

Mutagenic effect on seed germination, seedling growth and seedling survival of Bael (*Aegle marmelos* Correa.)

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ABSTRACT

There is no named variety or cultivar in bael like other fruit crops. Mostly local selections having desirable traits are utilized for planting. Therefore, its breeding is crucial for creating new improved varieties. An effective method for generating variety and choosing superior plants appears to be mutation breeding. The current study was conducted in July 2022 at the Uttar Banga Krishi Viswavidyalaya at Pundibari Faculty of Horticulture Pomology and Post-Harvest Technology Laboratory and Teaching Farm. 100 numbers of Bael (*Aegle marmelos* Correa.) seeds treated in different concentration of EMS (0.25%, 0.50%, 0.75% and 1.00%) and Colchicine (0.25%, 0.50%, 0.75% and 1.00%) along with control with 4 replications following randomized block design. The study indicated that the percentage of seed germination was decreased with increasing concentrations and doses, when compared to the control. On the basis of the percentage of germination of seeds, the LD_{50} (Lethal dosage) value was calculated. The 50% decrease in seed germination was seen in both the 0.75% of EMS (T_4) and 1.00% in Colchicine (T_3) treatments, and regarded as the LD_{50} value for both. The maximum shoot length, root length, seedling length and seedling survival % were observed in 0.25 % EMS (T_1) whereas, root length and seedling survival % in 0.25% of colchicines (T_2) but shoot length, total seedling length and numbers of leaves in 0.75% (T_3). The lowest were observed in 1.00% of Ethyl Methane Sulphonate and Colchicine.

Keywords: Bael, colchicine, EMS, lethal dose, mutagen, seedling survival

INTRODUCTION

Bael (*Aegle marmelos* Correa.), significant crop of Rutaceae family with high medicinal value. Plant is robust by nature, has strong nutritional content, and is well-suited for processing (Kundu and Ghosh., 2017). In addition to the fruit, the entire plant, including the leaves, wood, roots, and bark, is utilized in ayurveda medicine and for other purposes (Sharath *et al.*, 2016). The locally available genotypes of bael need to be identified and evaluate to develop a good cultivar. The high degree of variability with desirable quantity and qualitative characters are useful tools to identify new genotypes. Although bael is rarely grown in systematic orchards, it is typically grown in parks, backyards of houses, gardens and temples. Recently it has been produced for commercial orchards, making it one of India's underutilized fruits.

Genetic advancement by induced mutation in the agricultural sector, has a wide range of applications to create new varieties with better traits that are resistant/tolerant to disease, insects, drought, salinity, heat, and pests. When compared

to other chemical mutagens, EMS and Colchicine significantly increase the variability of plant materials. The frequency and saturation of mutations may be controlled by altering the dosage of the mutagenic agent (Menda *et al.*, 2004). Mutagen cause varied length fragments with sudden insertions or deletions (Kim *et al.*, 2006). EMS and Colchicine also improved plant morphological characteristics (Vikhe and Nehul, 2020). Ethyl Methane Sulphonate (EMS) is a mutagenic and carcinogenic organic compound that is thought to be the most potent and effective mutagen as it replaces nucleotide by abnormal base pairing (Waungh *et al.*, 2006). Colchicines is an efficient polyploidy mutagen to create variability by doubling the chromosome number (Liu and Guan, 2006). Colchicine, at a concentration of 0.1%, was found to accelerate the emergence of early seedlings, improve plant morphological traits, and increase the generation of total chlorophyll and total carbohydrates in Jamun leaves (Barman *et al.*, 2014). Considering high degree of mutagenic effect of EMS and Colchicine, these chemicals were used

to know their LD₅₀ value and the seedling behavior in bael for creating new genotype/s suitable for Terai region of West Bengal, India.

MATERIALS AND METHODS

The present investigation was carried out during July 2022 in the Laboratory and instructional Farm of Pomology and Post-Harvest Technology, Faculty of Horticulture at Uttar Banga Krishi Vishwavidyalaya, Pundibari. Mature fruits were collected from the trees which grown at the university farm. Seeds were extracted from ripe fruits, and washed thoroughly with running water. Immediately after extraction, the seeds of bael were treated with different doses of EMS (0.25%, 0.50%, 0.75% and 1.00%) and Colchicine (0.25%, 0.50%, 0.75% and 1.00%) along with control with 4 replications for studying the impact on seed germination. For EMS treatment, 100 numbers of healthy seeds were treated with 0.25%, 0.50%, 0.75% and 1.0% freshly prepared EMS solution in 0.1 M phosphate buffer maintaining pH-7.0 for 8

hours. Whereas for colchicine treatments, 100 numbers of healthy seeds were treated with 0.25%, 0.50%, 0.75% and 1.0% freshly prepared aqueous colchicine solution for 8 hours. After 8 hours the EMS and Colchicine treated seeds were washed thoroughly for 1 hour in running water to eliminate the residual effect of the chemicals. After that, the seeds were sown immediately in the black polythene bag containing a single seed per bag for germination. The treatments were arranged in randomized block design with four replications. 100 numbers of seeds were treated for each replication of each treatment. Single seed was sown in each polythene bag. Four seedlings per replication were randomly selected for shoot length (cm), root length (cm), total seedling length (cm), number of leaves and seedling survival at 60 days after seed sowing. The germination percentage, seedlings survival percentage, shoot length, root length and total seedling length were calculated as follows:

$$\text{Germination Percentage} = \frac{\text{Total number of emerged seedling}}{\text{Total number of planted seeds}} \times 100$$

$$\text{Seedling survival percentage} = \frac{\text{Total number of germinated seed}}{\text{Total number of seeds sown}} \times 100$$

Shoot length (cm) = Shoot length was calculated by measuring the shoot length with the help of a scale from the cut-base of soil line to the shoot tip of 4 plants in each replication and average value was calculated

Root length (cm) = Length of root was measured with a scale from the cut-base to the tip of taproot of 4 plants in each replication and average value was calculated.

Total seedling length (cm) = Total seedling length was calculated by measuring the full length of the seedling (shoot length + root length) after uprooting 60 days after germination.

RESULTS AND DISCUSSION

Germination percentage (%)

The data presented in Table 1 showed that seed germination was highest in control (untreated seeds) 91.00%. Regarding effect of EMS and Colchicine on seed germination, it was observed that germination percentage was decreased with the increase of concentrations irrespective of mutagens. Highest germination of 75.25 per cent was noted

with EMS 0.25% concentration and lowest of 39.75 per cent with 1.00 % EMS. Similar mutagenic effect of EMS was observed by Singh *et al.* (2021) in short day onion. In case of Colchicine, highest germination was with 0.25 per cent (83.50%) and lowest with 1.0% (50.25%). LD₅₀ (Lethal dosage) was for EMS was 0.75 % while 1.00 per cent was for Colchicine. From this investigation it was assumed that Colchicine was better mutagen than EMS for getting higher seedlings populations in bael. The preceding studies by El-Latif *et al.* (2018) in Papaya provided support for the findings. Interruptions at the molecular material may be responsible for the decreased seed germination with greater doses/concentrations of the mutagens. Similar findings were reported by Kumar and Mishra (2004) in where of germination was reduced with increasing concentrations of physical and chemical mutagen.

Seedling growth

The maximum shoot length (29.38 cm), root length (24.58 cm) and seedling length (53.95 cm)

Table 1: Effects of EMS and Colchicine on seed germination and seedling behavior of bael

| Treatment details | Germination percentage (%) | Shoot length (cm) | Root length (cm) | Total seedling length (cm) | Number of leaves | Seedling survival percentage (%) |
|-------------------|----------------------------|-------------------|------------------|----------------------------|------------------|----------------------------------|
| Control | 91.00 | 27.70 | 22.70 | 50.40 | 8.50 | 86.00 |
| 0.25% EMS | 75.25 | 29.38 | 24.58 | 53.95 | 7.50 | 70.75 |
| 0.50% EMS | 61.50 | 23.70 | 17.80 | 41.50 | 6.50 | 55.00 |
| 0.75% EMS | 50.50 | 20.48 | 13.58 | 34.05 | 6.75 | 42.00 |
| 1.00% EMS | 39.75 | 18.80 | 13.48 | 32.27 | 5.00 | 30.50 |
| 0.25% Colchicine | 83.50 | 22.10 | 25.28 | 47.38 | 6.25 | 74.25 |
| 0.50% Colchicine | 75.25 | 27.83 | 21.43 | 49.25 | 9.50 | 61.50 |
| 0.75% Colchicine | 61.00 | 22.30 | 16.70 | 39.00 | 7.50 | 52.75 |
| 1.00% Colchicine | 50.25 | 21.83 | 16.63 | 38.45 | 6.50 | 40.50 |
| CD (0.05%) | 3.90 | 3.60 | 4.79 | 6.48 | 1.66 | 4.73 |
| SEm (±) | 1.36 | 1.26 | 1.67 | 2.26 | 0.58 | 1.65 |

in 0.25% EMS treated plants (Table 1). It was decreased along with a similar rise in EMS dosages. The minimum shoot length (18.80 cm), root length (13.48 cm) and seedling length (32.27 cm) in 1.00% EMS. Singh *et al.* (2022) reported that higher dose of mutagen inhibits plant height in Mosambi. The highest shoot length (27.83 cm), root length (21.43 cm) and seedling length (49.25 cm) was observed in 0.50% Colchicine while the lowest shoot length (21.83 cm), root length (16.63 cm) and seedling length (38.45 cm) was noted in 1.00% Colchicine. When compared to EMS, Colchicine caused a smaller reduction in seedling height. Similar findings were reported by various researchers for sunflower (Jayakumar and Selvaraj, 2003) and gladiolus for colchicine (Manzoor *et al.*, 2018). The root and shoot length were reduced with increasing concentration of mutagen was indicative of the inhibitory impact of mutagens on seedling length. The activation of growth hormone, such as auxin, and an increase in cell division rates were theorized to be the causes of these stimulations by EMS and Colchicine treatments (Zaka *et al.*, 2004).

Number of leaves

From the Table 1 it was observed that the minimum numbers of leaves were obtained in 1.00% EMS (5.00), whereas the maximum leaves was recorded in 0.50% colchicine (9.50).

Seedling survival percentage (%)

After 60 days of germination the survival percentage was maximum (86.00%) in control followed by 74.25% in 0.25 % Colchicine and 70.75% in 0.25% EMS whereas the minimum was

reported in 1.00% EMS (30.50%) (Table 1). With a rise in the concentration of both mutagens, a progressive decline in plant survival was seen. It was maximum in Colchicine at 0.50% (74.25%) and lowest in Colchicine at 1.00% (40.50%). Auti (2005), Dhanavel *et al.* (2008), Kavithamni *et al.* (2008), Potdukhe and Narkhede (2002) found decreased survival rates as a result of mutagenesis treatments in different crops.

CONCLUSION

From the investigation it was concluded that the 50 per cent seed germination rate was observed in EMS (T_4) at 0.75% and Colchicine at 1.00%. The development of seedlings and the percentage of seeds that germinate were suppressed when mutagen concentrations/doses increased. As the concentration or dose of the mutagens increased, the survival rate significantly decreased. Almost every mutagenesis treatment reduced the length of the shoots, the roots, the entire seedling, and the number of leaves per seedling.

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