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INDUCTION OF GENETIC VARIABILITY IN GREEN GRAM (*VIGNA RADIATA* (L.) WILCZEK) VIA CHEMICAL MUTAGENESIS: DETERMINATION OF LD_{50} AND PRELIMINARY EFFECTS OF EMS ON SEED GERMINATION AND SURVIVAL

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ABSTRACT

Green gram (*Vigna radiata* (L.) Wilczek) is a nutritionally important legume with limited genetic variability, posing a challenge for crop improvement. This study aimed to determine the median lethal dose (LD_{50}) of ethyl methane sulphonate (EMS) and assess its preliminary effects on seed germination and survival. Seeds were treated with varying EMS concentrations (0.1%-0.5%) and germinated under controlled conditions. Germination and survival data were analysed using probit analysis in KAUGRAPES software. Results revealed a dose-dependent decline in germination, with the LD_{50} estimated at 0.38% EMS. Higher EMS concentrations significantly reduced seedling vigour and increased fungal contamination. Based on the LD_{50}, EMS doses of 0.3%–0.46% were selected to induce mutation in M_1 generation. The study highlights the importance of LD_{50} estimation for optimizing EMS protocols and establishes a reference for mutation breeding efforts in green gram variety CO 8.

Key words : Ethyl methane sulphonate, Mutation breeding, Mutagenesis, LD_{50} determination, Genetic variability.

Introduction

India is the largest producer, consumer and importer of pulses. Green gram or mung bean (*Vigna radiata* (L.) Wilczek) is the third most important food legume grown and consumed in India after chickpea and pigeon pea (Sofia *et al*., 2020; Kumar *et al*., 2021). Green gram is a powerhouse of nutrition as it is an excellent source of high quality, easily digestible protein, carbohydrates (Sen *et al*., 2022), dietary fibres, vitamins and minerals (Singh, 2017; Sarma *et al*., 2022). It occupies a unique position in the cropping system due to its short duration, wide adaptability, high per day productivity, low input requirements and diversified uses. It also plays a crucial role in sustainable agriculture due to its ability to fix atmospheric nitrogen (Singh *et al*., 2016).

Despite its importance, the genetic improvement is slow. According to many researchers, the limited genetic variability in green gram, largely due to its self-pollinating nature, represents a major bottleneck for crop improvement. Mutation breeding directly addresses this limitation, as it serves as a key breeding approach to artificially induce mutations, thereby creating the essential genetic diversity required for the development of new and improved genotypes (Kumar *et al*., 2021). Unlike conventional breeding methods, mutation breeding is effective and much faster (Udage, 2021). One of the advantages of using induced mutations is its ability to improve one or more specific traits without changing the rest of the genetic characteristics of a crop (Khan and Goyal, 2009).

In green gram, mutation breeding has been found to be advantageous as it can generate many different alleles with varying degrees of modification of traits (Sen *et al*., 2022). The selection of an effective and efficient mutagen

is very important in mutation breeding (Das *et al*., 2021). EMS is a widely preferred chemical mutagen due to its ability to induce point mutations (Raihan *et al*., 2018). The effect of EMS on the genome is also highly predictable. Without much damage to the genetic material, a high density of point mutations can be achieved (Varadaraju *et al*., 2017). Moreover, EMS is advantageous for its ease of use as well as ease of detoxification during disposal of the remnants (Udage, 2021).

In this context, the investigation is primarily focused on determination of LD_{50} and the preliminary effects of EMS on seed germination and survival. The evaluation of mutagenic effects in M_1 and M_2 generations is ongoing and will be reported separately.

Materials and Methods

The experiment was conducted at the Department of Genetics and Plant Breeding, College of Agriculture, Vellayani, Thiruvananthapuram. The green gram variety CO 8, released by Tamil Nadu Agricultural University (TNAU), was selected for this study due to its high-yielding nature, short duration and resistance to yellow mosaic disease and stem necrosis (TNAU, 2013). Good quality seeds were procured for the conduct of this experiment.

A completely randomized design (CRD) with 3 replications was employed. Thirty seeds per replication were pre-soaked in distilled water for six hours to enhance the efficacy of the EMS treatment and activate seed metabolism (Ali *et al*., 2024). The seeds were then treated with different concentrations of EMS *viz*., 0% (control), 0.1%, 0.2%, 0.3%, 0.4% and 0.5% for six hours with intermittent shaking to ensure uniform absorption of the mutagen (Sen *et al*., 2022). After treatment, the seeds were thoroughly rinsed under running water for one hour to remove any residual EMS.

The seeds were germinated on germination papers placed in Petri plates. Both germination percentage and seedling survival percentage (counted through day 8, after which seedlings failed to develop further on the plates) were recorded, since survival percentage reflects the toxic effects accurately. LD_{50} was calculated through probit analysis in the KAUGRAPES software, based on the reduction in surviving seedlings relative to the control. Based on the estimated LD_{50}, effective EMS doses were selected for treating seeds used to raise the M_1 generation. Observations were recorded accordingly.

Results

The LD_{50} value was determined via probit analysis in the KAUGRAPES software, using the reduction in seedling survival through day 8 compared to the untreated control. Some EMS treated seeds exhibited delayed germination, causing a gradual increase in observed germination. A consistent and nearly linear decline in seedling survival was observed with increasing EMS concentrations, indicating a clear dose-dependent toxic effect. The LD_{50}, defined as the concentration at which seedling survival is reduced by 50%, was estimated to be 0.38% EMS (Fig. 1).

This finding suggests that higher EMS concentrations progressively reduced seed viability and survival. At concentrations well above the LD_{50}, germination was severely delayed or inhibited, and seedling vigour was notably reduced. Additionally, a higher incidence of fungal contamination was recorded at these elevated EMS levels.

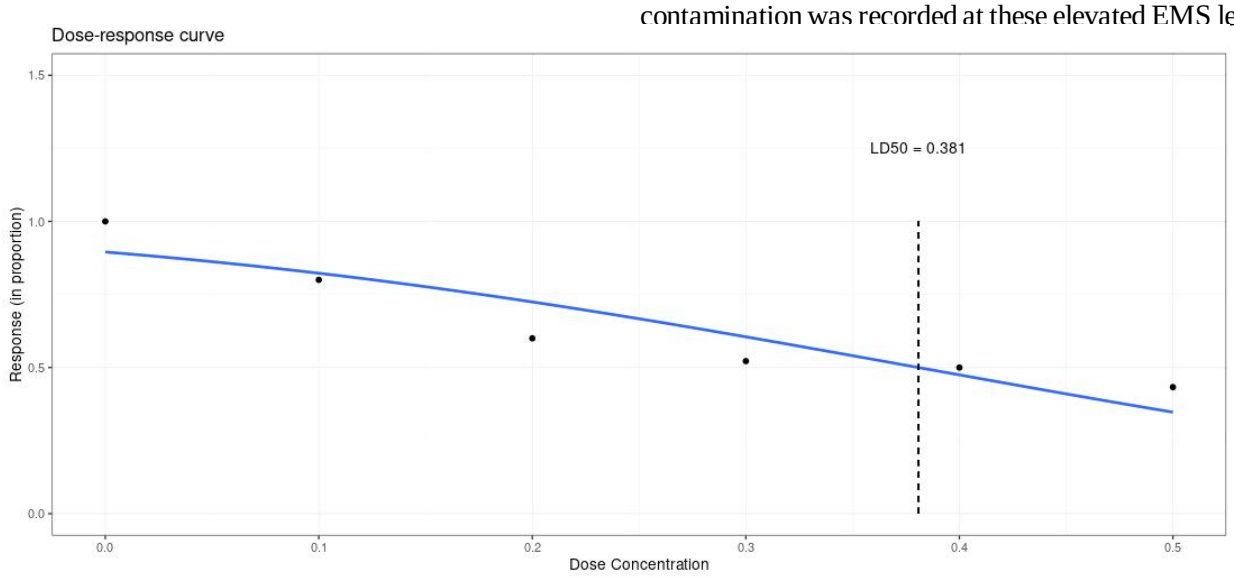

Fig. 1 : Probit analysis curve showing the relationship between EMS concentration and survival percentage in green gram variety CO 8.

Fig. 2 : Visual comparison of green gram (var. CO 8) seedling emergence on day 3 across EMS treatments (T_0:0%, T_1:0.1%, T_2:0.2%, T_3:0.3%, T_4:0.4%, T_5:0.5%).

This may be attributed to the compromised physiological integrity of the seeds, making them more susceptible to opportunistic fungal infections.

The identified LD_{50} value of 0.38% was used as a reference point for selecting EMS concentrations for M_1 generation mutagenesis. The chosen range of 0.3% to 0.46% is expected to induce a spectrum of mutagenic effects while maintaining an acceptable level of seedling survival and growth.

Discussion

Determining the LD_{50} of EMS in the green gram variety CO 8 is a crucial first step in mutation breeding. In the present study, the LD_{50} was estimated at 0.38% EMS using probit analysis of seedling survival, addressing the limitation that germination percentage alone can overestimate viability as germinated seeds often fail to survive. The observed near-linear decline in survival with increasing EMS agrees with similar trends in other green gram genotypes (Ali *et al*., 2024).

Reduced viability and vigour at higher EMS doses, together with increased fungal infestation, highlight the need for precise dose optimization. Excessive EMS levels not only impair seedling establishment but may also compromise physiological defenses, making seeds more susceptible to opportunistic pathogens. These observations are in line with previous reports highlighting the detrimental effects of high EMS concentrations on plant survival and physiological stability (Vairam *et al*., 2017; Ali *et al*., 2024).

The established LD_{50} value of 0.38% was used to define an effective mutagenic range (0.3%-0.46%) for inducing genetic variability in the M_1 generation. This calibrated range aims to achieve a balance between adequate mutagenic pressure and acceptable seedling survival. In conclusion, the findings affirm that EMS-induced mutagenesis in green gram is genotype-specific and necessitates precise dose standardization. The LD_{50} identified in this study provides a reliable basis for future mutation breeding programs targeting genetic improvement in this crop. Further analysis of the subsequent generations will deepen understanding of EMS-induced genetic variability.

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