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Assessment of Mutagenic Sensitivity and Lethal Dose of EMS in Papaya (*Carica papaya* L.) variety CO 7

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ABSTRACT

Background: Mutagenesis is the process through which an organism's genetic makeup is irreversibly altered. The objective of this study was to determine the optimal ethyl methanesulfonate (EMS) dosage to create desirable genetic variations in the papaya variety CO 7.

Methods: The pre-soaked papaya seeds were exposed to five different concentrations of EMS (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) for three hours. Non-treated seeds were used as a control. Data was recorded for germination per cent, seedling survival per cent, seedling length, girth, number of leaves, leaf length and width, petiole length and girth.

Result: A declining trend in germination and growth of seedlings with the increase in EMS doses was observed. The probit curve analysis based on the seed germination percentage revealed that the LD_{50} value was found to be 0.55% EMS which was fixed as an optimal dose for large-scale mutagenesis experiments in papaya variety CO 7. The R^2 value ranged from 0.73 to 0.99 and the growth reduction percentage (GR_{50}) varied from 0.69 to 1.16 for different seedling traits studied. Since the optimization of mutagen dose would be expected to create desirable mutations with nominal biological damage, this study might assist further mutagenesis experiments in papaya.

Key words: Ethyl methanesulfonate, GR_{50}, Induced mutation, LD_{50}, Papaya.

INTRODUCTION

Papaya (*Carica papaya* L.) is a tropical fruit that belongs to the family Caricaceae and originated in Central America. It is the fourth most important fruit due to its wide ecological adaptability, easiness in cultivation, high palatability, early fruiting and semi-perennial bearing nature. Papaya fetches higher productivity per unit area and fair economic returns in a short period, hence the crop is highly preferred by farmers. It is extensively grown for delicious fruits and various processed products. Papaya is a low-calorie and nutrient-rich fruit. About 100% daily requirement of vitamin C and 30% of vitamin A are met with serving of one papaya.

Additionally, it provides little amounts of vitamin E, K, thiamine, riboflavin, niacin, pyridoxine and folate (Ali *et al*., 2011). In addition, different plant parts of papaya such as leaves, fruits, roots, peel and seeds also possess significant medicinal uses. Apart from this, the enzyme papain, which is isolated from the latex of rpaw papaya fruits has numerous industrial applications in meat processing, leather, textile, cosmetic and pharmaceutical preparations.

Mutagenesis is the process through which an organism's genetic makeup is irreversibly altered. It can be effectively utilized in crop improvement programs to widen genetic variability. Compared to conventional methods, mutation breeding is an effective tool for improving and correcting certain characteristics without altering the other traits of crop plants in a short period, especially when the studied traits exhibit simple Mendelian inheritance. Mutations can be induced using different physical and chemical mutagens. Among various chemical mutagenic compounds, alkylating agents have found widespread use in mutation breeding, in which ethyl methanesulphonate (EMS) is being significantly used for mutagenic experiments in plants (Leitão., 2012). They have been the most successful in terms of developing new mutant cultivars due to their effectiveness, convenience and most importantly, the simple hydrolysis-based detoxification process for disposal. EMS mainly creates point mutations in the plants. Fruit crops have had comparatively few mutation breeding experiments. There

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are few mutagenic studies conducted on papaya to improve the yield and resistance to biotic and abiotic stresses (El-Latif *et al*., 2018; Kumar *et al*., 2016). India leads the world in papaya production and area however, there are still a few hurdles that curtail the production which need to be resolved to enhance the production (Auxcilia *et al*., 2020). Varieties resistant to Papaya ring spot virus with good yield, quality and dwarf stature are to be developed. To create desired mutants with useful variations, it is crucial to determine the optimal dosage of the mutagen. Since lower doses of mutagen have minimal effects on the genome which seldom cause phenotypic alterations and higher doses may frequently result in aberrations or unfavourable consequences, the determination of the LD_{50} and GR_{50} is the initial step in a mutation breeding approach. The present experiment, aims to study the biological sensitivity of papaya seeds to EMS and its impact on seed germination and seedling growth to optimise the lethal dose of EMS in papaya variety CO 7.

MATERIALS AND METHODS

The papaya variety CO 7, a high-yielding gynodioecious cultivar developed by Tamil Nadu Agricultural University, Coimbatore was used in the present study. The mutagenic experiment was carried out at University Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore in 2021. Papaya seeds were soaked in sterile water overnight and exposed to five different concentrations of EMS for 3 hours *i.e.*, Control 0.0% (3 hours soaking in water) EMS 0.2%, EMS 0.4%, EMS 0.6% and EMS 0.8% and EMS 1% and were sown in polybags filled with the mixture of red soil: FYM: sand in a ratio of 1:1:1. Seed germination, survival of seedlings and seedling growth were observed. Seed germination was recorded after four weeks of sowing whereas, survival percentage and seedling growth were recorded six weeks after sowing. Growth parameters such as seedling length, girth, number of leaves, leaf length and width, petiole length and girth were measured from randomly selected seedlings. The study was carried out as a Completely randomized design with six treatments and three replications. A total of 30 seeds per replication were treated with different doses of EMS. Using Probit Analysis, the lethal dosage (LD_{50}) was determined based on the germination percentage. The growth reduction percentage (GR_{50}) for different seedling attributes was derived from the linear regression line on the dose-response curve. AGRES statistical computing software was used to test the significance of different parameters under various treatments.

Germination percentage (%) =

$$\frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Survival percentage (%) =

$$\frac{\text{Number of plants survived after germination}}{\text{Total number of germinated seeds}} \times 100$$

Vigour index = Germination % × Mean of seedling length

RESULTS AND DISCUSSION

Effective induced mutagenesis experiments initiate the standardisation of mutagenic dosages. To determine the optimum dose to induce desirable biological variations in mutation breeding programmes, lethal dose 50 (LD_{50}) and the growth reduction percentage (GR_{50}) are considered as important parameters. In the present study, the germination of seeds treated with EMS was continually monitored. Germination was started in the 0.2% EMS-treated seeds after 10 days of sowing followed by control and 1% EMS-treated seeds in 11 days. In the remaining treatments, germination was started in 12 days. There was no particular trend observed in the number of days to germination with the rise in the concentration of EMS (Table 1). A significant difference in the germination percentage was noticed among different treatments. The germination percentage ranged from 30% to 90% and was thought to be influenced by EMS treatment (Table 1). As expected, germination and survival were highest in the control as no mutagenic treatment was applied. The highest germination percentage (65.33%) among the treated seeds was observed in 0.2% EMS followed by 0.4% EMS. Germination per cent was found to be the lowest (30%) in 1% EMS. A decrease in the germination trend was observed with an increase in the concentration of EMS. The highest seedling vigour (1176) among the treated plants was observed in 0.2% EMS and was declining with an increase in the EMS concentration. These results are in accordance with the EMS-based mutagenic studies on papaya (Singh *et al*., 2008; Rajbhar *et al*., 2014) and pumpkin (Chen *et al*., 2018). Seed germination and seed vigour index decreased significantly with an increase in doses of EMS in *okra* (Baghery *et al*., 2016). The major reasons for the reduction in seed germination by EMS might be due to the altered enzyme activity (Zou *et al*., 1999), metabolic disturbances (Ananthaswamy *et al*., 1971), inactivity of plant hormones (Sideris *et al*., 1971) and chromosomal aberrations (Nurmansyah *et al*., 2018).

The maximum seedlings survival percentage of 94.39 was observed in 0.2% EMS followed by 0.4% EMS and the minimum survival per cent (77.78) was observed in 1% EMS. The survival percentage varied significantly across various treatments (Table 1). The survival of seedlings declined with the rise in EMS concentration. These findings are in concurrence with earlier studies in cluster bean (Prasath *et al*., 2019), which confirmed that the survival rates declined with increasing EMS levels.

Different seedling growth attributes studied in response to EMS treatment showed significant differences among treatments (Table 2). The effect of various EMS concentrations was found significant for seedling length and girth. The seedlings subjected to EMS treatment displayed delayed and weak growth compared with the control. The maximum length and girth were recorded in control followed by 0.2% EMS. Seedling length and girth were reduced with an increase in doses of EMS and the minimum value was found upon 1% EMS treatment. Similar results were reported previously in papaya cv. Pusa Dwarf (Kumar *et al*., 2016) and onion cv. Bhima Dark Red (Singh *et al*., 2021), where a gradual decline in plant height was observed with the increase in the concentration of EMS. There was a significant difference among various EMS concentrations on petiole length and girth. The maximum petiole length was observed in 0.2% EMS which was on par with 0.4% and 0.6% EMS. A significant decrease in petiole length was observed in EMS-

Table 1: Germination, seedling survival percentage and number of days for germination in papaya variety CO 7 treated with Ethyl methanesulfonate.

EMS concentration (%)	Germination percentage (%)	Survival percentage (%)	Number of days for germination	Seedling vigour index
0.00	90.00	96.30	11.00	2178.00
0.20	65.33	94.39	10.00	1176.00
0.40	56.67	94.12	12.00	906.67
0.60	43.33	84.62	12.00	563.33
0.80	36.67	81.82	12.00	385.00
1.00	30.00	77.78	11.00	285.00
Mean	53.67	88.17	11.33	915.67
SEd	1.33	7.76	0.25	26.94
CD (0.05)	2.91	4.88	0.55	58.69

Table 2: Seedling growth parameters of papaya variety CO 7 treated with Ethyl methanesulfonate.

Concentration (%) of EMS	Seedling height (cm)	Seedling girth (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Petiole girth (cm)
0	15.2	1.9	10	8	7.2	6.2	0.79
0.2	11	1.4	p8	6.9	5.1	5.1	0.65
0.4	10	1.1	7	6.6	5.3	4.8	0.61
0.6	8	1.2	7	5.8	4.5	4.1	0.56
0.8	6.5	1.1	6	5	4.2	3.5	0.55
1	5.5	1	6	4.4	3.5	2.7	0.43
Mean	9.37	1.28	7.33	6.12	4.97	4.40	0.60
SEd	0.14	0.04	0.20	0.12	0.02	0.47	0.015
CD (0.05)	0.30	0.08	0.43	0.27	0.04	1.03	0.03

Table 3: Results of dose response curve of different seedling parameters.

	Seedling height (cm)	Seedling girth (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Petiole girth (cm)
Linearequation	y = -9.1429x +13.938	y = -0.7571x +1.6619	y = -3.7143x + 9.1905	y = -3.5x +7.8667	y = -3.1429x +6.5381	y = -3.2857x +6.0429	y = -0.3071x +0.7519
R^2 Value	0.9418	0.7318	0.8521	0.987	0.8564	0.984	0.9212
GR_{50}	0.69	0.94	1.12	1.1	0.93	0.89	1.16

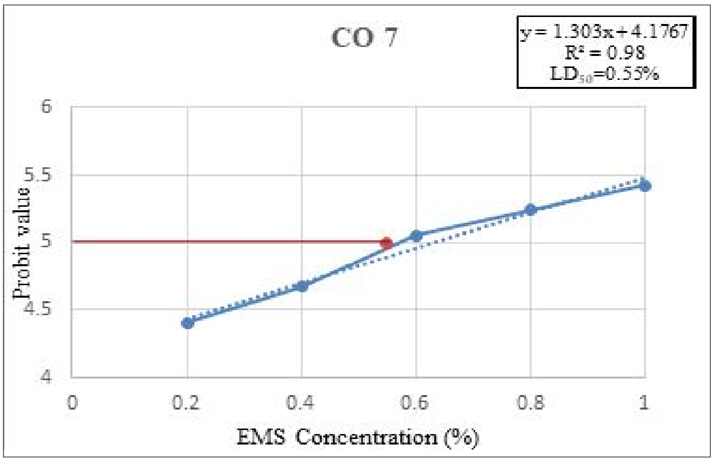

Fig 1: Dose response curve for germination percentage (Probit analysis).

induced variants of mango (Rime *et al*., 2019). The maximum number of leaves among the treatments was observed in 0.2% EMS. The highest leaf length and leaf width were observed in 0.2% and 0.4% EMS, respectively. This is in close agreement with the reports of Jyothsna *et al*., (2022) in which a steady descent was observed in the seed germination, growth and vigour of the fenugreek seedlings in response to the increase in EMS concentrations. The reduction in seedling growth due to EMS application might be caused by deterioration in assimilation mechanisms, destruction of growth inhibitors or inhibition of auxin synthesis (Roychowdhury and Tah, 2011).

The LD_{50} value reveals the biological sensitivity of a species, making it easier to fix the ideal doses of mutagens to achieve desirable mutations with less injury (Laskar *et al*., 2020). In the present experiment, the LD_{50} value of EMS

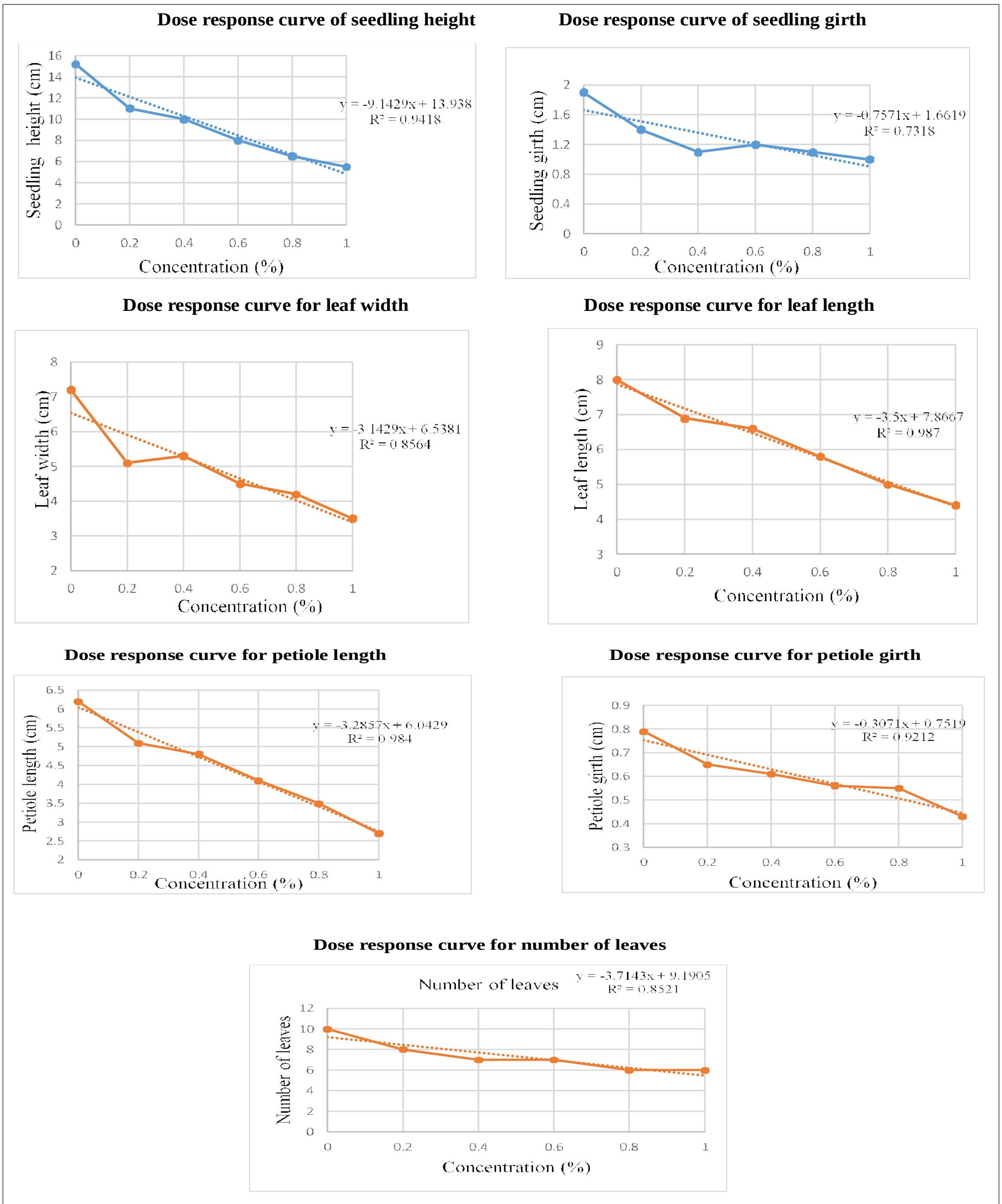

Fig 2: Dose response curve for different seedling parameters (Linear regression).

was determined to be 0.55% in CO 7 papaya based on the germination percentage (Fig.1). The LD_{50} value of EMS was reported in different fruit crops such as Mangosteen (0.43%) (Suwanseree, 2020) and strawberry (Murti *et al*., 2013). The LD_{50} value of EMS was determined as 0.25% in papaya variety Arka Prabhath (Pujar *et al*., 2019) and 0.5% in the variety Arka Surya (Santosh *et al*., 2008). In the present study, GR_{50} for different seedling growth parameters was determined using linear regression on the dose-response curve (Fig. 2). The R^2 value ranged from 0.73 to 0.99 and the growth reduction percentage (GR_{50}) varied from 0.69 to 1.16 for different seedling traits studied (Table 3). The GR_{50} value for seedling length and girth was determined as 0.69 % and 0.94% respectively. GR_{50} value of mutagens identified in different crops such as safflower (Shrivastava *et al*., 2021) and sorghum (Wanga *et al*., 2020). Since more desirable mutations occur at LD_{50} and GR_{50} doses, the optimized EMS concentrations reported in this study could be utilized in developing novel mutants in papaya.

CONCLUSION

In the present study, the biological sensitivity of papaya variety CO 7 to EMS was tested and the lethal dose was optimized. Following the exposure of papaya seeds to EMS, the germination and seedling growth traits showed a declining trend with the increase in EMS doses. The LD_{50} value of EMS for papaya variety CO 7 was determined as 0.55% and growth reduction dose values for different seedling traits ranged between 0.69 to 1.16%. The results of this study have demonstrated that induced mutagenesis using EMS could be employed to increase genetic variability and also would facilitate further mutation-based crop improvement programs in papaya.

Conflict of interest: None.

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