

A Study on Seed Germination and LD₅₀ by Chemical Mutagenesis in Cluster Bean (*Cyamopsis tetragonoloba* L. Taub) Pusa Navbahar Variety

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ABSTRACT

Cluster bean, often known as guar (*Cyamopsis tetragonoloba* L.), is a self-pollinated crop with chromosomal number $2n = 14$. Guar is a tough, deep-rooted leguminous herb native of dry and semi-arid regions of the world. It seems to have originated in Africa, but it is now grown throughout southern Asia, particularly in India, which produces 80% of the world's guar. Cluster bean is utilized for human consumption and cattle feed (30-40%), industrial applications (50-55%), medicinal purposes (5%), soil enhancement and other purposes. It is widely utilized in the paper, mining, food, cosmetic, textile, oil and pharmaceutical industries worldwide. Mutation breeding is an important strategy for developing new cultivars by utilizing physical and chemical factors for crop improvement. In this present investigation, to assess the efficiency and effectiveness of chemical mutagens; Ethyl Methane Sulphonate (EMS) and Diethyl Sulphate (DES) using different concentrations. According to results the 50% of seed germination in EMS in 40mM and DES in 30mM. The LD₅₀ values for 40mM EMS and 30mM DES were determined based on the percentage of seed germination on the seventh day.

Key words: *Cyamopsis tetragonoloba*, Ethylmethane sulphonate and Diethyl sulphate, Mutation breeding, LD₅₀ value.

INTRODUCTION

Cluster bean, often known as guar (*Cyamopsis tetragonoloba* L.), is a self-pollinated crop with chromosomal number $2n = 14$ [1]. Guar is a tough, deep-rooted leguminous herb native to dry and semi-arid regions of the world. It seems to have originated in Africa, but it is now grown throughout southern Asia, particularly in India, which produces 80% of the world's guar. It is additionally grown as a cash crop in Pakistan and, to a greater extent, in other regions of the world such as Australia, Bangladesh, Myanmar, South Africa, and Sri Lanka [2]. It is an ancient crop of the rainy ecosystem that provides nutrients to both humans and animals. Cluster bean is utilized for human consumption and cattle feed (30-40%), industrial applications (50-55%), medicinal purposes (5%), soil enhancement, and other purposes [3]. This plant is well-known for the gum extracted from the seeds' endosperm. Guar is a coarse, upright, bushy, drought-resistant summer annual plant that grows to a height of 2 to 4 feet. It has sharp, saw-toothed trifoliate leaves, little purplish-white flowers that bloom along the axis of a spikelet, and hairy pods that grow in clusters 3-4 inches long. There are miniature and tall varieties available. Guar blooms self-pollinate. A mature unopened bud begins white and gradually transforms to a light pink as the petals expand. Finally, the blossom is a dark blue color [4].

Galactomannan gum is a polysaccharide produced from guar seeds that are soluble in cold water and produces a thick solution at low concentrations. It is widely utilized in the paper, mining, food, cosmetic, textile, oil and pharmaceutical industries worldwide. Cluster bean endosperm contains a high concentration of excellent galactomannan (78-82%) [5]. It has medicinal purposes in the treatment of diabetes and high cholesterol cases, in addition to its industrial applications [6]. Tender pods are a good source of calcium, iron, and dietary fiber, which provide to improve bone health, blood circulation, and bowel function. It is also utilized as green manure since it fixes 50-60 kg ha/year of atmospheric nitrogen and adds organic matter to the soil. Through mutually beneficial relationships with bacteria, legumes can meet a large portion of their nitrogen requirements from atmospheric nitrogen [7]. As a result, global demand for cluster beans has risen significantly in recent years, prompting crop introductions in various countries, including the United States, South Africa, Australia, Brazil, Zaire, and Sudan. Despite its role in agricultural output, the productivity of guar is quite low [8].

Mutation is an excellent tool for studying the function and nature of genes and thus producing raw materials for the genetic improvement of economically important crops. Many mutagenic agents have been employed to generate beneficial mutations at a high frequency;

ionizing radiation, such as x-rays and gamma-rays, as well as chemical mutagens, has been extensively studied for causing variety [9]. Mutation breeding is an important strategy for developing new cultivars by utilizing physical and chemical factors for crop improvement. Through comparison experiments, induced mutagenesis as a breeding technique for cluster bean development was investigated. Mutation breeding is one of the most advanced plant breeding approaches [10]. It is important in several domains, including Plant Morphology, Cytogenetics, Plant Biotechnology, and Molecular Biology. Mutation breeding has been increasingly popular as an effective tool for developing crops in recent years [11]. Chemical mutagenesis is one of the most effective and convenient methods employed in various plant species. EMS and DES have been utilized as chemical mutagens in cluster beans. Because of its great potential to induce point mutations and deletions in chromosomal areas, EMS is the most common and widely utilized chemical mutagen in plants [12].

MATERIALS AND METHODS

Collection of seeds and mutagenic treatments

The healthy seeds of *Cyamopsis tetragonoloba* L. (Pusa Navbahar-variety) seeds were obtained from the Tamil Nadu Agricultural Research Institute (TNAU) in Coimbatore. Mutagens employed the Chemical mutagens namely Ethyl Methane Sulphonate (EMS) and Diethyl Sulphate (DES) were used at different concentrations to induced mutagenesis. The seeds were pre-soaked in distilled water for 6 hours to make them more susceptible to mutagenic activity. Pre-soaked seeds were treated with various concentrations viz., 10, 20, 30, 40, and 50mM of Ethyl Methane Sulphonate (EMS) and Diethyl sulphate (DES) solution for 4 hours with repeated stirring. Following treatment, the seeds were thoroughly washed under running tap water for 8-10 times to remove chemical residues. As a control, untreated seeds of *Cyamopsis tetragonoloba* L. Pusa Naubahar were pre-soaked in distilled water for 4 hours. The seeds were sowed in petri plates after pre-soaking. In this present investigation, to assess the efficiency and effectiveness of chemical mutagens; Ethyl Methane Sulphonate (EMS) and Diethyl Sulphate (DES) using different concentration.

Lethal Dose values and Percentage of seed Germination

The LD₅₀ is a measuring of the lethal dose of chemicals. The LD₅₀ was recognized by the amount of material, given all at once, which causes the death of 50% of plants treated with chemical mutagens in seedlings.

Seed Germination Percentage

Each treatment's seeds, along with the control, were placed on absorbent cotton-wet Petri dishes. Three replicates were studied for each treatment, and the number of seeds that germinated on the seventh day was recorded and the germination percentage was calculated. Based on a 50% reduction in seed germination, the LD₅₀ value was fixed, and the two treatments, EMS and DES, were placed around the LD₅₀ value for further studies.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of Seed sown}} \times 100$$

RESULTS AND DISCUSSION

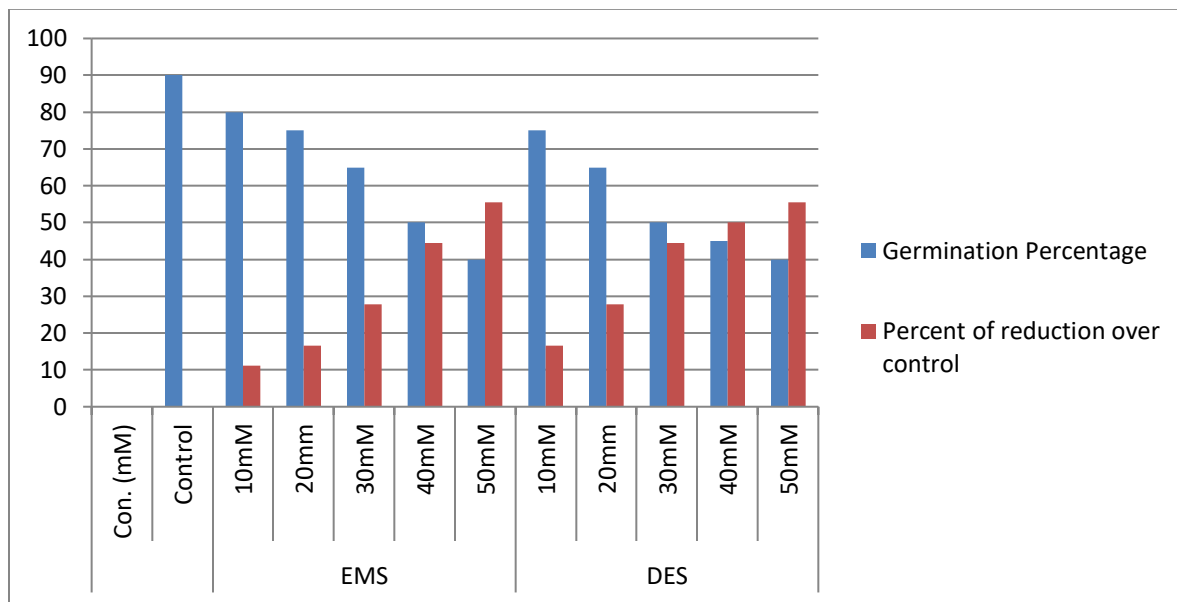
Seed germination is the process through which a plant grows from a seed. The table shows seed germination data analysis for *Cyamopsis tetragonoloba* L. Pusa Navbahar variety. The current study concentrated on induced chemical mutagenesis in the *Cyamopsis tetragonoloba* L. variety Pusa Naubahar was exposed to two different mutagens at varying concentrations in order to induce mutation. Under laboratory *in-vitro* conditions, the seed germination percentage of several harmful treatments revealed that the seed germination percentage decreased with increasing concentrations of EMS and DES. The percentage of seed germination in EMS 50% in 40mM and DES 50% in 30mM. The LD₅₀ values for 40mM EMS and 30mM DES were determined based on the percentage of seed germination on the seventh day.

The germination percentage of the seeds was determined with the effect of chemical mutagens in order to determine the optimum concentrations of the mutagens. The 50 percent lethality (LD₅₀) was calculated based on seed germination. To create the variability in this investigation, three mutagenic agents (EMS and DES) were utilized. The LD₅₀ value was found at

40mM EMS and 30mM DES among the mutagenic concentrations. In terms of germination percentage, higher concentrations had more lethality effects. Similar results were reported by Mahla *et al.*, (2010) are analyzing the EMS showed more effect of lethality the significant type results of cluster [13]. Ariraman *et al.*, (2014) reported that the Pigeon pea (*Cajanus cajan* (L.) seeds were exposed to various doses of gamma radiation and concentrations of Ethyl Methane Sulphonate in order to determine the LD₅₀ [14]. The seed germination percentage was used to calculate the LD₅₀ (lethal dose).

Table 1. Determination of LD50 for EMS and DES on *Cyamopsis tetragonoloba* L. Pusa Navbahar variety.

Mutagens	Treatments Con. (mM)	Germination Percentage	Percent of reduction over control
	Control	90	00
EMS	10mM	80	11.11
	20mm	75	16.66
	30mM	65	27.77
	40mM	50	44.44
	50mM	40	55.55
DES	10mM	75	16.66
	20mm	65	27.77
	30mM	50	44.44
	40mM	45	50.00
	50mM	40	55.55

Graph 1: Determination of LD₅₀ for EMS and DES on *Cyamopsis tetragonoloba* L.

CONCLUSION

By increasing the concentrations of Ethyl Methane Sulphonate (EMS) and Diethyl Sulphate (DES), the germination of cluster bean seeds was reduced. According to the findings of this study, lesser quantities of mutagens may be acceptable for creating genetic variety in the wild gene pool of this vegetable crop. Among the various chemical mutagen concentrations, 40mM of EMS and 30mM of DES were identified as a threshold doses for further analysis.

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