Propagation of Gamma – Irradiated and Salinity Tolerant Plants of *Mirabilis jalapa*,L. *in vitro* and *ex vitro* Establishment

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ABSTRACT

Micropropagation of *Mirabilis jalapa* , L. was achieved using a single node culture technique. Whereas, nodal explants of *in vivo* plants were sterilized and successfully cultured on MS medium supplemented with various levels of both BAP and NAA on three stages. In the initiation stage, the medium consisted of Murashinge and Skoog medium, supplemented with 30 g/l of sucrose and the auxin NAA at 0.0, 0.1 and 0.2 mg/l in combination with the cytokinin BAP at 0.0, 0.3 and 0. 6 mg/l. In the second stage (multiplication) the neoformed shoots on initiation medium were subcultured on the same medium used for stage I. Stage III for rooting of neoformed shoots was accomplished on MS medium augmented with NAA at 0.2 mg/l. Mature nodal segment was established aseptically and then multiplied *in vitro* and returned to *ex vitro* through several serial stages. During the initiation stage the increase of NAA, caused a decrease in the number of leaflets and grown buds /propagule against an increase in the number of roots/propagule .In the meantime, the increase in BAP was associated with a decrease in shoot length and the number of root/propagule, while the number of grown bud increased .In the multiplication stage the increase of NAA caused decrease in the number of grown buds against increase in the number of roots /propagule, meanwhile the increase of BAP lead to decrease in all traits except an increase in the number of grown buds/propagule.
Key words: *Mirabilis Jalapa* , L., **Micropropagation, plant tissue culture, initiation, multiplication, rhizogensis, establishment, In vitro, *in vivo* ,gamma rays ,salinity.**

INTRODUCTION

Mirabilis jalapa is conventionally propagated by seeds and no reports have been published about traditional techniques of vegetative propagation ,and this will be tedious when carry out on a large scale .Yet, no publication regarding *in vitro* propagation of this species ,unless- otherwise stated elsewhere. Nevertheless, tissue culture could greatly increase the normal multiplication rate of such selected plants inherited with desirable traits in short time (Malavasi and Predieri, 1988). Hence, can provide a source of clean material which has become increasingly important owing to its significance for horticultural industry or for pharmaceutical one as a medical plant, too. Furthermore, achievement such *in vitro* propagation could provide such preservation, also. Such rare articles have been

published regarding tissue culture of *Mirabilis jalapa*; where Michele *et al.* (2007) reported a protocol for *in vitro* regeneration and transformation system for *Mirabilis jalapa* (Nyctaginaceae). Among the types of explants and the different media tested, consistent shoot regeneration was obtained only from nodal segments grown in a regeneration medium consisting of MS medium (1962) supplemented with 2 mg/ l BA , 2 mg/l ZEA and 1 mg/ l IBA. Then, much of the literature will be relevant to some other ornamentals. For instance, Tamer and Mavituna (1997) reported that callus, suspension and immobilized cell cultures of *Mirabilis jalapa* synthesized both extracellular and intracellular proteinase. When compared with the leaves of the original plant, the proteolytic enzyme activity was 54- and 36- fold higher in cell suspension and callus cultures, respectively. Some of the sucrose was converted to glucose and fructose by the cultures, and all three sugars were consumed simultaneously. Suspension and immobilized cultures also produced ethanol during batch cultivation .Also, Gon *et al.* (2005) developed a protocol for *in vitro* culture and regeneration of *Bougainvillea glabra* . Whereas, MS medium + 3.0 mg /l + 0.2 mg 2,4-D/l+ 0.1 mg NAA/l ,was the best combination for callus induction where the induction and differentiation rates were 78.2 and 25.6%, respectively. Two types of shoots were observed, i.e the first type was directly induced from the bottom of the stem segment and the second type was from callus induced at the bottom of the stem segment. However, MS medium + 2.0 mg BA/l + 0.1 mg NAA/l was the best for clump shoot induction; where the rate of multiplication was 5.4, while an increase in BA and 2,4-D to 3.0 and 0.5-1.0 mg/ l, each in turn , resulted in leaf variation of the regenerated plants. Also, MS medium + 0.2 mg BA/l+ 0.5 mg GA_3 /l+ 0.1 mg NAA/l gave the best results for efficient seedling culture. Meanwhile, MS medium + 0.3 mg NAA/l was the best for root induction where rooting rate was 88.3%. Likewise, Dwivedi *et al.* (2000) found that treated rooted cuttings of *chrysanthemum* [*Dendranthema sp.*] with different doses of gamma rays (1.0,-2.0kr), demonstrated different types of morphological abnormalities in leaves and flowers. Chlorophyll variegation in leaves and flower colour mutations were the most prominent of the produced plants chimeric yellow ray florets were cultured in Murashige and Skoog medium(1962) supplemented with 0.2mg/l NAA, 1.0mg/l BAP [benzyladenine], 3% sucrose and 0.8% bactoagar at pH5.6. Regenerated plants were, successfully, transplanted to the field, where they flowered true to type flower colour.Also, Mandal *et al.* (2000) treated rooted cuttings of white flowered cv. "Purnima" and red flowered cv. "Colchi Bahar" of *Chrysanthemum morifolium* with gamma rays. Sectorial somatic mutations

in flower colour were detected for both the cultivars. Mutated ray florets (yellow colour for both cultivars) were cultured on agar-solidified Murashige & Skoog basal (1962) medium supplemented with sucrose and different combinations of BAP, kinetin and NAA. Direct shoot organogenesis was noticed within 2 weeks of culture initiation. The best regeneration was obtained on medium supplemented with 0.2 mg/l NAA and 0.5 mg/l BAP. Shoots regenerated from mutated ray florets were rooted *in vitro* and transferred to *in vivo*. These plants flowered true-to-type floret colour and shape. These isolated yellow mutants were maintained vegetative and have proved to be true-to-type in two successive generations, In the same year, Nagatomi *et al.* (2000) found that, in *Chrysanthemum morifolium,* the rate of flowers colour mutation was the highest in regenerants from petals, followed by those derived from buds and leaves. In combined irradiation and culture methods, chronic irradiation yielded a significantly higher flower colour mutation rate and a wider colour spectrum than acute irradiation. They, also, demonstrated that selection of a mutated coloured petal as explants under chronic irradiation promoted the induction of a wider colour spectrum on the regenerants. With acute irradiation, damage to the regenerated lines increased as the dose increased. Likewise, Kasumi *et al.* (2001) investigated the effects of gamma irradiation on cormel explants for inducing callus, somatic embryogenesis, and flower colour variations of the regenerated plants in *Gladiolus grandiflora* cvs. "Traveler and Topaz". The rate of somatic embryogenesis decreased as the exposure dosage was increased. The total dosages of 100-200 Gy caused a 50 percent reduction in the rate of callus formation and somatic embryogenesis. The flower colour variants occurred in cv. "Traveler "but not in "Topaz" one. The frequency of flower colour variants increased, resulting in the appearance of extremely deep colours and morphological variants when tissue culture was used in combination with gamma irradiation. Most flower colour variants were solid (i.e., nonsectorial-chimeric) variants. Their results showed that the application of tissue culture technique in combination with gamma irradiation could lead to a high frequency of variants, diversifying the germplasm pool for breeding of other vegetatively propagated ornamental plants.

Datta *et al.*(2005) investigated the effect of irradiation on *Chrysanthemum morifolium* cvs. "Flirt, Puja and Maghi" ,on their plant height and size of leaf and flower. Five solid flower colour/floret shape mutants with slight changes in ray floret morphology were detected and established. Khaing *et al.* (2007) reported that the shoot cuttings of *Gymnostachyum zeylanicum* were taken in order to subject them to

mutations by gamma irradiation and colchicine treatments. The treated plants had shorter, oval shaped and rounded-based leaves compared to the large, oblong and tapering-based leaves in control plant. They, also, showed early flowering and shortening of the flower stalk to 5-7 cm in length compared to the control (30-45 cm). However, all the dosages of gamma treatments resulted in retarded growth compare to the control plants. These plants showed dark green coloured leaves compared to the control ones.

Regarding, the effect of gamma radiation on the development of salt tolerant plants; Omar *et al.* (1988) investigated the effects of gamma rays and NaC1 on growth and cellular contents of soluble protein and nucleic acids of *Helianthus annuus.* The optimal caulogenesis was formed by stem segments cultured in MS medium enriched with 0.05 mg/l of 2, 4-dichloro phenoxyacetic (2,4-D) and 0.01 mg/l N-6 furfurylamino purine (kinetin). Regarding the radiation sensitivity, based on fresh weight changes, the presence of NaCl in the medium caused a significant reduction in callus fresh weight. In general, contents of protein soluble, carbohydrates and ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were increased at 2% NaC1 level. From viewpoint of agronomy, El -Halim *et al.* (1989) exposed the Egyptian wheat grains of cultivar GIZA-157 to 0, 2, 4, 8, 16, 32 and 64 K-rad gamma –radiation and germinated them in petri dishes containing 0, 2000 and 4000 ppm NaCl. They recorded increasing in germination rate, plant growth and yield characteristics after 2-8Kr seed treatment, whereas higher radiation doses decreased all the above mentioned characters .However, germination rate, plant growth and yield characteristics decreased with increasing salinity.

In addition, Yousef *et al.*, (2007) reported that *Alstroemeria* grown rhizome buds were cultured *in vitro* on MS basal medium (1962) with 3 different compositions of growth regulators (1, 0.2 mg /l NAA with 1 mg /1 BA and 0.2 mg/ l IAA with 1 mg /l BA. Cultures were incubated in 18± 2C° at 16 h photoperiod. Four subcultures of explants were done on the same fresh media with 3 weeks intervals. The results showed that *in vivo* rhizome bud produced the largest number of small rhizome and roots when the explants were cultured previously on medium containing 0.2

mg /l NAA with 1 mg 1/l BA. The aims of this study is to propagate *Mirabilis jalapa in vitro* via nodal cutting culture through initiation, multiplication ,rhizogenesis ,*ex vitro* and *in vitro* establishment.

MATERIALS AND METHODS

Initiation and maintenance of plant material (stage 0);the mother plant material obtained from irradiated seeds with 2.0Kr and treated with 50 mM NaCl, which germinated in pots of 30 cm diameter filled with peat moss and sand (2:1) and put in the plastic house . Nodal cuttings of 3 months-old - plants were cut and used to conduct culture experiments. Nodal cutting were surface sterilized initially by a brief passage through 80%(v/v) absolute alcohol followed by immersion for 15 minutes in 200ml of 1.5%(v/v) 'Chlorox' bleach containing one drop (0.1ml) of Tween 20 .The flasks were agitated, steadily, for 15 minutes manually and this process was repeated using three changes with sterilized distilled water, After decanting off the last change of water, a nodal cuttings were dissected from the explants in a laminar flow hood. Each explant was consisted of the lateral bud and small portion of stalk. These explants were cultured in glass jars containing 15ml nutrient medium for 35 days .The medium consisted of Murashinge and Skoog medium (1962), supplemented with 30 g/l of sucrose and the auxin NAA at three concentrations; 0.00 (nil), 0.1 and 0.2 mg/l in combination with the cytokinin BAP at three concentrations,0.0 (nil), 3 and 6 mg/l. This was referred to as the initiation medium (stage I). For proliferation or multiplication of shoots (stage II), the neoformed shoots on initiation medium were subcultured on the multiplication media which were MS medium supplement with NAA at three concentrations 0.00 (nil), 0.1 and 0.2 mg/l in combination with the cytokinin BAP at three concentrations 0.0(nil), 3 and 6 mg/l .For rooting of neoformed shoots (stage III), this stage was accomplished on MS medium augmented with NAA at 0.2 mg/l. Murashing and skoog (1962) medium was used in all studies. Media were prepared with MS medium *via* powder depending on the experiment .Stock solutions of plant growth regulators were prepared separately and stored in flasks in the cold room at (4C°).Sucrose concentration (3.0%,v/v) was added to the media at the time of preparation of the media.Purified agar (Agar –Agar technical) at 0.7% (w/v) was added to solidify the used media . For all experiments, media were adjusted to pH 5.7 using either 1.0M NaoH or 0.1M HCL prior to the addition of agar and before sterilization using a Seybold Wien pH meter. In all experiment's media were dispensed into glass jar using a syringe to dispense the nutrient solution and boiled agar per jar .All the autoclaving operations were carried out in an electric autoclave for 20 minutes at 1.1 Kg cm $^{-2}$ pressure and 121 **C °.** All instruments used for culture were autoclaved as above –mentioned. Subcultures instruments were immersed in 96 %(v/v) absolute alcohol. Culture jars were incubated in growth room at 25 C °, illuminated with 1.83

m (48 TDL) fluorescent lamps (Philips) located 40 cm above the culture jars, giving an average irradiance *ca.* 85 μ moles/m^2/s-1. These jars provided with a high total amount of light including a high proportion in the red wavelength which promote good leaf development. The following characters were determined per propagule :

1- Mean number of formed leaflets / propagule

2-Mean shoot length(cm)/ propagule

3-Mean number of formed grown buds propagule

4-Mean number of formed roots/ propagule

Illumination regimes were set at 16 h photoperiods. However, acclimatization of neoformed ramets *ex vitro* and establishment *in vivo,* the ramets (rooted shoots), in general, were washed out of solidified medium under running tap water, followed by immersing them into Rizolex-T50 WP(1g/l) [From Sumitomo Chemical Co. Ltd., Osak, Japan] fungicide for 30 sec. They were, then, transplanted *ex vitro* in small plastic pots (5x5), containing an autoclaved soil mixture of peatmoss and sand at (1:1); and finally placed under transparent plastic bags, to maintain high relative humidity, for hardening off. Twenty five days later , the plastic bags were perforated for gaseous exchange and continued for hardening .After two more weeks, the plastic bags were removed and the acclimatized plantlets were watered , as needed and fertilized , weekly, with N: P: K (2:1:1) to be ready for transplanting to the field as shown in Fig (1).

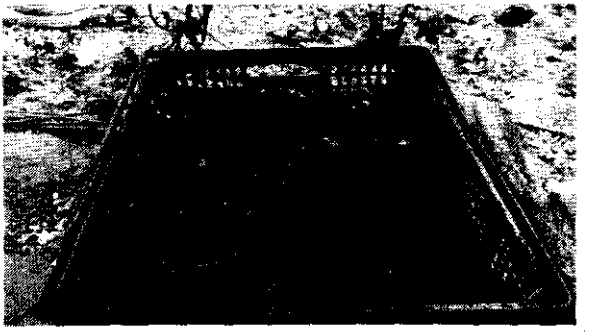


Fig.(1). Neoformed ramets of *Mirabilis jalapa* , L. plants grown after 2 weeks *ex vitro.*

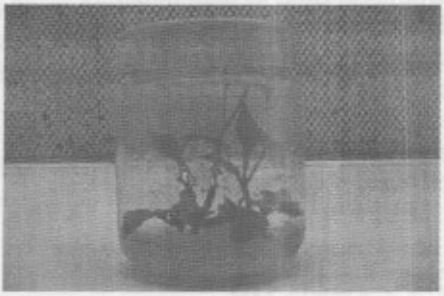
Experimental design

Complete randomized design was followed in all experiments. All the experiments were of factorial type. Data Collected and statistically analyzed according to Gomez and Gomez (1984) using SAS (Statistical Analysis System) computer program. Least significant different (LSD) was used to determine the significant difference between means . Probability levels of 1 and 5 present were taken ,generally ,for this respect .The significance levels were represented as follows : probability at 1% =**, 5% =* and not significant=NS.

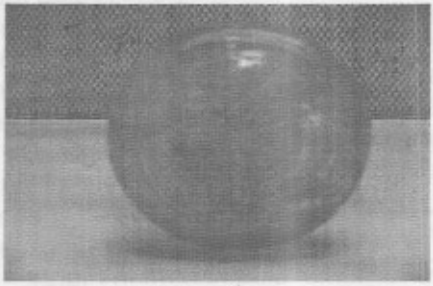
RESULTS AND DISCUSSIONS

As for initiation stage, results of Table (1) revealed that applied levels of NAA have exerted significant effects on the mean number of leaflets formed per propagule; meanwhile, BAP levels and their interaction with NAA levels was not significant. It is obvious that the mean value of the given trait decreased, significantly, with increasing NAA levels, especially at either 0.1 or 0.2 mg/l, meanwhile, its absence resulted in the highest mean value. This finding could be attributed to the role of auxins in organized tissue (such as nodal explants) being involved in the establishment and maintenance of polarity (bipolar growth) including leaflets and buds (George and Sherrington, 1984) and as shown in Fig(2).

Also, results of Table (1) exhibited that NAA levels have no significant effects on the mean shoot length (cm.)/propagule; meanwhile, BAP levels and their interactions exhibited significant effects on the given trait. However, as BAP level increased, the given trait decreased especially at the higher level (6.0mg/l). This finding could be attributed to the BAP mode of action on tissue culture growth where it encourages cell division but inhibition shoot elongation (George *et al* .,2008). Respecting the interaction between levels of both studied variables ,it showed significant effect – as indicated earlier – on the given trait .The highest mean value was recorded due to the interaction between BAP and NAA at 0.00 and 0.2 mg/l, respectively (Fig .2A); meanwhile, the lowest mean value of the given character was recorded due to the interaction of the between BAP and NAA at 6.0 and 0.1 mg/l , each in turn . This result may be taken place due supra – optimal level of BAP within the cells and tissue ,which reflected a



(A)



(B)

Fig. (2): Initiation stage using explants of ***Mirabilis jalapa*** **L.** during the experiments. The (A) morphogenesis and (B) rhizogensis on MS medium +0.0.1 mg/l of NAA.

negative balance between both growth regulators and subsequently recorded the lowest mean value (George *et al* .,2008) .

Results of Table (1) manifested that the mean number of formed grown buds /propagule was affected by both studied variables ,meanwhile, their interaction was not so .It was noticeable that as NAA level increased, the number of formed grown buds decreased ; whereas, MS - NAA free medium brought about the highest mean number of the given character compare to the other two levels, which were not ,significantly ,differed from each other. This finding could be due to the auxins mode of action, in this context, where they inhibited the growth of axillary buds (George *et al* ., 2008). On the other hand, there was a proportional relationship between BAP levels and the given trait. As BAP level increased the given character increased specially at 6.0mg/l (Fig. 3) compare to the lesser levels .This

finding could be attributed to the mode of action of cytokinin in this respect, where it stimulated of axillary's bud breaking and growth (Krikrian *et al.*, 1987)

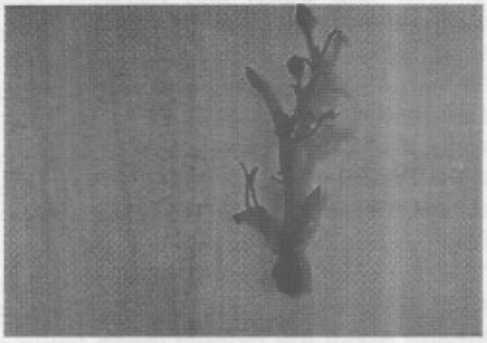


Fig. (3): Initiation stage using nodal explants of ***Mirabilis jalapa*** , L. during the experiments grown on NAA - free –MS medium and 6.0 mg/l BAP.

Results of Table (1) disclosed the effect of both applied growth regulator's levels and their interaction on the mean number of roots formed /propagule .It is obvious that both factor levels and their combination exerted highly significant on the given trait .Regarding the main effect of NAA levels, there was a proportional relationship between NAA levels and the given trait, meanwhile, as NAA level increased the given trait especially at 0.2 mg/l (Fig 2 B). This finding could be attributed to the major function of auxins for inducing rhizogenesis, whereas, they usually encourage adventitious root formation (Krikrian *et al* ., 1987). On the other hand, there was an inverse relationship between BAP levels and the given trait, in which as BAP level increased the given trait decreased, especially at either 3.0 or 6.0 mg /l. This result could be taken place due to the effect of cytokinins on inhibition of the root formation (George and Sherrington, 1984). Also ,the interaction between BAP and NAA at 0.00 and 0.2 mg/l ,respectively ,results in the highest mean value ;meanwhile ,the lowest mean value of the given trait was achieved due to the interaction between BAP and NAA at 6.0 and 0.00 mg/l ,each in turn .However the ratio between NAA and BAP played a crucial role for inducing the rhizogenesis of propagules grown *in vitro* (George and Sherrington,1984).Regarding multiplication stage; results of Table (2) demonstrated that BAP levels

alone exerted significant effect on mean number of leaflet formed /propagule on the multiplication stage, whereas, the auxin's levels and their interaction had no significant effect in this respect . Concerning the main effect of BAP; culturing the nodal cutting on BAP –free– medium resulted in the highest mean value, meanwhile as the BAP level increased, the given trait decreased as shown in Fig (4).This finding could be attributed to the carry- over the auxin and cytokinin levels within the cultured tissues (George *et al* .,2008). As for the mean value of shoot length character, data of Table (2) declared that levels of NAA have no significant effect on the mean value of shoot length of *Mirabilis jalapa* nodal cuttings cultured on multiplication medium ,but BAP levels and their interactions showed significant effect on the given trait ,in this context .Regarding BAP levels ,as it increased especially at the highest level (6.0 mg/l), the given trait decreased significantly, compare to the lesser levels as shown in Fig (3).This finding could be taken place due to the higher applied level of BAP which exerts inhibitory effect on the given trait (George *et al* .,2008).The interaction between both NAA and BAP at 0.1 and 3.0mg/l, respectively ,brought about the highest mean value of the defined character, while the lowest one was true under the combination of NAA and BAP at 0.1 and 6.0 mg/l, consecutively.

Results of Table (2) revealed that the different levels of NAA and BAP and their combinations affected significantly the mean number of buds formed /propagule. However, there was a proportional relationship between BAP levels and mean number of grown buds formed/propagule as shown in Fig.(3).This finding could be explained on the basis of mode of action of cytokinin's action in enhancing proliferation of axillary buds as reported earlier by (Krikrian *et al*.,1987) .On one hand ,there was an inverse relationship between NAA levels and the given trait as shown in Fig.(3) .Also, this finding could be attributed to the auxin's mode of action in affecting ,adversely ,the given trait as indicated earlier by (George *et al* .,2008) .However ,the interaction between NAA and BAP at 0.00 and 6.00mg/l achieved the highest mean value of the given trait ,meanwhile, the lowest mean value of the given trait was recorded due to the interaction NAA and BAP at 0.2 and 0.00 mg/l ,each in turn and as shown as Fig .(3) .Results of Table (2) elucidated that the different levels of NAA and BAP had significant effects on mean number of roots formed per propagule, but their interaction was insignificant. Again, there was a proportional relationship between NAA levels and the given trait, in which as NAA level increased, the given character increased, especially at 0.2 mg/l, but, there *vice versa* was true in case of BAP, notably at 6.0 mg/l, in which the lowest

mean value of the given trait. These findings confirm the above –related results in initiation stage, and the same interpretation is true too as shown in Fig.(3).

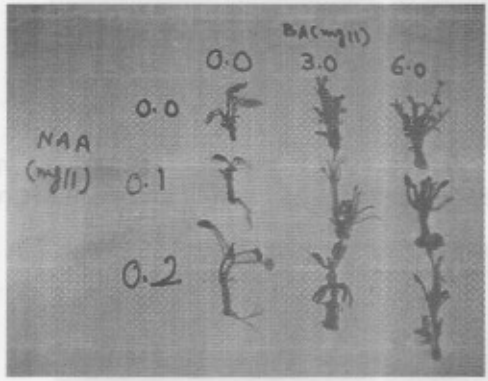


Fig. (4). Multiplication stage of ***Mirabilis jalapa*** **, L.** using nodal cutting explants after 35 days of growth *in vitro*.

Acclimatization of *Mirabilis jalapa*, L. plants *ex vitro* and establishment *in vivo*. Ramets (rooted shoots) of *Mirabilis jalapa* , L. that rooted readily after 5-7 days on MS-medium augmented with 0.2 mg/l NAA as reported earlier were transferred into *ex vitro* (Fig. 5) and *in vivo* (Figs. 6 a and b).The survival rate of transplants during the study was 100%.The transplanted *in vitro* grown plantlets were raised-up healthy, uniform and expressed normal appearance in their growth habit ,leaf shape, flowering ,flower colour(Fig.7).In conclusions ,the *in vitro* propagation of desirable *Mirabilis jalapa* , L. plants demonstrates the potentiality and possibility of initiation multiplication ,rhizogenesis ,and acclimatization *ex vitro* and *in vitvo of this species,* vegetatively successfully *via* nodal culture technique .Exploiting the advantages of this technique could lead to (a) stability of the mutants and /or desirable salt tolerant plants ,and (b) multiplying of this genus on a large –scale through direct organogenesis of organized tissue (using nodal culture) of specific and/or desirable genotypes.

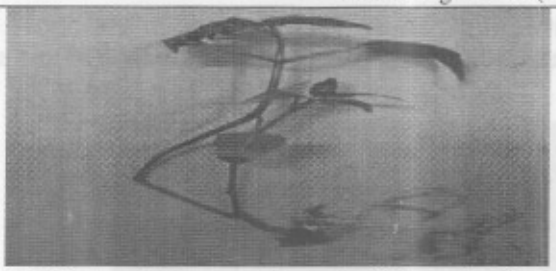


Fig. (5). ***Mirabilis jalapa* , L.** plantlet raised-up *in vitro*, after acclimatization, showing its healthy and normal growth.

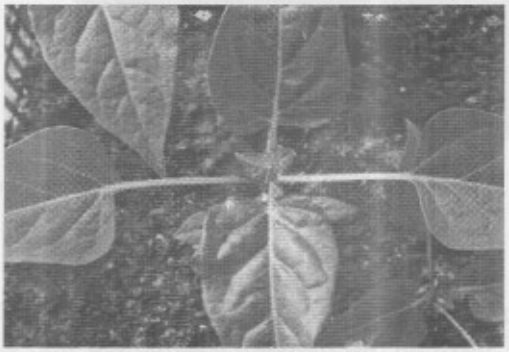


Fig. (6 a). acclimatization and establishment of neoformed ***Mirabilis jalapa* , L.** plantlets *ex vitro*.

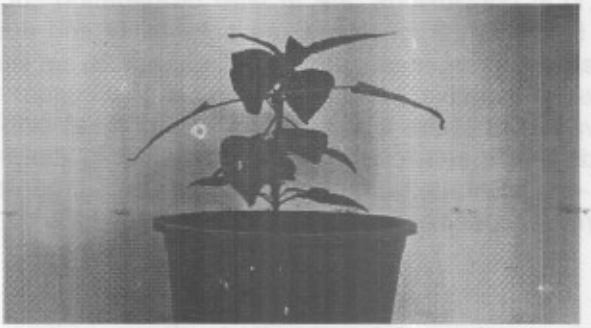


Fig.(6 b). **Vegetative growth of *Mirabilis jalapa* , L. plants grown after 10 weeks *ex vitro*.**

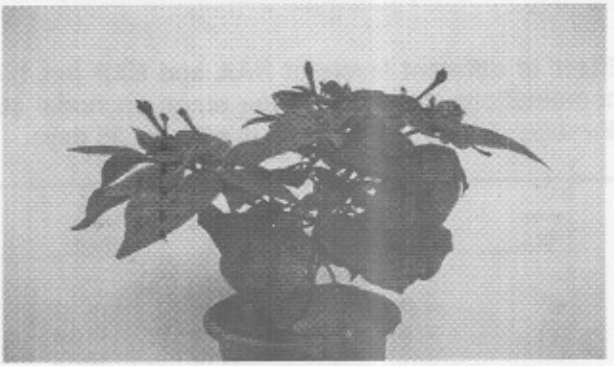


Fig.(7). **Flowering of *Mirabilis jalapa* , L. plants grown *in vivo* after 12 weeks *ex vitro*.**

In conclusion, the *in vitro* propagation of desirable *Mirabilis jalapa* , L. plants demonstrates the potentiality and possibility of initiation multiplication ,rizogenesis, and acclimatization *ex vitro* and *in vitro* vegetatively successfully *via* nodal culture technique. Exploiting the advantages of this technique could lead to (a) stability of the mutants and /or desirable salt tolerant plants ,and (b) multiplying of this genus on a large –scale through direct organogenesis of organized tissue (using nodal culture) of specific and/or desirable genotypes.

Table (1). Effect of different levels of NAA and BAP (mg/l) and their combinations during initiation stage on nodal cuttings of *Mirabilis jalapa* , L. cultured in *vitro* for 35 days.

Cha.	BAP Levels (mg/ l)	NAA levels (mg/l) 0.00	0.1	0.2	Mean BAP	Significance BAP x NAA	
Mean number of formed leaflets / propagule	0.0	14.64	12.31	11.33	12.76a	NS	
	3.0	12.60	12.33	11.31	12.08a		
	6.0	12.63	12.60	11.00	12.07a		
Mean(NAA)		13.29a		12.41b	11.21b		
L.S.D.(0.05)							-------
Mean shoot length(cm)/ propagule	0.0	4 .00		4.01	5.66	4.33a	**
	3.0	3.64		3.56	3.32	3.77 a	
	6.0	2.66		2.33	2.66	2.33b	
Mean(NAA)		3.43a		3.32a	3.77a		
L.S.D.(0.05)							1 176
Mean number of formed buds propagule	0.0	11.00	3.0	8.33	7.01	8.78c	NS
	13.33			8.65	9.66	10.55b	
	6.0	14.65		10.33	9.30	11.42a	
Mean(NAA)		13.00a		9.11b	8.65b		
L.S.D.(0.05)							-------
Mean number of formed roots/ propagule	0.0	10.01		10.33	11.07	10.44a	**
	3.0	6.6		10.30	10.00	8.96 b	
	6.0	2.32		5.33	9.32	5.65 c	
Mean(NAA)		6.31c		8.65 b	10.13a		
L.S.D.(0.05)							2.21

Values marked with the same alphabetical letters, within comparable group of means, do not differ significantly, using L.S.D. at 0.05 level of probability
** = Highly significant at the 0.01 level of probability.
NS = Not Significant

Table (2). Effect of different levels of NAA and BAP (mg/l) and their combinations during multiplication stage on nodal cuttings of *Mirabilis jalapa* , L. cultured *in vitro* .

Cha.	BAP Levels (mg/ l)	NAA levels (mg/l) 0.00	0.1	0.2	Mean BAP	Signific BAP x
Mean number of formed leaflets / propagule	0.0	14.33	14.65	14.64	14.51a	
	3.0	13.21	13.32	13.33	13.28b	
	6.0	13.19	13.10	13.00	13.09b	
Mean(NAA)		1357a	13.66a	13.65a		
L.S.D.(0.05)						-
Mean shoot length(cm)/ propagule	0.0	5 .00	4.65	4.33	4.66a	
	3.0	3.66	6.00	4.63	4.77 a	
	6.0	2.00	2.66	5.32	3.67b	
Mean(NAA)		4.55a	4.11a	4.42a		
L.S.D.(0.05)						
Mean number of formed buds propagule	0.0	11.32	10.66	7.32	9.74c	
	3.0	13.32	9.67	10.00	11.00b	
	6.0	15.65	11.00	10.65	12.44a	
Mean(NAA)		13.44a	10.42b	9.33b		
L.S.D.(0.05)						
Mean number of formed roots/ propagule	0.0	11.31	11.32	14.65	12.44a	
	3.0	8.61	10.32	10.01	9. 64 a	
	6.0	3.66	9.60	10.34	7.86b	
Mean(NAA)		7.85b	10.41a b	11.66a		
L.S.D.(0.05)						

Values marked with the same alphabetical letters, within comparable group of means, do not differ significantly, using L.S.D. at 0.05 level of probability.

** = Highly significant at the 0.01 level of probability.

NS = Not Significant.

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الملخص العربى

إكثار نبات شب الليل المعامل باشعه جاما والمتحمل للملوحه معمليا وتحقيق زراعته خارج المعمل

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أجريت التجارب الخاصة بالإكثار المعملي الدقيق في معمل زراعه الأنسجة النباتية بكلية زراعة سابا باشا- جامعة الإسكندرية عام 2009-2010 تمت زراعه الأنسجة النباتية المختارة على مرحلتين، الأولى مرحله التنشئة، ثم مرحله التضاعف تلاهما مرحلة الأقلمة . مرحلة التنشئة و تلخصت فى أخذ جزء من النبات الام (العقد الساقية) وتم تطهيرها ثم زرعت فى بيئة موراشينج وسكوج و التي زودت بأوكسين

نفثالين اسيتيك اسيد (NAA)بتركيزات (0.0, 0.1, 0.2 mg/L) وسيتوكينين بنزيل امينو بيورين(BAP) بتركيزات (0.0, 3.0, 6.0 mg/l). مضافا إليه 30 جرام/لتر سكروز، وأوكسين و لدنت باجار عند تركيز 7جم / لتر (وزن/جم) وتم ضبط درجه الحموضة و قلوية البيئة 5,7 وتم تعقيمها بالاتوكلاف . اما مرحلة التضاعف: حيث تم فيها استزراع البراعم الجانبية للنبتات الناتجة من مرحلة التنشئة في نفس الوسط السابق بنفس التركيزات السابقة .اما مرحلة التجذير: فقد أجريت فى نفس الوسط السابق، مع استخدام تركيز أوكسين 0.2 مليجرام /لتر من اجل التجذير ، ثم نقلت الأجزاء النباتية التى تم تجذيرها تم إكثارها إلى الصوبة لأقلمتها ثم زراعتها في الحقل.و أظهرت النتائج إن الزيادة في الأوكسين أثناء مرحله التنشئة أدى الى نقص عدد الوريقات مع وجود علاقة طردية للأوكسين مع عدد الجذور، حيث زاد العدد خاصة عند 0.2 مليجرام/لتر، و كذلك قد تبين وجود علاقة عكسية بين طول المجموع الخضري وتركيز السيتوكينين وعدد الجذور. فى حين أن الوسط الغذائي الخالي من السيتوكينين أظهر أعلى متوسط لعدد الوريقات ، فى مرحلة الأقلمة كما أظهرت النتائج أن نسبة النباتات الحية المنزرعة خارج المعمل كانت 100% وكانت النباتات الناتجة متشابهة فى شكل الورقة ولون الأزهار وطريقة النمو مع النبات الأصلي.