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COMPARISON OF MUTAGENIC EFFECTIVENESS OF ETHYL METHANE SULPHONATE AND SODIUM AZIDE IN M_2 PROGENIES OF HORSE GRAM (*MACROTILOMA UNIFLORUM* LAM. VERDC.)

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ABSTRACT

Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.), a drought tolerant pulse and fodder crop, thrives in temperatures between 20 to 30°C. It is crucial for climate resilient agriculture and has potential as a future food source. However, the genetic improvement of horse gram has been significantly constrained, primarily due to its narrow genetic base, which is a consequence of its self pollinating nature. To address this, mutation breeding using chemical mutagens can be an effective method to create new and improved varieties. This study assessed the effectiveness of two chemical mutagens, Ethyl Methane Sulphonate (EMS) and Sodium Azide (SA) on the GPM-6 horse gram variety. Viable mutant frequencies in the M_2 generation were evaluated across various concentrations of EMS (0.1%, 0.2%, 0.3%, 0.4%) and SA (0.03%, 0.05%, 0.07%, 0.1%). Mutagenic effectiveness varied between EMS and SA treatments and across concentrations, with no discernible trend in the induction of viable mutants. Higher effectiveness was observed at SA (0.07%) with value of 2.71 followed by EMS (0.1%) at 1.42. SA treatments demonstrated the highest average mutagenic effectiveness (1.21), outperforming EMS (0.64). The results suggest that SA is more effective than EMS in producing viable mutants with desirable traits and fewer deleterious effects leading to healthier and more viable plants.

Key words : Ethyl methane sulphonate, Mutagenic effectiveness, Horse gram, Mutagen, Sodium azide.

Introduction

Macrotyloma uniflorum (commonly known as horse gram, hurali, kulthi bean or Madras gram) is a pulse and fodder crop that grows optimally in temperatures between 20 and 30°C. The plant typically reaches a height of around 60 cm and features alternate trifoliate leaves with petioles. The leaflets are either elliptical or obovate in shape. Horse gram is a diploid species with a chromosome number of $2n = 20$ and its genome size is approximately 400 Mbp (Shirasawa *et al.*, 2021). Renowned for its excellent drought tolerance and nutrient rich seeds, horse gram is an important crop for climate resilient agriculture and holds promise as a future food source. For every successful crop improvement program genetic variability is a crucial prerequisite, which necessitates the implementation of an appropriate selection procedure. Techniques such as plant introduction, selection and

hybridization followed by selection can be employed to generate variability. However, the applicability of these methods is constrained when the desired traits are absent in the available germplasm. Despite its potential, genetic improvement efforts for horse gram have been minimal highlighting the necessity to expand research in this area. The self-pollinated nature of horse gram, combined with its small flower size, makes emasculation and pollination a highly challenging task. As a result, traditional plant breeding approaches have a restricted scope for improving horse gram. As a self pollinated crop, horse gram has limited genetic variation, making mutation breeding one of the fastest methods to introduce variability and develop new, improved varieties (Novak and Brunner, 1992). Chemical mutagens in particular have proven effective in inducing genetic variability, which facilitates the production of a diverse range of plant mutants.

Therefore, present investigation is proposed to assess the effectiveness of chemical mutagens on horse gram.

Materials and Methods

Experimental material for the present study was comprised of mutated GPM-6 M₂ progenies, total of 2400 (EMS=1200, SA=1200), progenies along with five checks (GPM-6, CRIDA-18R, CRHG-4, AK-42, Bailhongal local) were used. The seed material was developed and advancement was made in the Department of Genetics and Plant Breeding, College of Agriculture, Vijayapur. The experiment was conducted at 'C' block, College of Agriculture, Vijayapur (University of Agricultural Sciences, Dharwad) during late *Kharif* 2023-24.

In the present investigation, the chemical mutagens viz., Ethyl methane sulphonate (EMS) (mono functional alkylating agent) and sodium azide (SA) were employed for treating the seeds. The details of the mutagens employed along with their concentrations are given in Table 1. Seed treatment was performed using EMS and SA mutagens in a 0.1 M phosphate buffer (pH=7). The seeds were kept at room temperature for a period of four hours during the mutagenic treatment and subsequently, they were thoroughly rinsed under running tap water for two hours and air dried to remove excess water and were sown in the field. The viable plant count was taken and used for further calculation of mutagenic effectiveness.

Mutagenic effectiveness is a measure of the frequency of mutations induced by unit mutagen, the viable mutations were scored treatment wise to evaluate the mutagenic effectiveness of each treatment. The effectiveness of the mutagens was worked out by using the formula as proposed by Konzak *et al.* (1965). The viable mutation frequencies in M₂ generation were used to determine the mutagenic effectiveness.

$$\text{Effectiveness of the chemical mutagen} = \frac{\text{Mf}}{\text{C} \times \text{T}},$$

$$\text{Mf} = \frac{\text{Viable mutant plants observed}}{\text{Total plants examined}}$$

Where,

Mf = viable mutation frequency on M₂ plant basis

C = Concentration of mutagen

T = Duration of mutagenic treatment

Results and Discussion

The significance of a mutagen is contingent upon its effectiveness, which is measured by the frequency of mutations induced per unit dose of the mutagen (Konzak

Table 1 : Details of the mutagens employed along with their respective concentrations.

Chemical Mutagens:	Concentration (%)			
Ethyl methane sulphonate (EMS)	0.1 (T ₁)	0.2 (T ₂)	0.3 (T ₃)	0.4 (T ₄)
Sodium azide (SA)	0.03 (T ₁)	0.05 (T ₂)	0.07 (T ₃)	0.1 (T ₄)

et al., 1965). Consequently, mutagenic effectiveness serves as an index of the genotype's response to progressive doses of the mutagen.

Upon evaluating the mutants, it was apparent that almost all mutagenic treatments exhibited varying degrees of viable mutants with respective doses in the M₂ generation. Viable mutants were observed in both EMS and SA treated plants. In EMS treated plants, the number of viable mutants recorded was 172, 116, 244 and 12 in the concentrations of 0.1%, 0.2%, 0.3% and 0.4%, respectively. Similarly, in SA treated plants, the number of viable mutants was 49, 19, 230 and 63 in the concentrations of 0.03%, 0.05%, 0.07% and 0.1% respectively. The frequency of viable mutants was also noted in both EMS and SA treated plants. Within the EMS treated plants, the highest viable mutant frequency (0.81) was noted at 0.3%, followed by 0.57, 0.38 and 0.04 at concentrations of 0.1%, 0.2% and 0.4% respectively. Similarly, in SA treated plants, the highest viable mutant frequency (0.76) was observed at 0.07%, followed by 0.21, 0.16 and 0.06 at concentrations of 0.1%, 0.03% and 0.05% respectively (Table 2).

The mutagenic effectiveness in EMS treated plants was highest at 0.1% (1.42), followed by 0.67, 0.47 and 0.02 at concentrations of 0.3%, 0.2% and 0.4% respectively (Fig. 1). In SA treated plants, the highest mutagenic effectiveness was noted at 0.07% (2.71), followed by 1.33, 0.52 and 0.30 at concentrations of 0.03%, 0.1% and 0.05%, respectively (Fig. 2). In both EMS and SA treated M₂ plants, the mutagenic effectiveness of the treatments varied not only between the chemical mutagens but also between the treatments themselves. No discernible trend was noted in the induction of viable mutants in both EMS and SA treated M₂ progenies these findings align with prior studies by Dhasarathan (2014), Hemnani (2017) and Shaik (2021). Notably, higher values for effectiveness were recorded at SA (0.07%) (2.71) followed by EMS (0.1%) (1.42) (Table 2).

The EMS and SA treated M₂ progenies differed in the quantity of viable mutants induced. Furthermore, the increase in frequency of viable mutants in both EMS and

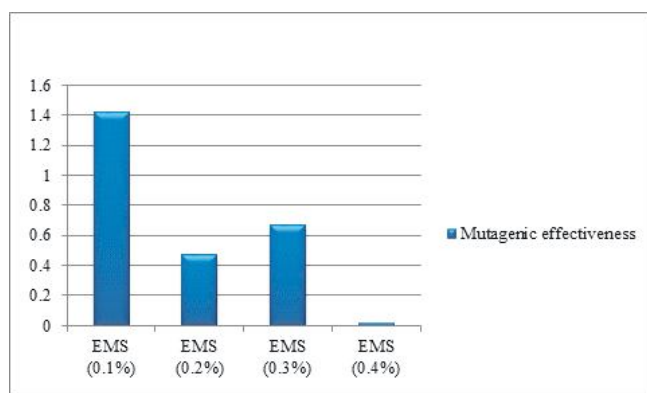


Fig. 1 : Mutagenic effectiveness of different concentrations of EMS in M₂ progenies of horse gram.

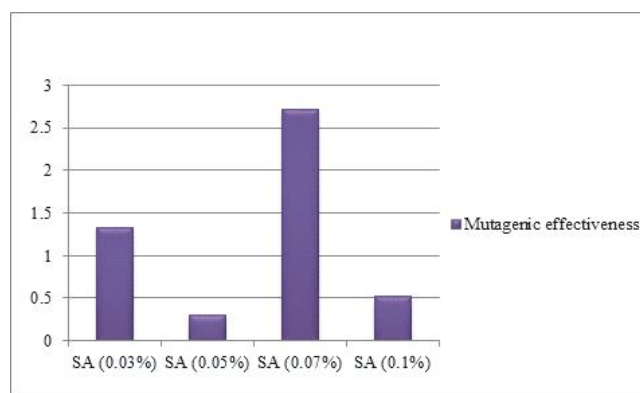


Fig. 2 : Mutagenic effectiveness of different concentrations of SA in M₂ progenies of horse gram.

Table 2 : Effectiveness of EMS and SA mutagens in M₂ generation of horse gram mutants.

Concentration(%)		Total plants	Viable mutants	Viable mutant frequency (Mf)	Mutagenic effectiveness
EMS	0.1	300	172	0.57	1.42
	0.2	300	116	0.38	0.47
	0.3	300	244	0.81	0.67
	0.4	300	12	0.04	0.02
Mean					0.64
SA	0.03	300	49	0.16	1.33
	0.05	300	19	0.06	0.30
	0.07	300	230	0.76	2.71
	0.1	300	63	0.21	0.52
Mean					1.21

SA treated M₂ plants was dose dependent, indicating a positive relationship between the dose of mutagenic treatment and the frequency of viable mutants. The frequency of viable mutants varied across different doses of EMS and SA treated M₂ plants, suggesting a differential response of GPM-6 horse gram to the mutagenic treatment. In present study the highest average mutagenic effectiveness value was recorded in SA treatments (1.21), followed by EMS treatments (0.64) similar findings were reported by Sreevalli (2021). The highest average mutagenic effectiveness value confirms that SA was more effective than EMS in generating viable mutants with desirable mutations and fewer deleterious effects, resulting in better overall plant health and viability. These findings align with prior studies by Shirsat *et al.* (2010), Wani *et al.* (2011), Jain *et al.* (2013) and Kulkarni and Mogle (2013).

Conclusion

The present investigation is proposed to assess the effective mutagens through induced mutation. It was concluded that sodium azide (SA) was more effective in inducing viable mutations in horsegram with more stable

and beneficial mutations with better overall plant health. Therefore, sodium azide is better mutagenic agent for creating genetic variability and may be preferred for its effectiveness and lower risk of adverse effects in horse gram.

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