

USING CHEMICAL MUTAGENS TO DEVELOP NEW GENOTYPES WITH IMPROVED FRUIT QUALITY OF THE ANNA APPLE CULTIVAR

N. Abd-Elhamed¹, M.F. Maklad,¹ N.A. Awad² and M.I Anwar²

1- Department of Horticulture, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

2- Breeding Research Department of Fruit Trees, Ornamental and Woody Plants, Horticulture Research Institute, ARC, Giza, Egypt

ABSTRACT

A field experiment was carried out during the two successive seasons of 2021 and 2022 in a private farm on Anna apple cultivar (*Malus domestica*) to study the effect of chemical mutagens (Ethyl methane sulphonate (EMS), Malic hydrazine (MH) and Colchicine) with concentration of (0.05, 0.1 and 0.2%) for 24 hours and grafting on *Malus* root stock on survival percentage after grafting, vegetative growth, fruit quality characteristics and total yield of tree. The chemical mutagens gave the best results in vegetative growth, fruit quality and total yield per tree compared with control. However, control gave the highest value of survival percentage after grafting. Also, studies on relationship and correlation coefficient based on phenotypic dendrograms showed that colchicine 0.05% and colchicine 0.1%, this reflects a similar effect of colchicine at the two concentrations on tested phenotype characteristics.

Key words: Anna apple cultivar, Ethyl methane sulphonate (EMS), malic hydrazine (MH), Colchicine, survival percentage, vegetative growth, Fruit quality.

INTRODUCTION

The genus *Malus* belongs to the subfamily domestica of *Rosacea* family which contains at least 22 widely recognized primary species. Apple is one of the deciduous fruits in Egypt the important species are Anna, Ein Shamir, Dorset Golden, E25 and *Malus communis*. All examined species of apple are diploid ($2n = 34$), a few of them are polyploidy. Mostly cultivated by Anna, Ein Shemer, and Dorset Golden, E25 and *Malus communis*, cultivar. Most of this area is concentrated in Nubaria, Matrouh, New valley, North and South Sinai Governorates. An Apple pom fruit belongs from Southeast Asia, and it is one of the trees in the temperate zone whose cultivation extends from Japan and China in the east to North America in the west. The most important determinant of the spread and success of its cultivation is the availability of the necessary to break the dormancy phase in the winter need about (300-350) cold hour.

Mutation with colchicine originally extracted from *colchicum autumnal*, may induce some morphological, cytological and histological changes, and even changes in the gene level and duplication in chromosome (Murali, *et al* 2013). Chemical mutagens such as ethyl methane sulphonate (EMS), a compound of the alkaline sulphonate series, is most frequently used for chemical mutagenesis in higher plants due to its potency and the ease with which it can be used, it usually causes high frequency of gene mutations and low frequency of chromosome aberrations (Mohamed *et al* 2014). Malic hydrazine induced a high frequency of somatic mutations and

high yield of chromosome aberrations (Tomas *et al* 2000). The present study aims to study the effect of some chemical substances i.e., (ethyl methane sulphonate (EMS), malic hydrazine (MH) and colchicine) at different concentrations on vegetative growth characteristics, fruit quality, chemical quality and survival percentage after grafting of an apple cultivar.

MATERIALS AND METHODS

This study was conducted at the private orchard, El-Sadat city, Monefeia governorate, during two successive seasons of 2021 and 2022. Anna Apple cultivar was used to study the effect of some chemical mutagens, ethyl methane sulfonate (EMS), colchicine, and maleic hydrazine (MH) on soaking buds of Anna apple cultivar for 24 hours and grafting on *Malus* root stock in the nursery one year old on the survival percentage after grafting and some vegetative growth and fruiting characteristics.

Chemical mutagens treatments with (EMS - MH -Colchicine)

A preliminary study was performed to subject the most promising new genotypes for restricted selection and evolution. Therefore, after the evolution of the mutated plants, the most ten promising ones were selected from the obtained data for statistical analysis. The experiment included 10 treatments as follow:

1. Soaking for 24 hours in ethyl methane sulphonate (EMS) at 0.05%
2. Soaking for 24 hours in ethyl methane sulphonate (EMS) at 0.1%
3. Soaking for 24 hours in ethyl methane sulphonate (EMS) at 0.2%
4. Soaking for 24 hours in malic hydrazine (MH) at 0.05%
5. Soaking for 24 hours in malic hydrazine (MH) at 0.1%
6. Soaking for 24 hours in malic hydrazine (MH) at 0.2%
7. Soaking for 24 hours in colchicine at 0.05%
8. Soaking for 24 hours in colchicine at 0.1%
9. Soaking for 24 hours in colchicine at 0.2%
10. Soaking in tap water for 24 hours (control)

Data recorded

Survival percentage after grafting: calculated after 15-30 days after treatment as follow: Number of survived plants/total number of grafted plants X100

Tree characteristic: Ten trees were taken from each treatment after grafting on March and April and the following data were recorded.

Tree height (cm): It was measured from the highest point of the plant up to the crown surface.

Trunk diameter (cm): The trunk diameter above soil surface by 20 cm was measured.

Total yield of tree: At harvested stage in June and July, total number of fruits was counted, a sample of three replicates of apple fruits were collected, each replicate composed of five fruits. Average fruit weight was calculated and the product was multiplied with the total number of fruits/tree to estimate yielding.

Vegetative growth characteristics

Leaf petiole length (cm): it was measured along the petioles.

Number of internodes/m: it was calculated in the longitudinal meter of branches.

Leaf length (cm): it was measured from leaf tip to the point of petiole intersection along the midrib.

Leaf width (cm): it was measured at the widest part of the leaf.

Leaf area (cm²): it was measured by using 5 border disks (4 cm²) of each sample dry weight for both of border disks and leaves. Leaf area = dry weight of leaf x area border disks/ dry weight border disks (Bleasdale, 1987).

Fruit quality: Physical and chemical fruit traits of mutated plants were estimated as follows:

Physical fruit properties of mutated plants

Fruit weight: It was determined by weighing the 10 fruit samples. Then the average fruit weight was calculated in grams.

Fruit volume: The average fruit volume in cm³ was measured using a graduated cylinder.

Fruit height: The average fruit length was calculated in centimeter.

Fruit diameter (cm): The average fruit diameter was calculated in centimeter.

Fruit pulp thickness: It was determined by using caliper.

Chemical fruit properties of mutated plants

Total soluble solids (T.S.S.): A random sample of 10 fruits from each experimental plot at full ripe stage was taken to determine the percentage of soluble solids content by using hand refractometer.

Total titratable acidity (T.T.A.): A random sample of 100g of fruit at full ripe stage from each experimental plot was taken to determine T.T.A. of juice by titration with 0.1N Na OH (Sodium hydroxide) solution using phenol phthalein indicator, according to the method described in **A. O. A.C. (1990)**.

Statistical analysis and experimental design: The experiment was arranged in a randomized complete block design (RCBD) each treatment contained three replicates. Data were recorded after two years and subjected to analysis of variance (ANOVA) according to Snedecor and Cochran, (1982) by using MSTAT C as follow:

The experimental design: Data were analyzed as randomized complete block design (RCBD) one factor in a simple experiment. The differences between treatment means were tested using Least Significant Difference (L.S.D.) at 5% level.

Relationship and correlation coefficient based on phenotypic dendrogram: All data obtained were tabulated and statistically analyzed according to Snedecor and Cochran (1980). Statics program of phonetic and chemical relationships among populations were evaluated by cluster analysis from the matrix of average values of morphometric and chemical traits. Each genotype was considered as an operational taxonomic unit. The relationships among the genotypes were estimated by the Pearson coefficient (r). Distance values (D) were defined as follows: $D = 1 - r$, and the distance matrix was represented in a phenogram by the UPGMA clustering method Sneath and Sokal (1980).

RESULTS AND DISCUSSION

Effects of different concentration of some chemical mutagens (EMS-MH - Colchicine) on survival percentage after grafting are presented in Fig. (1).

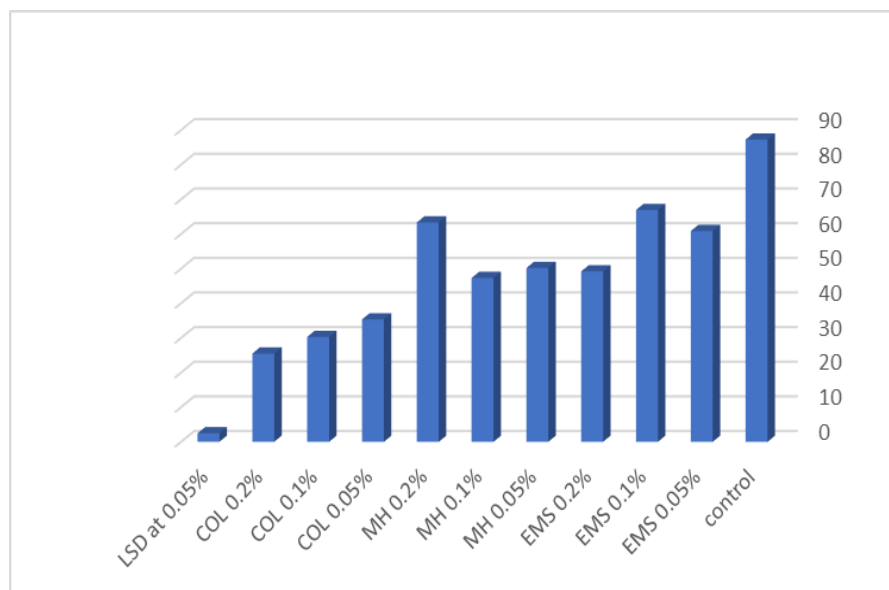


Fig. 1. Effect of chemical mutagens on Survival percentage after grafting.

Data in Fig. (1) revealed that survival percentage after grafting ranged from (25.47% to 87.25%). The control gave the highest significant survival percentage (87.25%) after grafting while colchicine 0.2% treatment gave the lowest (25.47%) in 2021 and 2022 seasons.

These results are similar to those obtained by Qingrong *et al* (2009) using colchicine treatment in pear at 0.4% (w/v) for 24, 48, or 72 h, who found that explants survival was significantly decreased by an increase in the length of colchicine treatment. In addition, Mahadevamma *et al* (2015) stated that survival percentage decreased with the increase in concentration of colchicine at 30 days after treatment. The survival percentage was the least in 1.00% colchicine treatment (48.33%) while it was 97.50% in untreated plants when LD₅₀ value was found to be 0.975%. The decrease in survival percentage with the increase in concentration of colchicine may be attributed to its toxic effects at the higher concentration of colchicine as observed earlier.

Effect of different concentration of some chemical mutagens (EMS - MH - colchicine) treatments on tree characteristic.

Data in Table (') illustrated the effect of chemical mutagen treatments on tree height. It was clear that there were significant differences among mutagen treatments. The highest significant value of average tree height was detected with colchicine mutagen at 0.2%; it was (174.11 cm.). While the lowest significant value of tree height was obtained by control; it was (130.00 cm) in the first season. The highest significant value of tree height was detected with colchicine's mutagen at 0.2% it was (183.11 cm). While the lowest significant value of tree height was obtained by MH at 0.1%; it was (159.89 cm) in the second season. It induces polyploidy by inhibiting the spindle fiber formation during cell division, chromosome gets multiplied but cell divisions do not occur. These results are similar to those obtained by Santosh *et al* (2010) using ethyl methane sulphonate (EMS); the LD50 was observed at 0.5% concentrations in papaya were recorded increased for plant height. In addition, Anil, *et al* (2013) reported that concentrations of 0.1% and 0.3% EMS treatment used significant variation in the morpho-metric characters such as height of the plant. Yogesh *et al* (2014) reported that maximum plant height (28.69 cm) was recorded in variety Pusa Giant and the minimum (18.78cm) in cultivar Pusa Dwarf. Maximum plant height (26.57 cm) was recorded under control which was followed by 2000 ppm, 1000 ppm and 3000 ppm of EMS solution. Sutaree, *et al* (2015) showed that, colchicine of 0.25% in the solution with seeds submerged for 24 h gave the highest percentage of tetraploid chromosomes in seedlings of tangerine citrus (63.64%). Colchicine significantly affected plant height.

Trunk diameter: Data in Table (') exhibited the effect of different mutagens on average trunk diameter. The highest significant value of average trunk diameter was achieved with mutagen a treatment of MH at 0.1%, as it raises values of 5.10 cm. and 5.31 cm, during the two seasons, respectively. While the lowest significant value of average trunk diameter was obtained by mutagen of colchicine at 0.05% treatment as it recorded 3.38 cm. and 3.97cm in the 1st and 2nd season respectively. These results are similar to those obtained by Santosh *et al* (2010) who reported that ethyl

methane sulphonate' (EMS) at 0.5% concentration increased trunk diameter in papaya. However, Anil Kumar *et al* (2013) reported that concentration of 0.1% and 0.3% EMS treatment were significantly affected the morphometric characters such as trunk diameter. Abd El-Latif *et al* (2018) studied that impact of some chemical substances (ethyl methane sulphonate (EMS) at 10, 20 and 30 ppm) and (colchicine at concentrations of 1%, 2% and 3%) on trunk diameter of *Carica papaya* cv. *solo* and found that EMS inhibited the process of cell division unlike addition of colchicine. This effect might be explained by the DNA-replication during mitosis by more damages, caused by EMS.

Table 1. Effect of mutation treatments (EMS - MH - Colchicine) in Anna apple cultivar on Tree height, Trunk diameter and Total yield of tree during two seasons of 2021 and 2022.

Treatment	Tree height (cm)		Trunk diameter (cm)		Total yield (kg)/tree	
	Season	Season	Season	Season	Season	Season
EMS 0.05%	174.00	178.00	4.66	5.00	4.49	5.48
EMS 0.1%	164.22	169.89	4.27	4.74	5.91	5.82
EMS 0.2%	170.44	173.78	3.76	4.34	5.21	5.58
MH 0.05%	167.11	167.11	4.44	4.79	4.46	5.39
MH 0.1%	158.67	159.89	5.10	5.31	4.79	5.66
MH 0.2%	165.33	169.89	3.87	4.37	4.67	5.39
Colchicine 0.05%	165.11	172.67	3.38	3.97	5.54	5.83
Colchicine 0.1%	166.44	168.89	4.57	4.87	4.76	5.43
Colchicine 0.2%	174.11	183.11	3.54	4.27	5.23	5.41
Control	130	175	4.2	5.2	3.34	3.46
LSD at 0.05%	10.83	9.06	0.47	0.50	0.50	0.51

EMS (Ethyl Methane Sulphonate), MH (Malic Hydrazine), COL (Colchicine)

Total yield per tree: Data presented in Table (1) revealed that all chemical mutagens treatments had significantly increased the total yield of Anna apple cv. than the control in both seasons. The maximum yield was recorded on Anna trees received by ethyl methane sulphonate (EMS)

followed by colchicine (COL) and then MH in descending order in both seasons.



EMS 0.1 %



Colchicine 0.05 %



EMS 0.2 %



MH 0.1 %

Fig. 2. Effect of chemical mutagens on total yield per tree of Anna apple.

The most pronounced increases in yield per tree were obtained with mutagen EMS 0.1 treatment (5.91 and 5.82 kg/tree) followed by COL 0.05 (5.54 and 5.83 kg/tree) compared with the control (3.34 and 3.46 kg/tree) in both seasons, respectively. The promotion on yield in response to application of these chemical mutagens (EMS - MH - colchicine) were mainly attributed to their beneficial effect on synthesizing changes which was accompanied with nutritional status of Anna trees in favor of producing more yield. These results are in agreement with those obtained by Anil, *et al* (2013) who stated that *Morus* species treated with EMS at 0.1% and 0.3% had affected in significantly maximum altering the total yield. In addition, Mahesh *et al* (2017) cleared that treated papaya with EMS at 0.50% caused maximum fruit yield per plant. Cline and Bakker (2016) pointed out that treated 'Empire'/M.26 apple trees with MH at 0.1% significantly increased total and marketable fruit yield and gave higher number of fruits per tree.

Effect of different concentration of some treatment with chemical mutagens (EMS - MH -Colchicine) on vegetative characteristics

Leaf petiole: Data in Table (2) exhibited the effect of chemical mutagen treatments on leaf petiole. There were significant differences among mutation treatments in average leaf petiole. The highest significant value (3.39 cm and 3.63 cm) of average leaf petiole was achieved by of MH mutagen at 0.1% during both seasons, respectively, while the lowest significant value was recorded with control (2.23 cm and 2.20 cm) of average leaf petiole in both seasons, respectively. These results are similar to those obtained by Mahesh, *et al* (2016), who observed that EMS 0.50% on papaya increased significantly the petiole length. However, Jome, *et al* (2019) characterize of the putative mutant of mango populations cv. Arka Puneet based on morphological, physiological and biochemical parameters. Morphological changes such as petiole length were observed and increased at 0.8 percent EMS treated mutants.

Number of internodes: Data in Table (2) showed the effect of different mutagen treatments on the average number of internodes. There were significant differences among tested treatments regarding number of internodes. The highest significant value of average internode number was detected by MH at 0.2% treatment; it was 41.04/m, while the lowest

significant average of internode number with colchicine at 0.1% was (35.74/m) in the first season.

Table 2. Effect of mutagen treatments (EMS - MH - Colchicine) in Anna apple cultivar on Leaf petiol, Number of internodes, Leaf length, Leaf width and Leaf area during 2021 and 2022 seasons.

Treatment	Leaf petiole (cm)		Number of internodes/m		Leaf length (cm)		Leaf width (cm)		Leaf area (cm ²)	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
EMS 0.05%	3.28	3.47	36.59	39.41	8.24	8.46	3.76	3.92	20.49	21.69
EMS 0.1%	3.15	3.38	40.74	44.30	8.12	8.39	3.70	3.98	20.12	22.03
EMS 0.2%	3.25	3.46	36.15	40.07	7.95	8.20	3.76	3.99	19.81	21.59
MH 0.05%	2.76	3.11	39.93	42.22	7.93	8.13	3.97	4.13	20.98	22.37
MH 0.1%	3.39	3.63	36.85	39.82	7.96	8.27	4.12	4.35	21.97	23.98
MH 0.2%	3.13	3.35	41.04	43.93	8.02	8.24	3.69	3.86	19.69	21.31
Colchicine 0.05%	2.58	2.91	37.07	41.15	7.82	8.07	4.30	4.41	22.42	23.62
Colchicine 0.1%	2.81	2.92	35.74	39.00	8.31	8.44	5.18	4.69	28.71	26.49
Colchicine 0.2%	2.71	2.94	37.74	40.85	7.95	8.25	4.59	4.77	24.31	26.89
Control	2.23	2.20	40.00	37.00	8.16	7.56	4.10	4.30	22.12	21.63
LSD at 0.05%	0.35	0.29	3.92	4.08	NS	0.44	0.45	0.43	2.13	2.35

EMS (Ethyl Methane Sulphonate), MH (Malic Hydrazine). COL (Colchicine)

The highest value with EMS at 0.1% was (44.30/m) compared with lowest value by control (37/m) in the second season. Sukhjit and Rattan (2010) found that using the ethyl-methane sulfonate at 0.6% with rough

lemon (*Citrus jambhiri* L.), number of days taken for seedling emergence increased with increasing dose of ethyl methane sulphonate. Seed germination, seedling height, internodal length, increased with increasing dose of ethyl methane sulphonate. In addition, Sukhjit (2015) found that treatment with ethyl methane sulfonate (EMS) at 0.3% on lemon (*Citrus jambhiri* L.) increased number of buds and internode length. Their study would be beneficial to induce desirable variations in plant growth characteristics of rough lemon by the use of mutagen treatment. However, Mei, *et al* (2017) found that treated with colchicine at 0.02% on rootstock of ‘Ruby Roman’ ‘Kober 5BB’ [‘5BB (2×)’], (*V. berlandieri* × *V. riparia*, decreased internode length.

Leaf length (cm): Average leaf length was influenced by the dose of these mutagens Table (2). The highest value (8.31 cm.) was recorded by colchicine’s 0.1% in the first season. On the other hand, the lowest leaf length (7.82 cm) was detected by colchicine’s 0.05%. While the highest value (8.46 cm) was recorded by EMS 0.05% while the control gave the lowest value (7.56 cm) in the second season. The presence of EMS inhibited the process of cell division; this effect might be explained with affected DNA-replication during mitosis by more damages. These results are similar to those obtained by Sutaree, *et al* (2015) who reported that colchicine’s treatment at 0.25% gave the highest percentages of significantly leaf length in citrus tangerine. In addition, Mahesh, *et al* (2016) indicated that in papaya treated with EMS 0.50%, a significant high percentage in leaf length was observed compared with control. In addition, Jome, *et al* (2019) characterized the putative mutant populations of mango cv. Arka Puneet based on morphological parameters changes such as leaf length, leaf width, an obvious increase was recorded with EMS mutagen at 0.8%.

Leaf width (cm): The effect of different mutation treatments on average leaf width is presented in Table (2). Highest significant value of average leaf width was achieved by colchicine 0.1% treatment (5.18 cm), while the lowest significant value of average leaf width was obtained by MH 0.2% (3.69 cm) in the first season, while the highest value with colchicine 0.2% (4.77cm) and lowest with MH 0.2% (3.86 cm) as compared with control in the second season. These results are similar to those obtained

by Sutaree, *et al* (2015) that showed that concentration of colchicine 0.2% for 12 h gave the highest value in leaf width (1.15 cm) compared with control. However, Jome, *et al* (2019) characterized the putative mutant populations of mango cv. Arka Puneet based on morphological parameters changes such as leaf length, leaf width and observed increase of these measurements with EMS mutagen at 0.8%.

Leaf area: Data in Table (2) showed that significant differences were observed with leaf area as colchicine's 0.1% treatment recorded the highest value (28.71 cm²) of average leaf area mean while MH 0.2% treatment recorded the lowest significant value (19.69 cm²) in the first season. On the other hand, colchicine's 0.2% gained the highest significant value of average leaf area (26.89 cm²). However, MH 0.2% scored the lowest significant value (21.31 cm²) compared with control in the second season. In this respect, Theiler (1991) found that cherry leaf area increased after colchicine treated plants than the untreated ones. Moreover, Bakry and Faten (2002) found that in papaya EMS at 200 ppm followed by 100 ppm gave the lowest value of leaf area at both of the studied seasons. However, Sharif, *et al* (2013) found that leaf area increased with colchicine's (0.10%) application. Leaf area was almost double in colchicine induced plants than their respective diploids. Maximum leaf area (19.84 cm²) was observed in tetraploid of Brazilian Sour orange (19.84 cm²) followed by (19.38 cm²) in *Sacaton citrumelo*. However, each rootstock showed the highest leaf area in tetraploid. On the other hand, Sutaree *et al* (2015) showed that colchicine's compound severely and significantly affected leaf area of *citrus tangerine*. Colchicine significantly affected leaf area when *citrus tangerine* buds submerged in 25% colchicine's solution for 24 h gave the highest percentage of tetraploid chromosomes (63.64%).

Effect of different concentration of some chemical mutagens (EMS - MH -Colchicine) on Physical fruit properties

Fruit weight (g): Data in Table (3) and Fig (3) indicate that all colchicine treatments showed the highest significant values (118.71, 101.80 and 101.60 g.) regarding average fruit weight with all of the tested concentrations (0.05%, 0.1% and 0.2% respectively) in first and the same trend in second season.

Table 3. Effect of mutagen treatments (EMS - MH -Colchicine) on Fruit weight, Fruit volume, Fruit length, Fruit diameter and Fruit pulp thickness in Anna apple cultivar during 2021 and 2022 seasons.

Treatment	Fruit weight (g)		Fruit volume (cm ³)		Fruit length (cm)		Fruit diameter (cm)		Fruit pulp thickness (cm)	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
EMS 0.05%	92.95	83.37	121.26	101.74	5.32	5.22	5.77	5.47	2.04	1.97
EMS 0.1%	99.91	86.68	128.04	105.31	6.24	5.93	5.95	5.62	1.91	1.86
EMS 0.2%	90.54	88.32	121.63	110.19	5.86	5.70	5.89	5.72	2.08	2.05
MH 0.05%	93.65	86.81	124.63	114.78	6.46	6.14	6.17	5.69	2.05	2.16
MH 0.1%	90.53	86.67	116.15	110.01	6.17	6.21	5.94	5.80	2.08	2.02
MH 0.2%	90.63	90.35	121.00	112.37	5.82	5.75	5.91	5.65	1.94	1.84
Colchicine 0.05%	118.71	112.18	140.06	133.44	5.95	5.77	5.80	5.61	1.97	2.03
Colchicine 0.1%	101.80	115.69	139.78	140.93	6.56	5.75	5.94	5.75	2.10	2.11
Colchicine 0.2	101.60	96.41	122.19	119.96	5.91	5.54	5.79	5.54	1.96	1.90
Control	89.80	88.40	100.00	98.17	5.62	5.23	5.55	5.25	1.63	1.36
LSD at 0.05%	6.23	5.45	6.44	6.82	0.38	0.63	0.29	0.38	0.27	0.19

EMS (Ethyl Methane Sulphonate), MH (Malic Hydrazine). COL (Colchicine).

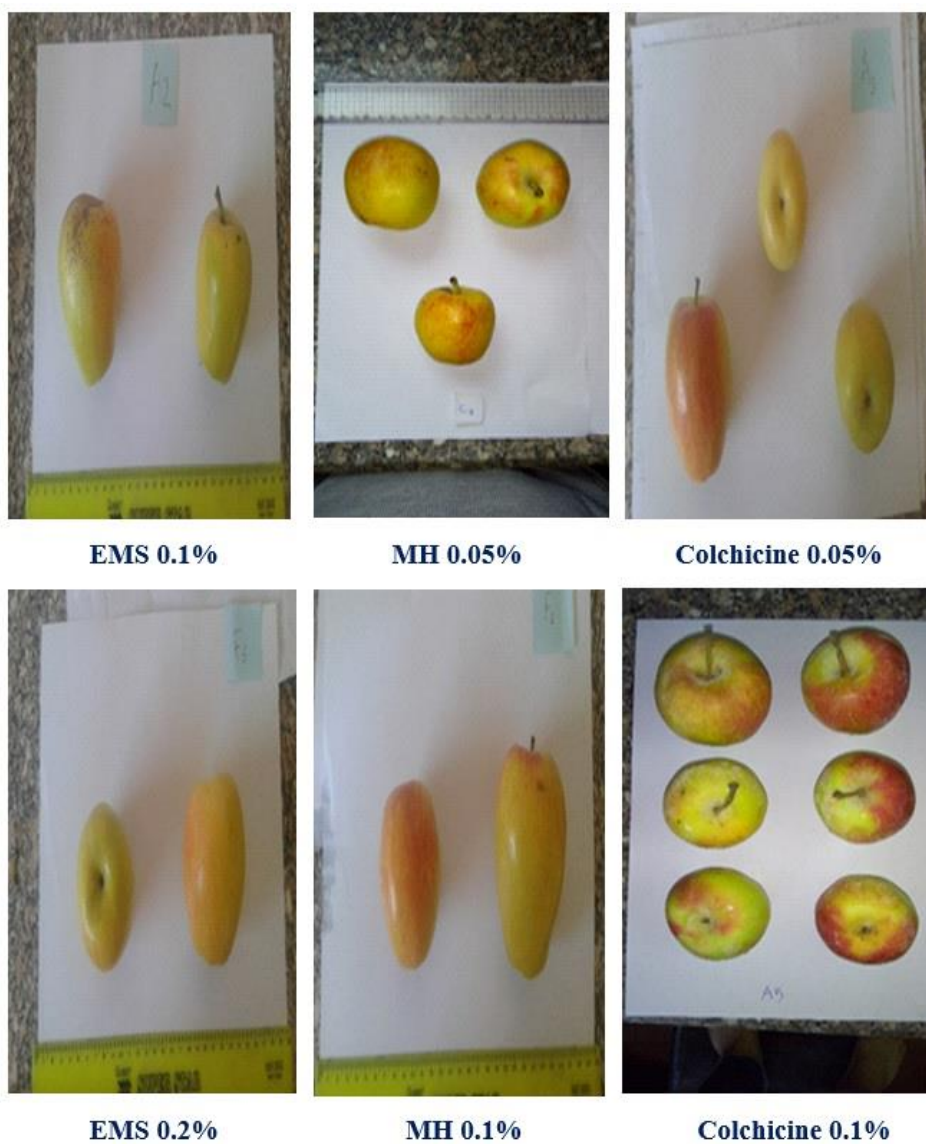


Fig. 3. Effect of chemical mutagens on physical fruit characteristics.

On the other hand, intermediate values of average fruit weight were shown by control (89.80 g.) in the first season. Meanwhile, EMS at 0.05% almost reveals the lowest significant values of average fruit weight (83.37 g) compared with control in the second season. These results are similar to those obtained by Mahesh (2017) who revealed that fruit attributes were effectively improved by seed treatment with ethyl methane sulphonate (EMS). EMS treatment 0.50% had fruits with minimum central cavity and maximum fruit weight as compared to the control.

Fruit volume (cm³): Data in Table (3) showed that the highest significant average value of fruit volume was achieved with mutagen of colchicine 0.05% (140.06 cm³) in the first season and colchicine 0.1% (140.93 cm³) in the second season, while the lowest significant value of fruit volume was obtained with control (100 cm³ and 98.17 respectively) in the both seasons. These results are similar to those obtained by Pusdekar and Pusdekar (2009) who reported that malic hydrazine at 1500 ppm with papaya was effective with fruit volume.

Fruit length (cm): Regarding fruit length, colchicine 0.1% and MH 0.05% scored the highest value (6.56 cm - 6.46 cm) in the first season, and MH 0.05% - MH 0.1% gave the highest fruit length in the second seasons, respectively. However, EMS at 0.05% showed the lowest value (5.32cm and 5.22 cm) compared with control which gave (5.62 - 5.23) in the first season and second seasons, respectively (Table 3). These results are similar to those obtained by Bakry and Fatten (2002) who found that EMS at 200 ppm caused significant increase in fruit length of papaya followed by EMS at 100 ppm; the control treatment gave the lowest values in this respect. However, Mahesh (2017) revealed that fruit attributes were effectively improved by seed treatment with Ethyl Methane Sulphonate (EMS). EMS treatment at 0.50 % had fruits with minimum central cavity, maximum fruit length.

Fruit diameter (cm): Data in Table (3) showed that the highest significant average value of fruit diameter was achieved with mutagen of MH 0.05% which gave the highest value (6.17 cm) in the first season and MH 0.1% (5.80 cm) in the second season, while the lowest significant value of fruit diameter was with control (5.55 cm and 5.25 cm, respectively) in the

both seasons. MH caused foliar phytotoxicity and damage to the apical meristem and cell division; these results are similar to those obtained by Bakry and Faten (2002) who found that EMS at (200 and 100 ppm) and colchicine at 1%, 0.5% caused increasing fruit diameter of papaya in both of the studied seasons.

Fruit pulp thickness (cm): Regarding fruit pulp thickness, colchicine's 0.1% (2.10 cm) in the first season and MH 0.05% (2.16 cm) in the second season scored the highest value. However, EMS at 0.1% and MH at 0.2% showed the lowest value (1.91 cm and 1.84 cm) in the first and second seasons, respectively (Table 3). While the lowest significant values of fruit pulp thickness was detected with control (1.63cm and 1.36 cm respectively) in the both seasons. These results are in accordance with Mahesh (2017) who reported that treatment of papaya with ethyl methane sulphonate (EMS) 0.50% had fruits with maximum pulp thickness compared with control which had minimum pulp thickness.

Effect of different concentration of some chemical mutagens (EMS - MH -Colchicine) on treatment chemical fruit properties

Titrateable acidity%: Results in Table (4) showed that the highest significant value of titrateable acidity was achieved by mutagen of colchicine at 0.1% (0.562% and 0.587%) in the first and second seasons, respectively, as compared with control which gave the lowest significant value of titrateable acidity by EMS at 0.05% (0.438% and 0.434% respectively) in both seasons. These results are similar to those obtained by Pusdekar and Pusdekar (2009) who mentioned that acidity content of papaya fruits was significantly lower (0.12- 0.15%) when treated with MH at 200,400 and 600 ppm as compared with control.

Total soluble solids (T.S.S): Data in Table (4) showed that the highest significant value of total soluble solids (T.S.S) was with MH at 0.05% (13.94% and 13.90%) in the first and second seasons, respectively, while the lowest significant value of total soluble solids (T.S.S) was obtained by control (11.83%) in the first season and colchicine 0.1% (12.12%) in the second season. These results are in accordance with Bakry and Faten (2002) who found that treated papaya with EMS at 200 or 100 ppm caused significant increase in T.S.S as compared with control.

Moreover, Mahesh (2017) stated that seeds of papaya treated with EMS at 0.5% had significant maximum T.S.S in both of the studied seasons.

Table 4. Effect of mutagens treatments (EMS - MH - Colchicine) in Anna apple cultivar on Titratable acidity and T.S.S during 2021 and 2022 seasons.

Treatment	Titratable acidity%		T.S.S%	
	Season 1	Season 2	Season 1	Season 2
EMS 0.05%	0.438	0.434	12.57	12.48
EMS 0.1%	0.540	0.530	13.68	13.47
EMS 0.2%	0.467	0.445	12.71	12.42
MH 0.05%	0.559	0.542	13.94	13.90
MH 0.1%	0.450	0.465	13.04	13.05
MH 0.2%	0.529	0.449	12.54	12.48
Colchicine 0.05%	0.533	0.535	12.54	12.59
Colchicine 0.1%	0.562	0.587	12.11	12.12
Colchicine 0.2%	0.514	0.520	13.08	13.02
Control	0.451	0.487	11.83	12.50
LSD at 0.05%	0.0571	0.051	0.69	0.50

EMS (Ethyl Methane Sulphonate), MH (Malic Hydrazine). COL (Colchicine).

Assessment of mutagenic substance relationship as revealed by variable characteristics:

Various methods are used for assessment of diversity and genetic relationship among the accessions. Morphological markers are widely used for estimation of diversity and characterization in some evaluation studies although it is affected by environment (Elameen, *et al* 2010). The morphological marker is known for its coverage in study of agronomic traits in addition to convenience. Further the technique is relatively cheaper and easier to conduct. Many previous studies (Koehler, *et al* 2003; Campos, *et al* 2005) reported that molecular and morphological diversity is independent and rather complementary to genetic diversity. Therefore, an assessment of mutagenic substance based on phenotypic characteristics was performed to determine the relationship among tested substances and its concentrations.

Relationship and correlation coefficient based on phenotypic dendrogram:

Regarding phenotypic dendrogram, morphological, physical and chemical fruit characteristics were used to generate phenotypic based dendrogram (Fig.4). In the first season, the obtained dendrogram was divided into two cluster; the first one, contains colchicine 0.05% and colchicine 0.1%, this reflects a similar effect of colchicine at the two concentrations on tested phenotype characteristics. On the other hand, the second was divided into two sub-clusters; one of them included EMS 0.1%, MH 0.05% and colchicine 0.2%. Meanwhile, the second sub-cluster was separated into two groups; one of these groups had only MH 0.1%, however, the second group collected EMS 0.2%, MH 0.2% and EMS 0.05%.

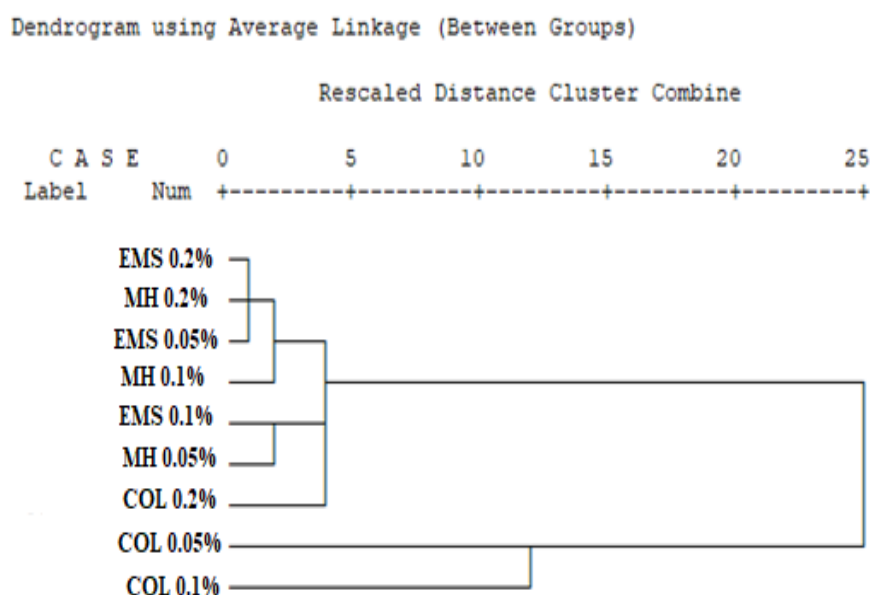


Fig. 4. UPGMA dendrogram based on phenotypic characteristics in the first season.

Three clusters were observed in the second season phenotypic dendrogram (Fig 5). As the dendrogram was isolated colchicine 0.05% and colchicine 0.1% in one cluster. However, the second cluster joined colchicine 0.2%. The third cluster was divided into two groups; the first group included EMS 0.05%. The second group binds EMS 0.1%, EMS 0.2%, MH 0.05%, MH 0.1% and MH 0.2%.

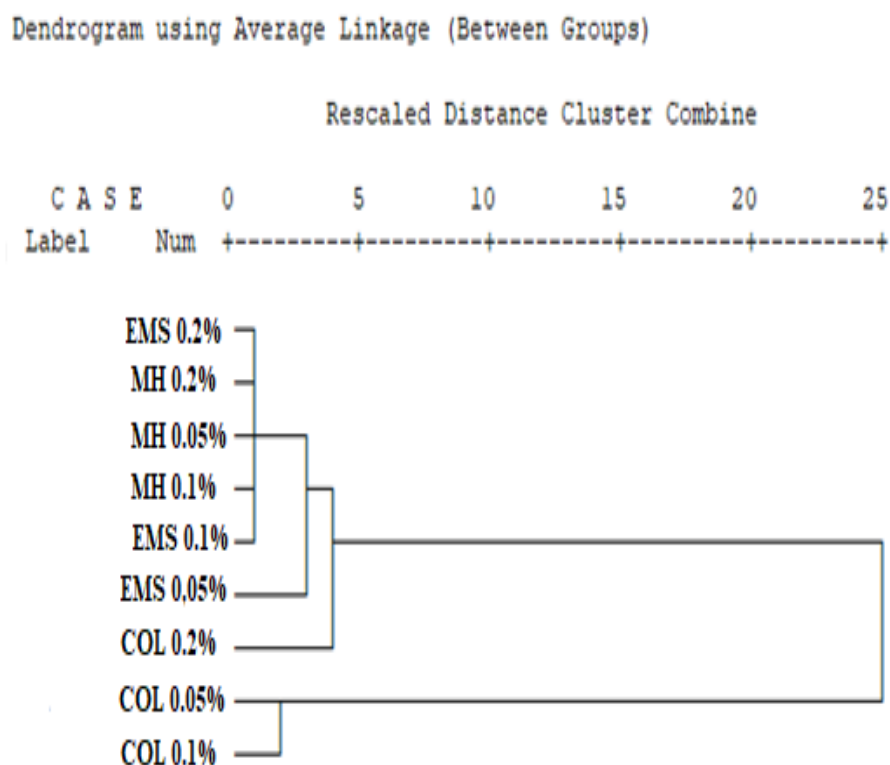


Fig. 5. UPGMA dendrogram based on phenotypic characteristics in the second season.

A combined data of the first and second seasons were employed to generate dendrogram based on phenotypic characteristics (Fig 6). The combined dendrogram reveals a great deviation relative to the dendrograms of both (٢٠٢١ and ٢٠٢٢) season.

Dendrogram using Average Linkage (Between Groups)

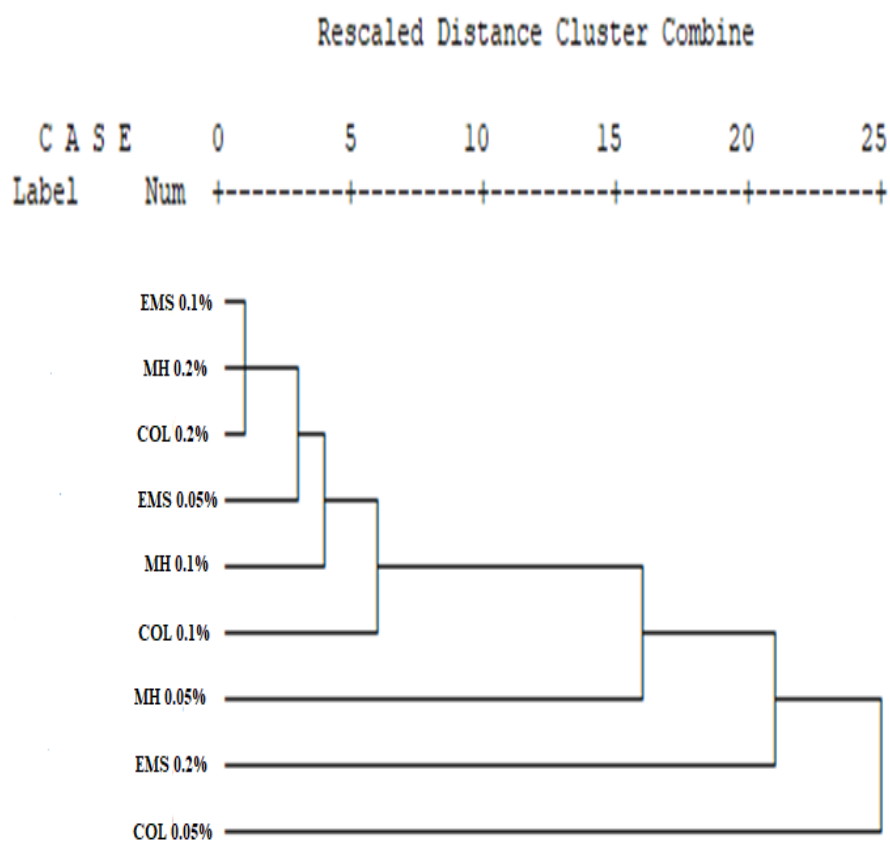


Fig. 6. UPGMA dendrogram based on phenotypic characteristics (combined the first and second seasons).

As it was classified into six clusters, the first one fastens colchicine 0.05%, EMS 0.2% was in the second cluster, however, MH 0.05% was in the third cluster, the fourth cluster included colchicine 0.1%, meanwhile, MH 0.1% was shown in the fifth cluster. On the other hand, the sixth cluster was divided into two groups; the first group bundle EMS 0.1%, MH 0.2% and colchicine 0.2%, while the second group contains only EMS 0.05%. Phenotypic characters revealed the existence of diverse relationship within tested mutagen in spite of genotypes exhibiting similar phenotypic qualitative characters. This could be due to mode of action of investigated mutagen against apple genetic model. In this respect, assessment of phenotypic traits on pummelo from uncontrolled field survey had shown that the environmental factors affected to the tune of 40% (Paudyal and Haq 2008). Since this is first of this kind in evaluation of phenotypes for mentioned genotype, further work on molecular analysis is essential for a clearer picture of mutagen diversity and relationship. It could be concluded that wide variation existed among the tested genotypes with respect to quantitative characteristics.

CONCLUSION

From present study, the effect of chemical substances (EMS, MH and colchicine) with concentration at (0.05, 0.1 and 0.2%) on survival percentage after grafting, vegetative growth, fruit quality and total yield of tree. The chemical substances gave the best results in vegetative growth, fruit quality and total yield of tree compared with control. However, control gave highest value on survival percentage after grafting. Relationship and correlation coefficient based on phenotypic dendrograms showed that colchicine 0.05% and colchicine 0.1%, which reflect a similar effect of colchicine at the two concentrations, on the tested phenotype characteristics.

REFERENCES

- A.O.A.C. (1990).** Association of Official of Agriculture Chemists, Official Methods of Analysis. 11th Ed. Published by the A.O.A.C. Washington D. C., U.S.A.
- Abd El-Latif, F. M., S. F. El-Gioushy, S. E. Islam and A. Z. Tahany (2018).** Impact of Papaya Seed Soaking in Different BA, Colchicine and EMS Solutions on Germination, Growth and Chromosomal Behavior. Asian Journal of Biotechnology and Genetic Engineering 1(1): 1-17
- Anil Kumar, H. V., T. S. Muralidhar, A. Sourav, J. Manas and J. Munira (2013).** EMS Induced Morphometric Biomass and Phytochemical Variations in *Morus* Species (Genotype RFS135). American Journal of Experimental Agriculture 3 (1): 43-55.
- Bakry, A. E. and H. M. I. Faten (2002).** Pre sowing treatments of papaya seeds as influenced by some chemicals and irradiation on germination, growth, flowering, sex expression and fruit quality. Inter. Conf.Hrot. Sci., 10-12 Sept, Kafr El-Shikh, Tanta Univ., Egypt.
- Bleasdale, L.K.A. (1987).** Plant physiology in relation to horticulture ELBS edition. The Macmillan press LTD. Associated companies in Delhi, Hong Kong Lagos and Singapore.
- Campos, E. T., M. A. G. Espinosa, M. L. Warburton, A. S. Varela and Á.V. Monter (2005).** "Characterization of madarin (CITRUS SPP.) Using morphological and AFLP markers." Formato Documento Electrónico, 30 (11): 687-693.
- Cline, J. A. and C. J. Bakker (2016).** Prohexadione-calcium, ethephon, trinexapac-ethyl, and maleic hydrazide reduce extension shoot growth of apple. Canadian. J. of Plant Science 97 (3): 457- 465
- Elameen, A., A. Larsen, S. K. Sonja, S. Fjellheim, S. Msolla, E. Masumba and O. A. Rognli (2010).** "Phenotypic diversity of plant morphological and root descriptor traits within a sweet potato, *Ipomoea batatas* (L.) Lam., germplasm collection from Tanzania." Genetic Resources and Crop Evolution.58 (3): 397-407.
- Jome, R., M. R. Dinesh, S. Murugan and K. S. Shivashankara (2019).** Evaluation and characterization of EMS derived mutant populations in mango. Scientia Horticulture 254:55-60.
- Koehler-Santos, P., A. L. C. Dornelles and L. Bd. Freitas (2003).** "Characterization of mandarin citrus germplasm from Southern Brazil by morphological and molecular analyses." Pesquisa Agropecuttria Brasileira Pesq. agropec. Bras. 38 (7): 797-806.
- Mahadevamma, M., M. R. Dinesh, R. V. Kumari and T. H. Shankarappa (2015).** Optimization of gamma irradiation and colchicine on induction of variability in Coorg honey dew papaya (*Carica papaya* L.).Environment, Ecology 33 (1):513-516
- Mahesh, K., P. Dk. G. Satya, and R. Sanjeev (2016).** Effect of seed treatment by ethyl methane sulphonate on growth, flowering and yield of papaya cv. Pusa Dwarf. Journal of Hill Agriculture 7(1): 64-67.

- Mahesh, K., K. Mukesh and C. Veena (2017).** Effect of seed treatment by ethyl methane sulphonate (EMS) on fruit quality of papaya (*Carica papaya* L.)cv. Pusa Dwarf International Journal of Applied Chemistry Volume 13, Number 1 pp. 145-150
- Mei Gao, T., K. K. Ayako, N. Shinichi, M. Kenichi and M. Hino (2017).**Vegetative Growth and Fruit Quality of ‘Ruby Roman’ Grapevines Grafted on Two Species of Rootstock and Their Tetrapod's. Horticulture Journal 86 (2): 171–182
- Mohamed, Z., W. S. Ho,S. L. Pang andF. B. Ahmad (2014).** EMS induced mutagenesis and DNA polymorphism assessment through ISSR markers in *Neolamarckia cadamba* (kelampayan) and *Leucaena Leucocephala* (petaibelalang), Eur. J. Exp. Biol 4(4):156-163.
- Murali, K.M., V. Jeevanandam, J. Shye and R. Srinivasan (2013).** Impact of colchicine treatment on sorghum bicolor BT 623, Mol. Plant Breed. 4 (15) 128-135.
- Paudyal, K. and N. Haq (2008).** "Variation of pomelo {*Citrus grandis* (L.) Osbeck} in Nepal and participatory selection of strains for further improvement." Agroforestry Systems 72(3): 195-204.
- Pusdekar, G. G. and M. G. Pusdekar (2009).** Effect of plant growth regulators on flowering and fruit quality in papaya (*Caricapapaya* L.) cv. CO-2. Journal article: New Agriculturist , 20 (1):107-110.
- Qingrong, S., S. Hongyan, L. Linguang and I. B. Richard (2009).** *In vitro* colchicine-induced polyploid plantlet production and regeneration from leaf explants of the diploid pear (*Pyruscommunis* L.) cultivar, ‘Fertility’ Journal of Horticultural Science & Biotechnology 84 (5) 548–552.
- Santosh, L. C., M. R. Dinesh and A. Rekha (2010).** Mutagenic studies in papaya (*Carica papaya* L.) Acta Horticulturae 851(851):109-112 ·
- Sharif, N., M.J.jaskaniandN. Memon (2013).** Responses of citrus rootstock ovules to colchicine applications, In vitro International Journal of Agricultural Technology Vol. 9(1): 201-209.
- Sneath, P. H. A. and R. R. Sokal (1980).** Numerical taxonomy: the principles and practice of numerical classification. Freeman WH. San Francisco, California, USA; 1973.
- Snedecor, G. W. and W. G. Cochran (1980).** Statistical methods. 7th Ed, " The Iowa state Univ. press, Press Amers., Iowa, U.S.A. 1980;507. 20.
- Snedecor, G. W. and W. G. Cochran (1982).** Statistical Methods. 6thed. TheIowa State Univ., Press, Iowa, U. S.A.p.593.
- Sukhjit, K. (2015).** Effect of mutagens on regeneration and growth of in vitro grown epicotyl segments of rough lemon seedlings (*Citrusjambhiri* Lush.) Journal of Applied and Natural Science, 7 (1): 459 - 465.
- Sukhjit, K. and H. S. Rattan (2010).** Effect of mutagens on *in vitro* seed germination and growth of rough lemon (*Citrusjambhiri*) seedlings. Indian Journal of Agricultural Sciences 80 (9): 773–6.

- Sutaree, S., S. Suphasit and W. Nattapong (2015). *In vivo* induction of tetraploid in tangerine citrus plants (*Citrus reticulata* Blanco) with the use of colchicines. Pakistan Journal of Biological Sciences 18(1): 37-41
- Theiler, H. R. (1991). Induction of dwarf F-121 cherry rootstocks by *in vitro* mutagenesis. Acta Horticulture 280:367-374.
- Tomas, G., M. Merten, D. A. Stavreva and S. Ingo (2000). Maleic hydrazine induces genotoxic effects but no DNA damage detectable by the Comet assay in tobacco and field beans. Mutagenesis, 15 (5): 385-389.
- Yogesh, P. R., L. Sahab, K. Mahesh, S. Gopal, K. Anil and S.U. Syed (2014). Studies on Effect of EMS (Ethyl Methane sulphonate) on Papaya (*Carica papaya* L) Seeds under *in-vitro* Culture. International Journal of Agriculture and Food Science Technology. 5(4): 315-324.

استخدام المطفرات الكيميائية لإستنباط تراكيب وراثية جديدة ذات جودة محسنة

لثمار صنف التفاح آنا

نظمى عبدالحميد^١؛ محمود مقلد^١؛ نهلة عوض^٢ و مى أنور^٢

١- قسم البساتين، كلية الزراعة، جامعة عين شمس، القاهرة، مصر

٢- قسم بحوث تربية أشجار الفاكهة ونباتات الزينة والنباتات الخشبية، معهد بحوث البساتين، مركز البحوث

الزراعية، الجيزة، مصر

أُجريت تجربة حقليّة خلال موسمي ٢٠٢١ و ٢٠٢٢ في مزرعة خاصّة على صنف التفاح آنا (*Malus domestica*). بهدف دراسة تأثير المعاملة بكل من (إيثيل ميثان سلفونات (EMS)، وماليك هيدرازين (MH)، والكولشيسين) بتركيزات (٠,٠٥، ٠,١، ٠,٢، ٠,٥) % لمدة ٢٤ ساعة، مع التطعيم على أصل التفاح (*Malus*)، و تأثير ذلك على نسبة البقاء بعد التطعيم، والنمو الخضري، وخصائص جودة الثمار، والمحصول الكلي للشجرة. أظهرت النتائج أن هذه المعاملات قد أعطت أفضل النتائج في النمو الخضري، وجودة الثمار، والمحصول الكلي للشجرة مقارنةً بالكنترول وفي المقابل، أعطى الكنترول أعلى قيمة في نسبة البقاء بعد التطعيم. كما أظهرت دراسات العلاقة ومعامل الارتباط، المستندة إلى مخططات الشجرة الظاهرية، أن تركيز الكولشيسين ٠,٥ % و ٠,١ % على التوالي، يعكس تأثيراً مشابهاً لهما على خصائص النمط الظاهري المختبر.

المجلة المصرية لتربية النبات ٢٩ (٢): ١٥٣- ١٧٦ (٢٠٢٥)