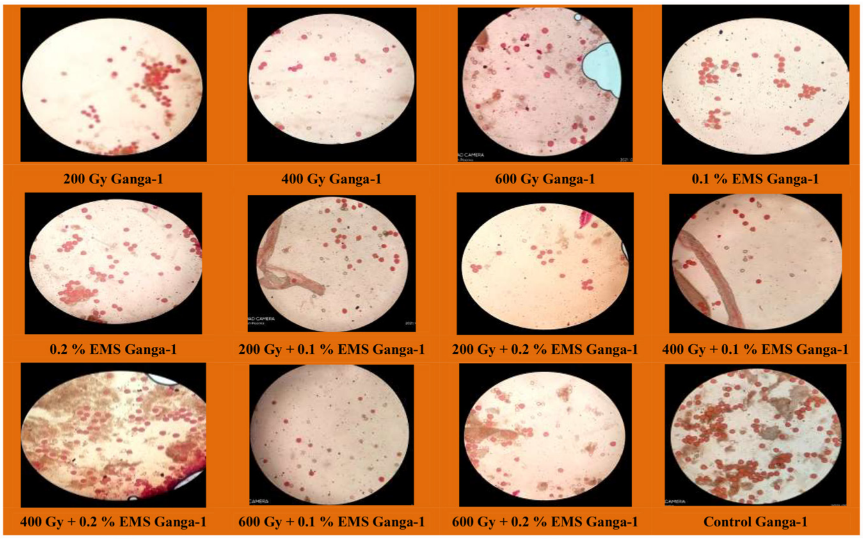

Fig 3. Differences in pollen fertility percentage at different doses of physical and chemical.

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 and 600 Gy) alone and their combined application with EMS were found lethal to mungbean and maximum lethality was exhibited with combined application of gamma radiation 600 Gy along with 0.2% EMS in both varieties (Table 2), however, lower dose of gamma radiation (200 Gy) and solitary application of EMS were not found lethal to mungbean.

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 found by Rukesh *et al.* (2017).

CONCLUSION

The narrow genetic base is a major impediment to breeding progress in mungbean. The application of gamma radiation and EMS may be helpful in regeneration and restoration of genetic variability in self-pollinated crops. Both mungbean varieties Ganga -1 and GM-4 responded to mutagen treatments but expressed in a different way under various treatment combinations of gamma radiation and ethyl methane sulphonate. Variety Ganga-1 was found to be more sensitive to mutagen treatment as compared to GM-4. 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 mungbean and provides scope of selection of desirable genotypes.

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