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Research Article

EMS - Chemical Mutagen for Induction of Mutations

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Abstract

Chemical and physical mutagenesis has been used to increase genetic variability in crop plants. More than 300 new varieties have been derived as mutants of urdbean (*Vigna mungo* L. Hepper) via the application of different mutagenic agents. Chemical mutagens such as ethyl methane sulphonate (EMS), diepoxybutane-derived (DEB), sodium azide and irradiation (gamma rays, X-rays and fast neutrons) have been widely used to induce a large number of functional variations in urdbean and others crops. Among chemical mutagens, the alkylating agent, EMS is the most commonly used in plants as it causes a high frequency of nucleotide substitutions, as detected in different genomes. In this study, seeds of potential genotype of the popular variety, VBN 4 (Vamban-4) were treated with EMS at concentrations of 30mM, 40mM, 50mM, 60mM and 70mM. Sensitivity to EMS was determined by germination percentage. As concentration of applied EMS increased, will decrease in germination, under laboratory conditions was observed as compared to the non-treatment control. The LD_{50} values were observed based on growth reduction of seedlings after EMS treatment with 60mM on the blakgram variety VBN 4.

Keywords: *EMS, Lethal Dose, Chemical Mutagenesis, Germination*.

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Introduction

Chemical mutagens (EMS, DEB and sodium azide) and irradiation (gamma rays, X-rays and fast neutrons) have been widely used to induce a large number of functional variations in urdbean. Chemicals induce mainly point mutations, and are thus ideal for producing missense and nonsense mutations, which would provide a series of change-of-function mutations. On the other hand, ionizing radiations normally induce chromosomal rearrangements and deletions [1].

Alkylating agents were the first class of chemical mutagens to be discovered when Auerbach and Robson [2] found the mutagenic effects of mustard gas and related compounds during World War II. Alkylating agents such as mustard gas, methylmethanesulfonate (MMS), ethylmethanesulfonate (EMS), and nitrosoguanidine have several effects on DNA. Because of its potency and ease with which it can be used, EMS is the most commonly used chemical mutagen in plants. EMS alkylates guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C-to-A/T transitions [1]. Since EMS produces a large number (genome-wide) of non-lethal point mutations, a relatively small mutant population (approximately 10,000) is sufficient to saturate the genome with mutations.

An important advantage of using a common mutagen, such as EMS, is that a substantial body of literature has confirmed its utility in forward genetic screens in a variety of organisms. These include the favorite model animal and model plant for mutagenesis studies in *Drosophila melanogaster* and *Arabidopsis thaliana*, respectively. EMS is remarkably consistent, in that apparently similar levels of mutagenesis have been achieved in these organisms, despite the approximately 1 billion years of divergence between them. For example, recessive lethal mutations are estimated to occur at similar rates in both cases, with EMS doses causing acceptable levels of sterility and lethality [1]. In addition, direct estimates confirm that base substitution rates are comparable for Arabidopsis seeds soaked in EMS [3-4] and EMS-fed Drosophila males [5], and approximately similar rates were found in a reverse-genetic screen of zebrafish progeny exposed to N-ethyl-N-nitrosourea (ENU) [6]. Thus, chemical mutagenesis causes a high frequency of nucleotide substitutions in a variety of organisms.

Genome size does not appear to be an important factor in EMS mutagenesis because estimates of per gene mutational density found for Arabidopsis appear to be similar for maize [7], which has a 20-fold larger genome size. Therefore, EMS may likely be the mutagen of choice for TILLING (Target Induce Local Lesion in Genome) in plants. However, the toxicity of EMS may vary depending on the species, and other mutagens or post-treatments with antitoxicants may be worth considering [8].

Over the last few years, several new projects have been done with the aim of producing EMS-induced urdbean mutant populations in the research institutes [1]. Initially LD_{50} dose is determined, which is used as an optimal dose for mutation induction. By ignoring this step, mutagen dose can either be high or low resulting mutation frequency [9]. The dose assessment for chemicals is determined by varying the concentration and duration of treatment, the solvent used [e.g. dimethyl sulfoxide (DMSO)], or the pH of the solution [9]. Chemical mutagens (EMS, DES, sodium azide) were also used by treating banana shoot tips to produce variants for tolerance to Fusarium wilt [9]. EMS has been successfully used on chrysanthemum, yielding a frequency of 5.2% mutants. A wide range of variations in petal color (pink-salmon, light-pink, bronze, white, yellow and salmon color) have been recorded [9]. Luan *et al.* [10] treated sweet potato (*Ipomoea batatas* L. callus) with EMS and obtained salt tolerant lines. At that time, the result was attributed to differences in the chemical composition of the chromosomes near the centromere, making them more sensitive to chemical mutagens. While it may indeed be the case, other explanations are possible. For example, genes near the centromere are less likely to be involved in recombination and hence mutations in those genes are less likely to be eliminated through selection. Mutants need at least two generations of meiosis involving chromosome segregation and recombination [11].

Lethal dose, the percentage of test organisms that killed by a specific dosage (of chemicals or radiation), half will die at LD_{50} [12].

Materials and Methods

Plant Materials

In this research, the promising blackgram variety namely VBN 4 (Vamban-4) was chosen for EMS-induced mutagenesis. Selfed seeds were obtained from the germplasm collection maintained by the National Pulses Research Centre, Vamban.

EMS Mutagenesis

Seeds of VBN 4 were placed in a 500 ml flask and ultrapure water was added to about 5 cm level above the seeds (~100 ml). Five different concentrations of EMS ranging from 30mM to 70mM with 10mM interval were used to fix LD_{50} value. The presoaked seeds after removal from the water were placed between folds of blotting paper to remove water adhering on the surface. Then the seeds were immersed for six hours in the requisite concentration of EMS with intermittent shaking. Immediately after the completion of treatment duration, the treated seeds were thoroughly washed in running tap water for half an hour to eliminate the residual effect of the EMS and the excess moisture in the seed coat was removed by using folds of blotting paper. The seeds were then subjected to germination test. Based on the effect of chemical mutagen on germination, LD_{50} value was obtained.

Results and Discussion

Data analysis on number of seed that germinated showed an attendant decrease in germination with applied increases in concentration of EMS. According to **Figure 1**, the results obtained indicate that reduction in seed germination occurred with corresponding increase in EMS concentration ($P < 0.01$). The mean germination percentage ranged from 42.67 (70mM) to 84.00 (30mM) in EMS treatment. In all the treatments, the germination percentage recorded was lesser than their respective control. The per cent reduction was low at 30mM which was 8.91 and high at 70mM which recorded 53.73 per cent and 50 per cent reduction was obtained at 60mM.

It can be observed in the laboratory conditions, EMS mutagenesis caused significant reduction in the germination. This was manifested as significantly decline ($P < 0.01$) in seed germination as the EMS concentration was increased. Among the chemical mutagens and alkylating agents, EMS has especially been demonstrated to be the most potent.

Previous studies affirm that while the mutagenic response is more or less linear with the dose, polyploids are more tolerant than diploids [11]. According to **Figure 1**, the current research results showed that after EMS treatment was applied, seed germination was decreased significantly with increasing EMS ($P < 0.01$).

In concert with a previous study on radiation mutation [13], survival of plants to maturity depends on the nature and extent of chromosomal damage. Increasing frequency of chromosomal harm with increasing radiation dose may be responsible for reduction in germination inability, plant growth and survival. In another study, changes in the germination percentage were attributed to gamma rays treatments [13].

Jain and Khandelwal [14] calculated the LD_{50} value on the basis of germination percent, seedling height and seedling dry weight was observed at 0.4 per cent of EMS and 0.04 per cent DMS respectively. They also observed gradual reduction in germination per cent, seedling height and seedling dry weight. Arulbalachandran and Mullainathan [15] subjected VBN 1 blackgram variety to gamma rays (20 to 120kR) and EMS (0.02 to 0.18%) treatments. They reported that the LD_{50} for survival was found to be 80kR for gamma rays and 0.1 % for EMS.

The chemo mutagenic studies of mungbean cultivar K 851 treated with ten different concentrations (0.01 to 0.1 per cent) of Hydrazine hydrate and Sodium azide was carried out by Rehman *et al.* [16]. Based on germination, he reported the LD_{50} for Hydrazine hydrate and Sodium azide as 0.052 and 0.067 per cent respectively. Rupinder Singh and Kole [17] treated the seeds of Pusa-9072 with five doses of EMS such as 0.1, 0.25, 0.5, 0.75, 1.0% and the LD_{50} for germination was computed to be 0.66% of EMS.

Furthermore, genes near the centromere are more prone to mutagenic treatment than those located farther away. In another study, chlorophyll mutants were frequently observed among EMS treatment group but were rare among those treated with physical mutagens [11]. The stimulating effect of physical mutation on germination may be credited to the activation of RNA or protein synthesis. It may occur during the early stage of germination after the seeds are treated [1].

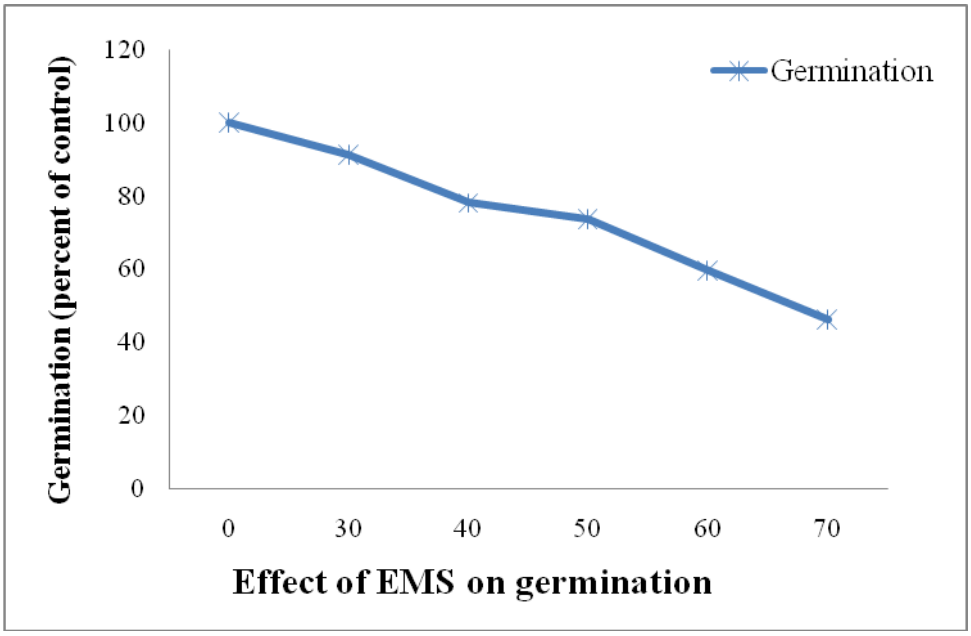

Figure 1 Effect of EMS on Germination percentage under laboratory condition.

Conclusion

Lethal Dose was determined by measuring the seed germination under laboratory conditions. In this experiment, quantitative determinations were applied as a regular procedure. The related data about percent of germination were collected and recorded. Variability on observed means was calculated. On the whole, differences between concentrations of EMS treatments significantly affected germination ($p < 0.01$). So, the LD_{50} values observed based on the growth reduction of seedlings after treatments occurred during the application of 60mM concentrations of EMS for the variety VBN 4.

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