



Optimization of Chemical Mutagen Treatment Techniques and Determination of Absorption Dose in *Jasminum auriculatum* Ecotype “Muthu Mullai” for Inducing Variation

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ABSTRACT

Background: Chemical mutagenesis is one of the effective tools for the induction of variation in plants. The biological effect of the chemical mutagen on plant tissues is determined by the absorption dose which is calculated by the product of the concentration of the mutagen and the treatment duration. Standardizing the treatment conditions for the best possible mutagenesis is the first prerequisite in chemical mutagenesis.

Methods: Five concentrations of EMS (5 mM, 10 mM, 15 mM, 20 mM and 25 mM), three treatment durations (1 hr, 2 hr and 3 hr) and two pre-treatment conditions (soaking in water and non-soaking in water) were taken into consideration in the current study to examine the significance of each factor in regulating the mutation and to determine the unknown value of “Absorption dose” from the known survival rate.

Result: The present study revealed that pretreatment with water affected the absorption of EMS into plant tissues and an increase in the EMS concentration and treatment duration decreased the survival rate of the cuttings. The study led to the inference that pretreatment of cuttings of *Jasminum auriculatum* ecotype Muthu Mullai with water for 2 hours and treatment with EMS at different concentrations of EMS ranging from 5 mM to 30 mM for a duration of 2 hours is suggested for higher mutation frequency.

Key words: Absorption dose, EMS concentration, EMS treatment method, EMS treatment time, Pre-treatment.

INTRODUCTION

One of the commercially significant jasmine species that is indigenous to Southeast Asia is *Jasminum auriculatum* Vahl. It is commonly grown in the southern regions of India including Tamil Nadu, Karnataka, Andhra Pradesh as well as in Uttar Pradesh and some areas of Bihar and West Bengal (Yamaguchi *et al.*, 2008).

J. auriculatum is used to make jasmine concrete, which is widely used in aromatherapy due to its potential to treat depression, nervous exhaustion and stress. It is also used in the cosmetic, perfumery, medicinal and pharmaceutical industries in addition to being a well-known fragrant loose flower and a highly favoured garden plant (Amini, 2014).

J. auriculatum is a crop that is vegetatively propagated and has very little natural variability. Due to the limitations of conventional jasmine hybridization, induced mutation may be a useful technique to produce desired differences in flower quality and quantity as well as to improve the level of gall mite resistance.

Induced mutagenesis is a potential tool to create novel genetic variability in ornamental crops, helping in evolving mutants which have direct utilization as improved varieties or as potential breeding materials for crop improvement without changing the genetic background. To achieve the ideal results in mutation experiments, optimizing the ideal mutagen dose is an essential prerequisite.

Mutant populations have been created in many flower crops using physical mutagens. Gamma radiation and fast neutrons can, however, induce larger DNA inversions and

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deletions that make it difficult to pinpoint the genes responsible for a mutant phenotype (Yan *et al.*, 2020). As an alternative, the chemical mutagen EMS has frequently been used to cause mutations (Unan *et al.*, 2022). EMS usually causes random point mutations, some of which can lead to novel stop codons in important genes (Dhanavel *et al.*, 2008).

Genetic diversity is fundamental to the success of breeding programs. Hence, it is imperative to maximize the effectiveness and efficiency of mutagens used to generate diversity. The effect of a mutagen completely depends on the concentration, treatment time and the pre-treatment of cuttings and the germplasm treated (Arun *et al.*, 2016). Therefore, it is necessary to optimize the treatment conditions for inducing variability by mutagens to assure a high mutation frequency without compromising the survival rate (Porch *et al.*, 2009).

With this background, the present research work was undertaken to standardize pre-treatment requirements and EMS treatment method, estimate the absorption dose of EMS by propagules treated with different concentrations of EMS and optimize the treating duration for Muthu Mullai, a promising ecotype of *J. auriculatum*.

MATERIALS AND METHODS

Plant material

The present study was carried out at Tamil Nadu Agricultural University, Coimbatore during 2020 to 2022 in a promising ecotype 'Muthu Mullai' of *J. auriculatum* identified from Jasmine growing regions of Coimbatore district. Semi hardwood cuttings of 15 cm length were prepared and 50 cuttings were treated per treatment. The treated plants along with the untreated control were placed under a controlled environment maintained with 90% RH to enable efficient rooting (Picture 1).

Chemical mutagen

EMS is a common chemical mutagen which is used in mutation breeding studies. For the optimization of a suitable treatment method for EMS, the freshly collected cuttings of Muthu Mullai ecotype were subjected to pre-treatments comprising of either soaking in distilled water for 2 hr at room temperature or non-soaking control. Six different EMS concentrations including 5 mM, 10 mM, 15 mM, 20 mM, 25 mM and three different treatment durations (0.5 hr, 1 hr and 1.5 hr) were adopted. EMS solution was prepared in 0.1 M phosphate buffer, pH 7.0 and cuttings were completely immersed in the solution for different treating durations with frequent turning of the cuttings (Siddique *et al.*, 2020). After the treatment duration, the cuttings were washed completely with running water for 1 hr. Each treatment was replicated three times with 50 cuttings per treatment and an equal number of cuttings was soaked in the distilled water without EMS treatment subsequently which was maintained as control. After the 45th day of planting in the mist chamber maintained with an RH of 90 to 95%, the survival rate was calculated.

Survival and mortality rates and morphological traits

Treated cuttings were placed under the mist chamber and the survival rate, mortality rate and morphological data including plant height, number of leaves and root length were measured on the 45th day of planting to assess the impact

of EMS on the growth of the cuttings. Observations were also taken for the control plants.

Statistical analysis

The influence of EMS on morphological characteristics was investigated using linear regression. The absorption dose was determined as per the method of Ke *et al.* (2019) with a few minor modifications. The survival rate for each treatment was standardised in each replicate, where,

x1 = S,

x2 = C,

x3 = T,

x4 = S2,

x5 = C2,

x6 = T2,

x7 = SC,

x8 = ST,

x9 = CT;

S = Represents the pre-treatment.

T = Represents the treatment period.

C = Represents the EMS concentration.

Then, the mean of the three replicates of each treatment was determined for step-wise multiple regression analysis and the values were fitted using weighted step-wise regression at a 0.5 per cent level of significance, where the weighted y was calculated by the reciprocal of the variance of the y as the weight. SPSS software was utilised as the statistical instrument. The data were fitted using the subsequent regression model:

$$y = \sum_{i=1} b_i x_i + \varepsilon \quad \dots(1)$$

Where,

y = Mean of normalized SR of three replicates.

b = Corresponding coefficient of x_i .

ε = Residual error.

RESULTS AND DISCUSSION

Results of the present study indicated that the regression model is highly significant, with a high coefficient of determination ($R^2=0.923$), implying that the experimental data match the model well. As a result, the model could accurately and reliably describe the relationship between the SR and EMS treatment within the experimental parameters like the pretreatment (S), Treatment time (T) and concentration (C). The following regression model was obtained by fitting the experimental data using the above equation.

$$y = 0.477S^2 + 0.932SC - 1.568CT \quad \dots(2)$$

Effect of EMS treatment and the treatment time

The model includes the three parameters (S, C and T), implying that they all had a considerable impact on the EMS treatment and the treatment time. With increasing EMS concentration and treatment time, the survival rate dropped. Also based on the kill curve analysis of EMS treatment on the vegetative parameters like shoot length, root length and

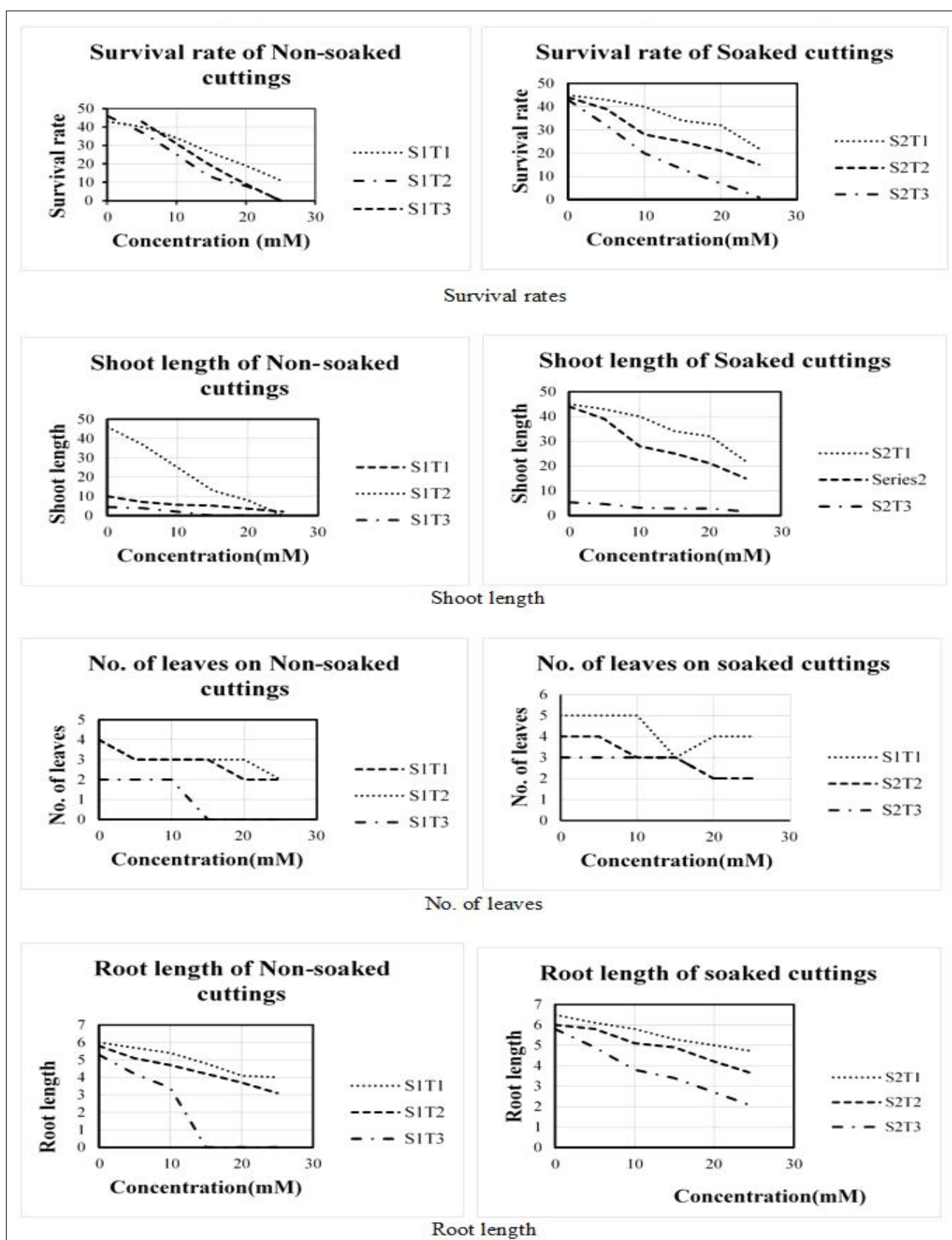


Fig 1: Response of different growth parameters in non-soaked and water-soaked cuttings.

no. of leaves (Fig 1), a decreasing curve was obtained towards the increase in the concentration of EMS, suggesting that the higher concentration of EMS and treatment time will decrease the growth of the plant. Longer treatment time will increase the infusion of the EMS into the cuttings causing more DNA damage leading to mortality of the cuttings. Therefore, the optimum dose of EMS and the optimum treatment time should be standardized before the initiation of mutagenesis to prevent the cuttings from heavy damage due to the higher level of EMS concentration and treatment time (Espina Mary *et al.*, 2018).

Effect of pre-treatments

The survival rate was also affected by pretreatment (soaking the cuttings in water vs. not soaking the cuttings in water), suggesting that pretreatment seems to have a considerable impact on the survival rate.

Let $S=1$ and 2 , for the non-soaked cuttings and cuttings soaked with water then equation (2) becomes,

$$y = 0.477 + 0.932C - 1.568CT \quad (S=1) \quad \dots(3)$$

$$y = 1.908 + 1.864C - 1.568CT \quad (S=2) \quad \dots(4)$$

On comparing the equation (3 and 4), the difference in the survival rate (SR) of the cuttings between the two pre-treatment methods depends completely on the EMS concentration and the treatment time. It is also seen from the graph (Fig 1) that the pretreatments including non-soaked cutting and cuttings soaked in water are rather similar in shape suggesting that the EMS concentration and the treatment time have a similar influence on the cuttings irrespective of the pretreatment.

Considering the equ (3 and 4) and the curve (Fig 2), the pretreatments also have a considerable impact on the survival rate of the cuttings and also on the vegetative parameters. The non-soaked cuttings were found to be more susceptible to EMS compared to cuttings presoaked with water. In the non-soaked cuttings, the penetration of mutagens was higher, leading to a greater effect on metabolic activities, thereby increasing the effectiveness of EMS at relatively low concentrations (Arisha 2014). In addition, the interdependence of treatment variables that influence the degree of lethality induced by a mutagen is clearly illustrated by the interactions between EMS concentration, treatment period and presoaking (Emrani *et al.*, 2011).

Determination of absorption dose, absorption time and effective time

From the exposure dosage, treatment period and concentration, the absorption dose may be derived as (4) and (5),

$$D = CT \quad \dots(5)$$

Where,

D - Exposure dose.

C - Concentration.

T - Treatment time

The absorption process needs a particular time (T_a), so the time that the mutagen acted upon the cuttings (T_e) is given as

$$T_e = T - T_a \quad \dots(6)$$

Hence, Equ (5) becomes,

$$D_a = CT_e = C(T - T_a) \quad \dots(7)$$

Therefore, the Equ (3 and 4) becomes

$$y = 0.477 - 1.568C(T - 0.594) \quad \dots(8)$$

$$y = 1.908 - 1.568C(T - 1.188) \quad \dots(9)$$

When comparing equations (10) and (11), it appears that the estimated absorption time for both pre-treatments (water soaked and non-soaked cuttings) is 0.594 and 1.188, implying that the EMS concentration and treatment duration influence the absorption dose, in a similar pattern for both the pretreatments and based on this value, difference between the pretreatments can also be observed (Fig 2). Ke *et al.*, (2019) observed the absorption doses D_{a1} and D_{a2} for the non-soaked and pre-soaked seeds in the Cauliflower as 4.089 and 3.823. Therefore, the absorption dose varies with the crop, cultivar and nature of the propagation material which should be further evaluated.

If this is correct, the corresponding effective time would be,

$$T_{e1} = T - 0.594 \quad \dots(10)$$

$$T_{e2} = T - 1.188 \quad \dots(11)$$

And the absorption doses will be

$$D_{a1} = CT_{e1} \quad \dots(12)$$

$$D_{a2} = CT_{e2} \quad \dots(13)$$

Absorption dose: The absorption dose would be

$$y = 0.477 - 1.568D_{a1} \quad (S=1) \quad \dots(14)$$

$$y = 1.908 - 1.568D_{a2} \quad (S=2) \quad \dots(15)$$

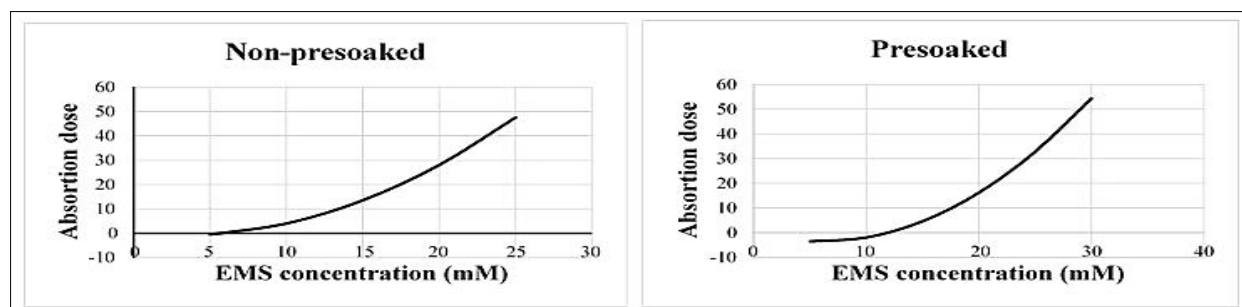
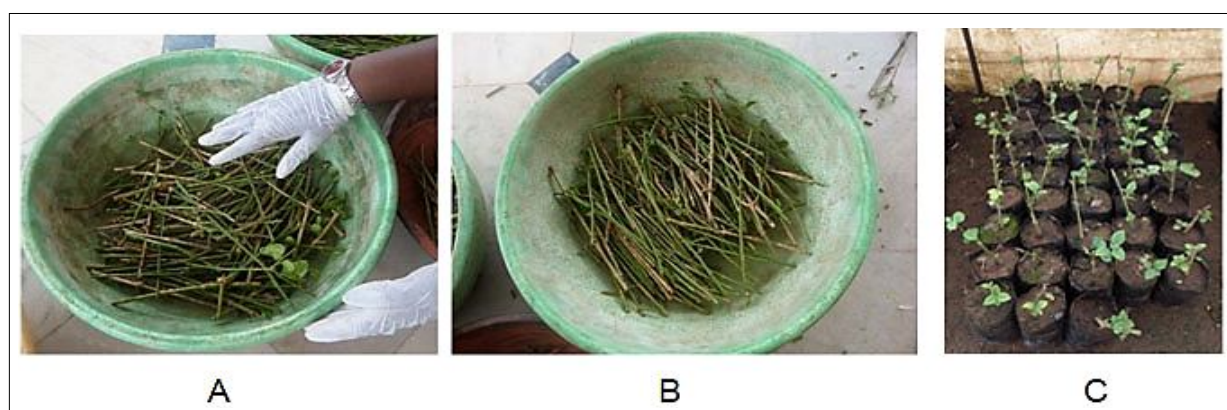


Fig 2: Absorption doses of different EMS concentrations in non-soaked and water soaked cuttings.



Picture 1: A): Treatment of cuttings with EMS; B): pre-soaking of cuttings with water; C): planting of the cuttings under the mist chamber.

From equ (14 and 15) the absorption doses were higher with the higher level of treating time and a higher absorption dose will lower the survival rate. This suggests that the equ (14 and 15) were in a similar form but with a slight variation in the slope indicating that the EMS treatment method influences the survival rate. Similar finding was reported by Ke *et al.* (2019) in cauliflower seeds where differences between presoaked and non-soaked seeds were observed.

Standardization of the optimum treatment conditions

In the present study, the kill curve analysis was helpful to fix the optimum treatment conditions for the induction of mutagenesis in *J. auriculatum* ecotype Muthu Mullai. Based on the kill curve analysis, the cuttings pre-treated with water followed by treatment with 25 mM concentration of EMS for 3 hrs showed a very low survival rate (2%) and poor growth parameters including shoot length (1.733 cm), number of leaves (1) and root length (2.03 cm) indicating that 3 hrs of treatment time is too high to maintain the survival rate and the plant population. Considering the cuttings treated for 2 hrs of treatment time, even at the higher concentration of 25 mM, the survival rate (30%) and the vegetative growth parameters including shoot length (3.9 cm), root length (3.56 cm) and number of leaves (2.33) were favorable to maintain the plant population and induce mutagenesis. Reduced growth with respect to the treatment time and EMS concentration against the control is due to the destruction in the auxin content, ascorbic acid content and other physiological and biochemical abnormalities (Yusuff *et al.*, 2016). In contrast, treatment of cuttings for 1hr recorded a survival rate of 44% at 25 mM concentration indicating that the EMS caused weak mutation suggesting that less treatment time maintains the higher survival rate but maintains low mutation rate (Lee *et al.*, 2017).

Further, the LD₅₀ value might be determined with the comparative study of higher concentration of EMS with pretreatment of cuttings with water for 2 hrs followed by the EMS treatment time for 2 hrs.

CONCLUSION

Based on the results, it is concluded that the pre-treatment of cuttings for 2 hr with water followed by the EMS treatment for 2hr would be the optimum treatment combination for inducing mutations in Muthu Mullai ecotype of *J. auriculatum*. While higher time duration might increase the mutation frequency, it is found to be too detrimental to the genetic material resulting in a very low survival rate.

Conflict of interest: None.

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