

Effect Of Different Doses Of EMS On Success, Survivability And Growth Of Lemon Using Leaf Cut Method Under Glass House And Field Condition

Md. Shamsul. Alam¹, Kazi Tahmina Akhter² And Md. Rafiqul.Islam³

Horticulture Division, Bangladesh Institute Of Nuclear Agriculture (BINA), Mymensingh

¹Senior Scientific Officer, Horticulture Division, BINA; ²Scientific Officer, Horticulture Division, BINA, ³Chief Scientific Officer And Head, Horticulture Division, BINA.

Abstract

An experiment was conducted at the Horticulture division of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during the period from March, 2024 to June, 2024 to develop mutant from lemon leaves by using EMS under glass house and field condition to find out the appropriate combination of EMS and suitable environment on success, survivability and growth of lemon. The experiment was consisted of two factors such as Environmental condition: (i) glass house and field condition and (ii) different doses of EMS: D₀ (Control) D₁ (2%EMS for 3hrs) D₂ (2.5 %EMS for 3hrs) D₃ (3%EMS for 3hrs) D₄ (4%EMS for 3hrs) and D₅ (5%EMS for 3hrs). Mature leaves were collected from BINA Lebu-1 mother plants. Collected leaves were exposed by different doses EMS solution separately and also a control. The experiment was laid out in RCBD with three replications. Total 36 treatment combinations of the environmental condition and EMS levels. EMS exposed leaves of lemon were planted in the unit plots on 8 March, 2024 in the afternoon with spacing of 15cmx15cm (row to row and leaf to leaf distance). Different environmental condition showed significantly influenced on almost all the mentioned parameters studied concerned with growth and growth contributing characteristics. The maximum success (62.33%), survivability (58.36%) and canopy volume (0.052m³) were found from glass house condition and the lowest values were found on all the mentioned parameters from control treatment. The glasshouse condition took the minimum time for root and shoot (42.82 and 68.88 days respectfully) initiation. The application of 3%EMS for 3hrs took the minimum time for root and shoot (40.50 and 63.00 days respectfully) initiation and the maximum success (67.10 %), survivability (60.52%) and canopy volume (0.094m³). The maximum time took for root and shoot (50.00 and 68.00 days respectfully) initiation and the minimum success (49.30 %), survivability (44.54%) and canopy volume (0.004m³) were found from control treatment. The combined effect of environmental condition and EMS was statistically significant on the success, survivability and growth of lemon. The treatment in glasshouse condition took the minimum time for root and shoot (38.00 and 70.00 days respectfully) initiation and the maximum success (69.30 %), survivability (65.40%) and canopy volume (0.194m³) as compare to control. Therefore, the overall results indicate that glasshouse condition with 3%EMS for 3hrs might be an efficient and eligible practice for developing mutant from leaves.

Keywords: EMS, Mutant, Environmental condition, Lemon leaf

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I. Introduction

Citrus Species including lemon (*Citrus limon*) have played a vital role in human culture and agriculture for centuries (Nicolosi *et al.*, 2000). However, the propagation and cultivation of lemon species present complex challenges that demand attention in modern agriculture. Approximately 95411.89 metric tons of lime and lemons are produced year from 30248.22 acres of land, yielding 18.34 kgs per fruit bearing trees (BBS,2021-22) whereas global production was 215,000 tons including 26.5 million tons in china, 3 million tons in EU and 1.9 million in turkey. In comparison to other countries, the yield is quite poor. Lemon is very important in respect of its nutritional values especially in Vitamin C. Different environmental conditions have significant effect of lemon production. The excessive heat and low temperature can retard the growth of lemon (Singh *et al.*, 2003). High temperatures affect citrus plants increasing transpiration, photosynthesis, destabilizing their cell membrane and increasing oxidative damage. The optimum temperature range for citrus sapling production is estimated to be 22-34°C. Moreover low rainfall can also be an effect of lemon production. Induce mutation through gamma irradiation can cause changes in chromosome and genome, which bring to successful variation in the morphology of plant. EMS represents an alternative for improving genotypes for

which breeding through hybridization involves many difficulties because of their complex reproductive biology, as in the case of citrus (Smith, 2022). EMS, which is a chemical mutagen, is a widely used method of having diversity on many plant species and creating new variations (Smith & Brown, 2017). A cutting is a piece of the part of plants used to propagate which regenerate there missing part is called cutting. Leaf cuttings have been used for the vegetative propagation of citrus (Platt and Opitz, 1973; Debnath *et al.*, 1986; Singh *et al.*, 2013). Roots were formed at the petiole end. Adventitious bud formation occurred at a very low frequency and could not be stimulated by application of growth regulators. Leaf cuttings with auxiliary buds formed plants at a higher frequency but development of buds into shoots was slow and irregular. As no vascular connections were found between petiole and auxiliary bud, the slow growth of the buds was attributed to insufficient supply of assimilates from the leaves. In order to achieve the following goals, the current study was conducted i) to investigate the effect of EMS on the success, survivability, growth of lemon; ii) to find out the suitable environment for maximum success, survivability on the growth of lemon sapling; and iii) to find out the appropriate combination of EMS doses and suitable environment on success, survivability, growth and yield of lemon sapling.

II. Materials And Methods

An experiment was conducted at the Horticulture division of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during the period from March, 2024 to June, 2024 to develop mutant from lemon leaves by using EMS under glass house and field condition to find out the appropriate combination of EMS and suitable environment on success, survivability and growth of lemon. The experiment was consisted of two factors such as Environmental condition: (i) glass house and field condition and (ii) different doses of EMS: D₀ (Control) D₁ (2%EMS for 3hrs) D₂ (2.5 %EMS for 3hrs) D₃(3%EMS for 3hrs) D₄ (4%EMS for 3hrs) and D₅ (5%EMS for 3hrs). Mature leaves were collected from BINA Lebu-1 mother plants. Collected leaves were exposed by different doses of EMS solution separately and also a control. The experiment was laid out in RCBD with three replications. Total 36 treatment combinations of the environmental condition and EMS levels. EMS exposed leaves of lemon were planted in the unit plots on 8 March, 2024 in the afternoon with spacing of 15cmx15cm (row to row and leaf to leaf distance). The spaces between the plots and blocks served to facilitate quick drainage of rain or irrigation water. Flood irrigation was given after 15 days of planting the leaf cuttings and in later period necessary irrigation was given by watering can whenever needed. Weeds, whenever appeared were removed very carefully by holding the base and pulling without disturbing cuttings. Data on the following parameters were recorded from each plot after 40 days of planting cuttings. Periodical data were taken from 10 cuttings on time of root initiation, time of shoot initiation, canopy volume of per plant at 150 days after planting of cutting, percent success & percent survivability at 150 days after plant of cutting. The recorded data for each parameter from the present experiment was analyzed statistically to find out the variation resulting from experimental treatment using MSTAT package program. The means for all treatments were calculated and analyses of variances of parameters under study were performed by F variance test at 5% and 1% levels of significance. The means of the parameter were separated by least significant difference test.

Plate3. Photograph showing the general view of experimental plat and collection of data



a. Length of root



b. Initiation of shoot



c. Plant in field condition



d. Plant in glasshouse condition



e. Measuring Plant height



f. Measuring canopy volume

Plate3. Photograph showing the general view of experimental plat and collection of data

III. Results

EMS (ethyl methane sulphonate) has been widely used for producing mutations in crop plants. Among them EMS has been frequently used to create variation in gene pools of crop plants on their cytological characteristics like cell division, resulting in cytological abnormalities and in a reduced frequency of dividing cells, which are ultimately reflected in reduced seedling growth and other morphological aberrations which vary from species to species and among different genotypes within the same species. To verify this concept a study was conducted in respect of growth and quality of lemon as influenced by two different environmental condition and gamma radiation doses.

Main effect of environmental condition

Time of root initiation

Time of root and shoot initiation, canopy volume, percent success and percent survivability were significantly influenced by the environmental condition. The minimum time required to root and shoot initiation (41.81 and 73.88 days) in the glasshouse condition than the maximum time took in the field condition (46.89 and 76.80days) respectively. The earliest root and shoot initiation under glass house condition might be due to the favorable environment conditions with abundant supply of carbohydrate and other food materials prevailed at the time. The canopy volume was found higher in glass house condition (0.052m^3) and the lowest canopy volume was obtained in the field condition (0.026m^3). The highest % success and % survivability (62.33% and 58.36%) were recorded from glasshouse condition and the lowest % success and % survivability (52.33% and 48.25%) were recorded from field condition (Table 1). This is might be due to the quick root and shoot initiation that enhanced the translocation of food materials from leaves which also enhanced excellent emergence of new leaf.

Table 1. Effect of environmental condition on the time of root and shoot initiation, canopy volume, % Success and % survivability of lemon

Variety	Time of root initiation at DAP	Time of shoot initiation	Canopy(m ³)	% Success	% survivability
C ₁	42.82	68.88	0.052	62.33	58.36
C ₂	49.89	76.80	0.026	52.33	48.25
LSD _{0.05}	0.61	0.93	0.0014	1.12	2.23
Level of significance	**	**	**	**	**
CV (%)	4.98	4.77	6.66	7.12	5.47

C₁ = Glasshouse condition, C₂ = Field Condition
 ** = Significant at 1% level of probability

Main effect of different doses of EMS

The effect of different doses of EMS on time of root and shoot initiation, canopy volume, percent success and percent survivability were influenced significantly. The minimum time required to root and shoot initiation (40.50 and 63.00 days) in D₃ treatment than the maximum time took to root and shoot initiation in D₅ treatment (51.50 and 69.50 days respectively). The canopy volume was found higher in D₃ treatment (0.094m³) and the lowest canopy volume was obtained in D₅ treatment (0.002 m³). The highest % success and % survivability (67.10% and 60.52%) were recorded from D₃ treatment and the lowest % success and % survivability (41.70% and 37.27%) were recorded from D₅ treatment (Table 2). This is might be due to the quick root and shoot initiation that enhanced the translocation of food materials from leaves which also enhanced excellent emergence of new leaf (Khan et al., 2017)

Table 2. Effect of different doses of EMS on time of root and shoot initiation, canopy volume, % Success and % survivability of lemon

Radiation	Time of root initiation	Time of shoot initiation	Canopy(m ³)	% Success	% survivability
D ₀ (Control)	50.00	63.00	0.004	49.30	44.54
D ₁ (2%EMS for 3hrs)	49.16	64.00	0.057	65.60	61.87
D ₂ (2.5 %EMS for 3hrs)	43.00	67.50	0.05	64.90	56.55
D ₃ (3%EMS for 3hrs)	40.50b	63.00	0.09	67.10	60.52
D ₄ (4%EMS for 3hrs)	42.00b	65.00	0.017	53.50	53.50
D ₅ (5%EMS for 3hrs)	51.50	69.50	0.07	41.70	37.27
LSD _{0.05}	2.29	2.99	0.002	3.78	4.45
Level of significance	**	**	**	**	**
CV (%)	5.65	4.90	6.66	7.87	9.87

** = Significant at 1% level of probability

Combined effect of environmental condition and different levels of EMS

Combined effect of different doses of EMS with environmental condition on time of root and shoot initiation, canopy volume, percent success and percent survivability were significantly. The minimum time required to root and shoot initiation (35.24.50 and 70.22 days) in the glasshouse condition than the maximum time took in D₅ treatment (50.21 and 88.00 days). The canopy volume was found higher in glass house condition (0.097m³) and the lowest canopy volume was obtained in D₅ treatment (0.009 m³). The highest % success and % survivability (63.10% and 61.50%) were recorded from glasshouse condition and the lowest % success and % survivability (40.70% and 38.22%) were recorded from field condition (Table 3). The application of use of physical and chemical in the tissue culture laboratory of Fruit Science mutagens in the plant tissue culture (Thapa, 1999) showed that relatively low-doses ionizing irradiation on plants accelerated cell proliferation, germination rate, cell growth, enzyme activity, stress resistance and crop yields (Chakravarty and Sen, 2001).

Table 4.3. Combined effects of EMS growing condition on the time of root and shoot initiation and canopy volume of lemon under glass house and field condition

Environmental Condition	Doses	Time of root initiation(Days)	Time of shoot initiation (Days)	Canopy(m ³)	% Success	% survivability
C ₁ (Field condition)	D ₀ (Control)	49.00	70.00	0.015	47.50	45.50
	D ₁ (2%EMS for 3hrs)	47.00	72.00d	0.069	66.80	63.10
	D ₂ (2.5 %EMS for 3hrs)	48.00	72.00	0.060	68.20	61.70
	D ₃ (3%EMS for 3hrs)	38.00	70.00	0.019	69.30	65.40
	D ₄ (4%EMS for 3hrs)	39.33	81.00	0.018	56.70	52.50
	D ₅ (5%EMS for 3hrs)	45.00	82.00	0.090	55.10	52.30

C ₂ (glass house condition)	D ₀ (Control)	41.00	78.00	0.023	48.30	45.50
	D ₁ (2%EMS for 3hrs)	39.23	77.14	0.036	64.60	60.80
	D ₂ (2.5 %EMS for 3hrs)	37.25	72.26	0.036	61.90	58.50
	D ₃ (3%EMS for 3hrs)	35.24	70.22	0.097	63.10	61.50
	D ₄ (4%EMS for 3hrs)	41.10	75.22	0.063	52.50	50.30
	D ₅ (5%EMS for 3hrs)	50.21	88.00	0.009g	40.70	38.22
	LSD _{0.05}	1.80	2.90	0.0055	3.54	4.54
	Level of significance	**	**	**	**	**
	CV (%)	4.45	3.78	5.69	6.68	7.87

** = Significant at 1% level of probability

IV. Discussion

Time of root and shoot initiation, canopy volume, percent success and percent survivability were significantly influenced by the environmental condition. The minimum time required rooting and shooting initiation as well as maximum canopy volume, percent success and percent survivability were obtained in the glasshouse condition. On the other hand maximum time took for rooting and shooting initiation as well as minimum canopy volume, percent success and percent survivability were found in the field condition. It is revealed that glass house conditions resulted in more canopy volume of plant which was significantly higher than that under open field conditions. This might be due to modification in environmental factors like temperature and soil moisture in the glass house condition due to partial shade. The absolute light conditions may have an injurious effect on growth of seedlings due to increase in temperature and decreased moisture in soil. The present findings are in agreement with the findings of Patel & Singh. (2019) and Thapa (1999) who reported that maximum plant height was recorded in citrus in 40 per cent shade.

The effect of different doses of EMS on time of root and shoot initiation, canopy volume, percent success and percent survivability were significantly. The minimum time required to root and shoot initiation in D₃ treatment than the maximum time took in D₅ treatment. The canopy volume was found higher in D₃ treatment and the lowest canopy volume was obtained in D₅ treatment. The highest % success and % survivability were recorded from D₃ treatment and the lowest % success and % survivability were recorded from D₅ treatment. This might be inhibition of auxin synthesis due to low doses apparently while larger doses can destroy auxin activity directly (Dwelle, 1975; Chervin *et al.*, 1992; Liu *et al.*, 2008). As a result, plant growth retarded by the high dose of radiation.

Combined effect of different doses of EMS with environmental condition on time of root and shoot initiation, canopy volume, percent success and percent survivability were significantly. The minimum time required to root and shoot initiation in the glasshouse condition with D3 treatment than the maximum time took in D₅ treatment in field condition. The canopy volume was found higher in glass house condition (0.097m³) with D3 treatment and the lowest canopy volume was obtained in D₅ treatment in field condition. The highest % success and % survivability were recorded from glasshouse condition with D3 treatment and the lowest % success and % survivability were recorded from field condition with D₅ treatment. The inhibition of propagation at high doses could be due to the damage in seed tissue, chromosomes and subsequent mitotic retardation and the severity of the damage depend on the doses used (Thapa,1999).

V. Conclusion

The percentage of success and percentage of survivability were positively influenced by different environmental conditions. Between the environmental factors tested glasshouse condition had given the higher success. In case of different doses of EMS, the success percentage and percentage of survivability were the highest in D₃ treatment. The treatment combination with the best glasshouse condition and the D₃ treatment gave the maximum values for most of all the parameters studied in the propagation of lemon plant from lemon leaves.

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