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Impact of Different Mutagens on *Vigna mungo* (L.) Hepper

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ABSTRACT: The present study was conducted to assess the impact of chemical (sodium azide and ethyl methane sulfonate) and physical (gamma rays) mutagens on the growth, yield attributes, and protein content of *Vigna mungo* (L.) Hepper in the M_1 generation. Significant variability was observed among the treatments for germination rate, plant height, number of pods and seeds, and seed yield. Among chemical treatments, SA at 0.02% and EMS at 0.10% showed the most promising results, with EMS 0.10% recording the highest protein content (26.04%) and SA 0.02% producing the highest seed yield (6.86 g per plant). Gamma radiation at 10 kR also showed favourable effects on yield traits but was less effective than chemical treatments in enhancing protein levels. Higher doses of both mutagen types generally had adverse effects on plant performance. The study concludes that low to moderate doses of EMS and SA are effective in improving both agronomic performance and nutritional quality, and recommends their use in black gram breeding programs to develop high-yielding, protein-rich cultivars.

Keywords: *Vigna mungo*, chemical mutagens, physical mutagens, high-yield, protein-rich.

INTRODUCTION

Pulses, ranking second in importance only to cereals, are a crucial crop group classified within the Leguminosae or Fabaceae family. This category encompasses species such as *Cicer arietinum* (chickpea), *Cajanus cajan* (pigeonpea), *Lens culinaris* (lentil), *Vigna radiata* (mungbean), *Vigna mungo* (urdbean), and *Pisum sativum* (fieldpea) (Mandal and Mandalm 2000). A distinctive characteristic of these crops is their inherent ability to fix atmospheric nitrogen. *Vigna mungo* (L.) Hepper, commonly known as black gram, exhibits a diverse range of vernacular names across various regions and languages, including "Mash" (Arabic), "Uddu" (Kannada), "Uḍid" (Marathi), "Uṛad" (Hindi), "Ulundu/Ulunthu" (Tamil), "Mash-kalai" (Bengali), "Uẓhunnu/Ulnnu" (Malayalam), "Aḍad/Arad" (Gujarati), "Masah" (Sanskrit), "Bano Māsh" (Persian), "Urad" (Urdu), and "Minumulu" (Telugu) (Khan *et al*., 2021).

Due to its high vegetable protein content, *Vigna mungo* (black gram) is a significant component of the Indian diet. Furthermore, it is a rich source of vitamins and minerals and is reported to contain key phytochemicals associated with disease prevention and health promotion (Misal and Chavan 2022). *Vigna mungo* seeds, sprouts, and green pods are edible and highly valued for their digestibility and minimal flatulence-inducing properties. However, the relatively high cost of the seeds typically precludes their use as animal feed, even in major production areas (Arulabalachandran and Mullaninathan 2009).

Vigna mungo is considered beneficial for individuals with diabetes and may also address sexual health concerns, including impotence, premature ejaculation, and low semen volume. Mutation breeding remains a significant conventional technique in plant breeding. The chlorophyll mutation frequency observed in the M2 generation serves as a reliable indicator for evaluating the genetic effects of mutagenic treatments (Lal *et al*., 2009).

There is a lack of molecular-level analysis to identify specific gene expressions or mutations responsible for the induced traits. Furthermore, long-term stability, agronomic performance in different environments, and nutritional or biochemical profiling of the mutant lines remain largely unexplored. This presents an opportunity to combine molecular breeding tools with mutation breeding to better understand and utilize these mutants for sustainable black gram improvement.

Induced mutagenesis, utilizing mutagens, is a cost-effective method for generating genetic variation, leading to the development of improved varieties with desirable traits. In *Vigna mungo* breeding, the application of mutagens has yielded substantial variation in yield per plant and nutritional quality, particularly in protein, methionine, and total sugar content. This study aimed to assess the impact of mutagens, specifically sodium azide (SA), ethyl methane sulfonate (EMS), and gamma rays, on *Vigna mungo*.

MATERIAL AND METHODS

All the field work has been carried out at Pangari, Dist. Parbhani while experimental laboratory work in Government Institute of Forensic Science and Maulana Azad College of Arts, Science and Commerce, Chh. Sambhajinagar (MS) India. The seed samples used in present investigation were obtained from Agriculture Research Station Badnapur, Dist. Jalna, Maharashtra, India.

Sodium azide (NaN_3) is recognized as a potent mutagenic agent in various organisms, including plants and animals, and is known to induce chromosomal aberrations. Ethyl methane sulfonate ($CH_3SO_2OC_2H_5$) is a highly carcinogenic chemical mutagen and a significant alkylating agent. It interacts with DNA through alkylation of the phosphate groups and purine/pyrimidine bases. In these experiments, sodium azide and ethyl methane sulfonate were employed as chemical mutagens while Gamma rays as a physical mutagen. Treatment concentrations included 0.01%, 0.02%, and 0.03% sodium azide, and 0.05%, 0.10%, and 0.15% ethyl methane sulfonate, and 10, 20, 30, 40, 50 and 60 KR gamma radiations.

Chemical treatment

(a) Surface sterilization: Healthy and well dried seeds with uniform size were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for about one minute and washed thoroughly with distilled water.

(b) Mutagenic treatment: The dry seeds were exposed to 10, 20, 30, 40, 50 and 60 KR gamma radiations at Government Institute of Forensic Science, Aurangabad. Different concentrations of SA 0.01, 0.02, 0.03% and EMS 0.05, 0.10, 0.15% were made for the mutagenic treatments. Seeds were pre-soaked in distilled water for 6 hours. The mutagenic chemicals were prepared freshly in aqueous medium at room temperature of 25 ± 2°C prior to treatment. Pre-soaked seeds were immersed in the mutagenic solution and conical flasks were kept on electric shaker. The treatment was given for 6 hours of SA and 5 hours of EMS with intermittent shaking.

Sowing of Mutagen Treated Seeds: After soaking for 2 hours, seeds were dried using blotting paper. Seeds from each treatment group, along with an equal number of untreated control seeds, were planted in a randomized block design to evaluate the M1 generation during the 2022 Kharif (rainy) season. All surviving M1 plants were self-pollinated, and seeds were harvested individually to establish the M2 generation. The M2 population was then screened during the subsequent rainy season.

RESULTS AND DISCUSSION

The present investigation was conducted to evaluate the effects of different chemical (SA and EMS) and physical (Gamma rays) mutagenic treatments on various growth and yield parameters of black gram (*Vigna mungo* L.) in the M1 generation (Table 1). The results revealed notable variations in germination percentage, seedling and plant height, number of leaves and pods per plant, seeds per pod, 100-seed weight, and seed yield per plant across all treatments compared to the control. Among chemical mutagens, EMS at 0.05% concentration showed a significant increase in plant height (49.9 cm), while SA at 0.02% concentration resulted in the highest number of seeds per pod (6.1) and pods per plant (21.5), indicating its effectiveness in enhancing yield-contributing traits. The maximum seed yield per plant (6.86 g) was also recorded under SA 0.02%, followed closely by the control and Gamma ray 10 kR treatments.

In the case of physical mutagens, lower doses of gamma radiation (10–20 kR) generally showed favourable effects on plant growth and yield, with 10 kR resulting in the highest seed yield (6.81 g) and enhanced number of pods (21.1) and seeds per pod (5.66). However, higher doses of gamma rays (50–60 kR) led to a decline in most observed traits, suggesting detrimental effects on plant vigour and productivity. Germination percentage remained unaffected in most treatments but showed a slight reduction at higher mutagen concentrations. Overall, the findings suggest that SA at 0.02% and Gamma rays at 10 kR were the most promising treatments for inducing favourable mutations and improving yield components in black gram during the M1 generation.

The study aimed to evaluate the effect of chemical and physical mutagens on the protein content in Vigna mungo (black gram) seeds. In present investigation it was found that lower to moderate doses of chemical mutagens are effective in enhancing protein biosynthesis or accumulation in black gram, indicating their potential for improving the nutritional quality of the crop.

In contrast, physical mutagenesis through gamma rays showed variable effects on protein content. The treatment with 10 kR gamma rays resulted in a slight decrease (22.98%), while the lowest protein content (20.75%) was observed at 40 kR. However, 50 kR and 60 kR doses showed relatively improved protein values (24.84% and 24.99%, respectively), though still lower than the best chemical treatments. These results demonstrate that while gamma irradiation can induce changes in protein content, chemical mutagens—particularly EMS and SA—are more consistent and effective in enhancing protein levels in black gram. The data supports the use of specific mutagenic treatments in breeding programs aimed at nutritional improvement.

Misal and Kharat (2022) conducted a study to examine the impact of chemical mutagens—Sodium Azide (SA) and Ethyl Methane Sulphonate (EMS)—on *Pisum sativum* L. for the purpose of inducing beneficial genetic variations. The researchers applied varying concentrations of both mutagens and found that SA at 0.02% and EMS at 0.1% were particularly effective in generating morphological mutants. Their findings highlighted notable changes in critical agronomic traits such as seed germination rate, plant height, pollen fertility, pod length, and yield. The study emphasizes the utility of these induced mutants as promising genetic resources for future pea crop improvement programs.

Similar kind of results noted by Budhavant and Ambhore (2023). This study evaluates the effects of physical (gamma rays) and chemical (SA and EMS) mutagens on *Vigna mungo* to induce useful mutations. Treatments with SA 0.02%, EMS 0.05%, and 10 kR gamma rays resulted in significant improvements in germination, growth parameters, and yield-related traits. The M2 generation showed a high frequency of morphological and chlorophyll mutations. These promising mutant lines offer potential for crop improvement in black gram breeding programs.

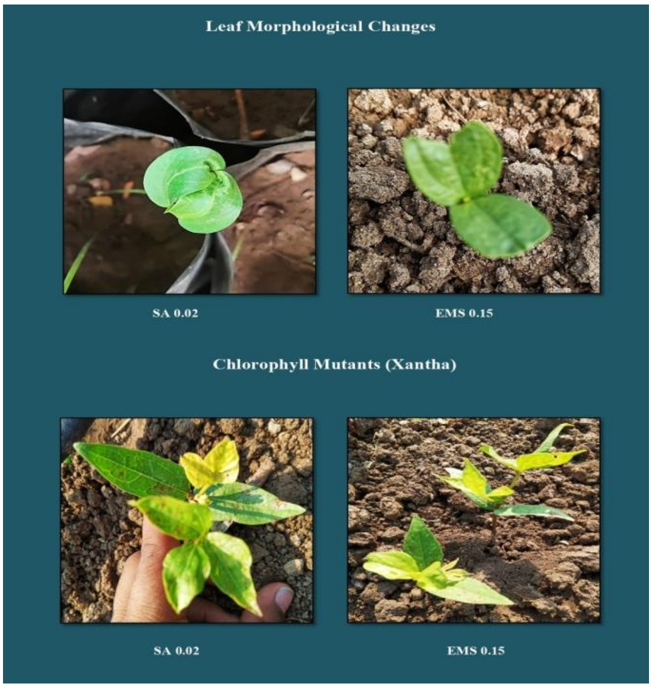

Fig. 1. Impact of mutagens on morphology of *Vigna mungo*.

Table 1: Effect of different Chemical and Physical Mutagenic treatments in M_1 of Black gram.

Treatments	Concentration used	Character Observed								
		Germination %	Seedling height (cm)	Plant height (cm)	No. of leaves/ plant	No. of pods/ plant	No. of seeds/ pod	100-seed weight	Seed yield per plant (g)	Protein content (%)
Control	-	90	5.46±0.335	38.2±0.372	11.00±0.281	17.55±0.342	4.55±0.284	4.96	6.84±0.321	23.61
Chemical mutagens	SA 0.01 (%)	90	6.73±0.346	45.7±0.341	12.62±0.277	13.87±0.323	5.12±0.256	4.89	6.60±0.365	23.48
	SA 0.02 (%)	100	6.46±0.424	40.8±0.352	15.6±0.214	21.5±0.387	6.1±0.245	4.92	6.86±0.266	26.02
	SA 0.03 (%)	90	6.31±0.247	42.3±0.435	13.77±0.263	15.55±0.351	4.77±0.278	4.78	6.71±0.367	24.75
	EMS 0.05(%)	100	6.01±0.375	49.9±0.337	11.22±0.255	17.55±0.320	4.88±0.266	4.90	5.98±0.227	25.63
	EMS 0.10(%)	80	6.66±0.332	45.6±0.330	11.11±0.218	18.55±0.337	4.77±0.234	4.71	6.80±0.286	26.04
	EMS 0.15(%)	80	6.85±0.431	48.7±0.346	9.71±0.233	16.28±0.357	4.85±0.256	4.69	5.88±0.220	25.69
Physical mutagens	Gamma Ray 10 kR	90	6.95±0.264	37.5±0.384	15.22±0.331	21.1±0.359	5.66±0.431	4.87	6.81±0.364	22.98
	Gamma Ray 20 kR	90	6.13±0.245	45.8±0.312	11.88±0.361	16.77±0.311	4.77±0.394	4.84	6.74±0.378	22.10
	Gamma Ray 30kR	90	5.33±0.212	42.8±0.343	12±0.342	17.7±0.348	3.77±0.386	4.81	6.67±0.322	23.39
	Gamma Ray 40 kR	80	5.42±0.234	47.5±0.322	10.75±0.384	20.62±0.391	4.75±0.367	4.69	6.40±0.347	20.75
	Gamma Ray 50 kR	80	5.46±0.289	38.4±0.362	9.25±0.361	16.87±0.363	4.37±0.384	4.72	5.94±0.364	24.84
	Gamma Ray 60 kR	80	5.35±0.241	35.9±0.385	9.75±0.330	16.25±0.310	4.25±0.386	4.66	5.88±0.319	24.99

CONCLUSIONS

From the present study, it can be concluded that both chemical and physical mutagens induced significant variability in growth and yield-related traits in the M1 generation of black gram. Among the treatments, SA at 0.02% proved most effective in enhancing yield parameters, such as number of pods per plant (21.5), seeds per pod (6.1), and seed yield per plant (6.86 g), while EMS at 0.05% resulted in the highest plant height (49.9 cm). Gamma radiation at 10 kR was also effective, producing a high number of pods (21.1) and seeds per pod (5.66), along with a notable seed yield (6.81 g). In contrast, higher doses of EMS and gamma rays showed a reduction in most traits, indicating possible cytotoxic effects.

Chemical mutagens, particularly EMS at 0.10% and SA at 0.02%, significantly enhanced protein content in *Vigna mungo*, reaching 26.04% and 26.02%, respectively. In contrast, higher doses of gamma rays, such as 40 kR, reduced protein content to 20.75%. These results suggest that chemical treatments are more effective than physical mutagens for improving nutritional quality. Therefore, lower concentrations of SA and gamma rays are recommended for inducing beneficial mutations in black gram breeding programs.

FUTURE SCOPE

The findings of this study highlight the potential of using chemical mutagens like SA (0.02%) and physical mutagens like gamma rays (10 kR) to induce beneficial mutations in black gram. Future research can focus on advancing these promising M1 mutants to subsequent generations (M2, M3, etc.) to evaluate the stability and heritability of the observed traits. Molecular characterization and marker-assisted selection can further help identify genetic changes and link them to specific phenotypic traits. Additionally, combining mutagenic treatments with modern breeding techniques could accelerate the development of high-yielding, stress-tolerant black gram varieties. Long-term field trials under different agro-climatic conditions will also be crucial to validate the practical utility and adaptability of these mutant lines for commercial cultivation.

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Conflict of Interest. None.

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