In Vitro Propagation and Somatic Embryogenesis of Marvel of Peru (*Mirabilis jalapa* L.) from Nodal and Leaf Explants

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ABSTRACT

Nowadays, there is a tremendous demands for *Mirabilis jalapa* L., it is a popular ornamental landscape plant grown worldwide for the beauty of its flowers, also earned its place in herbal medicine practices around the world for traditional medicine by the people from different countries. Therefore, *in vitro* propagation and somatic embryogenesis of *Mirabilis jalapa* was achieved during both seasons of 2012 and 2013, from nodal and leaf explants on MS medium supplemented with different concentrations and combinations of NAA, KIN, IBA and 2,4-D. Respecting *in vitro* propagation, the highest mean number of shoots (2.67) formed per propagule were recorded on MS medium supplemented with 3.0 mg/l KIN and 0.250 mg/l NAA. Regarding to rhizogenesis stage, it is obvious that, fortifying MS-medium with a combination of both IBA at 0.00 mg/l, and NAA at 0.500 mg/l, gave the highest mean value of roots formed per propagule (6.11). On the other end, the best results of embryonic callus induction response (100%) and embrogenic callus size per propagule (++++) in whole area of leaf explant was observed on MS medium augmented with 4.0 mg/l 2,4-D. Neoformed plantlets were successfully acclimated and established in peat and sand in ratio of 1:1, and flowered successfully under field conditions.

Keywords: *Mirabilis jalapa*, somatic embryogenesis, rhizogenesis, embryonic callus.

INTRODUCTION

Marvel of Peru (*Mirabilis jalapa* L.,) belongs to family Nyctaginceae, also known as four o'clock flower. It is a popular ornamental landscape plant grown worldwide for the beauty of its flowers and their sweet fragrance, color-changing phenomenon as it can display flowers with different color when it matures Ling *et al*. (2009). Apart from its ornamental value, it has also earned its place in herbal medicine practices around the world. Whereas, it is used for control of viruses, fungi and yeast. It is used in traditional medicine by the people from different countries for the treatment of diarrhea, dysentery, conjunctivitis, edema, inflammation, swellings, muscular pain and abdominal colics (Daniel, 2006; Holdsworth, 1992) and its extract has antibacterial, antiviral, and antifungal activities (Oladunmoye, 2007). In recent years much interest has been evinced in the propagation of medicinal plants by tissue culture (Tiwari, *et al*., 1998; Laparra *et al*., 1977). There are only a few reports in the members of Nyctaginaceae (Zaccai*et al*., 2007) and less in those members which are medicinal (Siddiqui *et al*., 2004). Besides, by maintaining genetic stability, tissue cultured plants are, also, valuable in speeding up conventional breeding and propagation, reducing space and labor requirement and achieving manipulative goals that cannot be carried out *viain vivo* conditions (Ling *et al*., 2009). On the other hand, the production of secondary metabolites *via* biotechnological tools (e.g. plant tissue cultures techniques) became an urgent need. As the regenerative potentiality is maximum in the leaf explants (Prasad and Chaturvedi, 1978), this was used as the explant material to determine the efficacy of the various media to induce callus and organogenesis. There are a few reports about micropropagation and callus induction of *Mirabilis jalapa* Ling *et al*. (2009) mentioned that the best callus induction response was obtained on half strength (½) MS media supplemented with 20.0 µM picloram which produced healthy and friable callus. On the other hand,Tamer and Mavituna (1997) were induced callus from leaf explants using Murashige-Skoog medium fortified with 1 mg/l 2,4-D, 1 mg/l NAA, 0.15 mg/l KIN and 0.05 mg/l BAP. The aim of this study was investigating the effect of explant types and plant growth regulators on the different stages of micropropagation and induction of callus and somatic embryogenesis from *Mirabilis jalapa* plants to micropropagateand manipulate this species efficiently.

MATERIALS AND METHODS

Investigation was carried out during both seasons of 2012 and 2013 at Plant Tissue Culture Lab, Plant Production Department, The Faculty of Agriculture Saba-Basha, Alexandria University.

Plant material and culture conditions

Nodal explants with axillary buds were collected from field-grown plants of 9 O'clock plants. The excised nodal segments (1-1.5 cm long) without leaves were surface sterilized initially in 70% (v/v) ethanol for 30 to 40 s, followed with 15 min in 15% commercial bleach (25% sodium hypochlorite), and rinsed four times with autoclaved distilled water. The sterilized nodal explants were cultured on an initiation medium supplemented with plant growth regulators (PGRs) as the starting plant material. After 3 months of culturing, *in vitro*nodal stems as well as leaflets of the induced plantlets were used for micro cuttings *in vitro* and callus

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induction on Murashige and Skoog's medium, (1962) supplemented with 30 g/l sucrose and 3 g/l gelrite was used for callus induction. For *in vitro* propagation, 6-benzyladenine (BA) and a-naphthalene acetic acid (NAA) and their combinations were added to the medium. The pH was adjusted to 5.8 ±0.2, and the medium was autoclaved at 121°C under 100 kPa for 20 min. The cultures were incubated in the growth room at 25±2°C, 50 to 60% relative humidity under 16/ 8 h photoperiod with light intensity of *ca* 30 µmol $m^{-2}s^{-1}$.

Initiation stage: the sterilized explants were cultured into the given MS medium which supplemented with different concentrations and combinations of auxin (NAA) at four concentrations: 0.0 (nil), 0.250, 0.500 and 1.000 mg/l, in combinations with cytokinin (KIN) at three concentrations: 0.0 (nil), 0.125 and 0.250 mg/l.

Multiplication stage: On the basis of the previous stage results, produced shoots from the treatments were subcultured and transferred to multiplication stage medium contained MS salts and two types of hormones were tested, KIN at four concentrations: 0.0 (nil), 1.0, 2.0 and 3.0 mg/l, in combinations with NAA at three concentrations: 0.000 (nil), 0.125 and 0.250 mg/l.

For rooting induction and acclimatization: microshoots raised were harvested after 4 weeks and each shoot was transferred to jars containing 20 ml of MS medium supplemented with different levels of IBA at 0, 0.250, 0.500 and 1.000 mg/l and NAA at 0, 0.250 and 0.500 mg/l. Data was recorded for different parameters including mean numbers of shoots, shoot length (cm), leaflets, nodes and roots formed/propagule. Some of the rooted plantlets were taken-off from rooting media, washed, and then transferred to pots containing a mixture of sterile peat and sand in a ratio of 1:1. Newly potted plantlets were covered with polythene bags for 1 week before they were transferred to a research greenhouse, and the acclimatized plantlets were watered and fertilized as needed.

Somatic embryogenesis from leaf explants experiment

From *in vitro* propagules, nodal cuttings and wounded leaflets of propagule were used as explants, which were cultured on MS medium supplemented with different concentrations of 2,4-D at 0.00 (nil), 0.50, 1.00 and 2.00 mg/l in combination with KIN at 0.00 (nil), 0.50 and 1.00 mg/l. The followed characters were recorded per propagule, the percentage of embryogenic calli, size of embryogenic calli and mean number of hairy roots per propagule. Embryonic calli size and hairy roots were, assessed and recorded macroscopically, for each jar and for statistically convenience, the minus or plus symbols were converted to a numerical code as follows: (-), 0; (+), 1; (++), 2; (+++), 3; (++++), 4. These symbols refer to no callus, low, moderate, high, and intensive embryonic callus formed per propagule, respectively.

Data analysis

All the experiments carried out during this study were designed as factorial experiments layout in completely randomized design (Gomez and Gomez, 1984). Recorded data were analyzed, statistically, using analysis of variance technique (ANOVA) and means were compared by Duncan's multiple range test (Steel *et al*., 1997) and significance was determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Results in Table (1) and Fig. (1) describe the effect of various levels of both growth regulators (NAA and KIN) and their combinations on the studied characters of Four O'clock plants. Concerning the main effect of NAA, mean number of shoots formed per propagule, NAA had no significant effect on mean number of shoots formed per propagule. On the other hand, KIN levels had a significant effect on the given trait. The highest mean value (1.56) was recorded due to augmenting the culture medium with 0.250 mg/l KIN, but, the least response was observed with the absence of KIN. Meanwhile, the interaction between both growth regulators exerted non-significant effect on the given trait, too. With respect to the shoot length per propagule, both growth regulators and their interactions, exerted significant effects on the given trait. In case of NAA main effect, the absence of NAA from the culture medium brought about the highest mean value (3.37). On the other side, the main effect of KIN, cleared a proportional relationship between the given trait and KIN levels; whereas, the highest level of KIN (0.250 mg/l) gave the maximum mean value of shoot length per propagule (3.37). However, the interaction between both added levels of NAA and KIN at 0.0 and 0.250 mg/l, respectively, resulted in the highest mean value (4.17). Concerning the number of leaflets and the number of nodes formed per propagule; the effect of KIN exerted non-significant effect on the given trait. On the other hand, the main effect of NAA and the interaction between both growth regulator levels exerted a significant effect on the leaflets and nodes number formed per propagule. In case of NAA as a main effect, its absence from the culture medium achieved the highest mean value (7.19 and 3.81, respectively). The interaction between both NAA and KIN at 0.0 and 0.125 mg/l, respectively, resulted in the highest mean value (8.00 and 4.33, respectively). Respecting the number of roots formed per propagule, both growth regulators hadn't significant effect on the given trait.

Table 1. Effect of different levels of NAA and KIN (mg/l) and their combinations on the initiation stage of Mirabilis jalapa L. explants

Characters	KIN levels (mg/l)	NAA levels (mg/l)				Mean KIN	Significance		NAAXKIN
		0.000	0.250	0.500	1.000		NAA	KIN	
(a) Mean number of shoots formed/propagule:							N.S.	*	N.S.
	0.000	1.33	1.44	1.44	1.33	1.39			
	0.125	1.56	1.56	1.67	1.33	1.53			
	0.250	1.78	1.67	1.67	1.78	1.72			
Mean (NAA)		1.56	1.56	1.59	1.48				
L.S.D. (0.05)							0.27	0.23	0.47
(b) Mean shoot length (cm)/propagule:							*	**	*
	0.000	2.44	2.39	2.56	2.11	2.38			
	0.125	3.50	2.78	2.72	3.39	3.10			
	0.250	4.17	3.33	3.89	3.67	3.76			
Mean (NAA)		3.37	2.83	3.06	3.06				
L.S.D. (0.05)							0.42	0.36	0.73
(c) Mean number of leaflets formed/propagule:							*	N.S.	*
	0.000	6.89	6.89	7.11	6.00	6.72			
	0.125	8.00	6.89	6.44	6.67	7.00			
	0.250	6.67	6.67	6.89	6.44	6.67			
Mean (NAA)		7.19	6.81	6.81	6.37				
L.S.D. (0.05)							0.63	0.54	1.08
(d) Mean number of nodes formed/propagule:							*	N.S.	*
	0.000	3.78	3.44	3.56	3.22	3.50			
	0.125	4.33	3.89	3.22	3.33	3.69			
	0.250	3.33	3.44	3.89	3.22	3.47			
Mean (NAA)		3.81	3.59	3.56	3.26				
L.S.D. (0.05)							0.44	0.38	0.77
(e) Mean number of roots formed/propagule:							N.S.	N.S.	**
	0.000	0.00	1.33	2.00	1.67	1.25			
	0.125	2.33	0.00	0.00	0.00	0.58			
	0.250	2.00	0.00	1.67	0.00	0.92			
Mean (NAA)		1.44	0.44	1.22	0.56				
L.S.D. (0.05)							1.02	0.89	1.77

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.
*, **: Significant or highly significant.

Fig. 1. Initiation of *Mirabilis jalapa* L. nodal explants cultured on MS medium supplemented with NAA and KIN at 0.0 and 0.250 mg/l, respectively

While, the combinations between NAA at 0.000 mg/l and KIN at 0.125 mg/l, led to formation the highest mean number of roots per propagule (2.33).

Data presented in Table (2) and Figure (2) showed that different levels of both applied growth regulators and their combinations had highly significant effects on the given traits of Four O'clock explants. Regarding the mean number of shoot formed per propagule, the highest values (2.07) was recorded due to the presence of KIN at 3.0 mg/l followed by KIN at either 1.0 or 2.0 mg/l (1.85). On the other extreme, the main effect of NAA declared that providing the MS medium with NAA at 0.250 mg/l, brought about the highest value on this trait (2.00). While, the interaction between KIN at 3.0 mg/l and NAA at 0.250 mg/l, resulted in the highest mean number of shoot formed per propagule (2.67). The above-mentioned results indicated, generally, that

the highest shoot number was attained due to the presence of KIN at 0.250 mg/l, in initiation stage (Table 1) and 3.0 mg/l, in multiplication stage as showed in Tables (1 and 2, respectively) which expressed significantly higher mean value compared to other treatments. In this respect, Davies (1995) reported that kinetin considers as in favour of stimulation cell division, morphogenesis (shoot initiation/bud formation) in tissue culture, and break of apical dominance and release growth of lateral buds. Khan *et al*. (2004), also, observed the highest profuse adventitious shoot in *Cordyline terminalis* cultured on medium containing a combination of Kinetin at 4.0 mg/l and NAA at 0.5 mg/l. Shah *et al*. (2006) found that the kinetin at concentration 0.250 mg/l, gave rise to 36 plants in *Bougainvillea spectabilis*. Ragi and Shibu (2014) reported that the greatest frequency of multiple shoot formation for *Boerhavia difusa* (Nyctaginaceae) was observed with 1.00 mg/l kinetin treatment. As for the shoot length per propagule, the treatment receiving KIN at either 0.0 or 1.0 mg/l, brought about the highest shoot length. Similar performance was noticed with the main effect of NAA at 0.125 mg/l. Likewise, the combinations between KIN at either 0.0 or 1.0 mg/l, and NAA at 0.125 mg/l, exerted significant effects on the given trait. On the other side, the results showed that, generally, KIN enhanced the shoot length of *Mirabilis jalapa* in the low concentration as shown in (Table 1), but the superaoptimal concentration resulted in inhibition of shoot length as tabulated in Table (2). When Kinetin was applied there was an inhibition for the length of shoot accompanied by the expansion in diameter. Also, Shah *et al*. (2006) reported that the MS medium fortified with 2 mg/l kinetin led to inhibition of shoot length of *Bougainvillea spectabili*. Cytokinins promote growth by swelling rather than elongation in soybean (Fatima and Bano, 1998). Zadoo (1986) confirmed that cytokinin induced expansion of growth in hypocotyl segments of morning glory and inhibited the extension growth. With regard to the main effect of NAA, the data tabulated in Table (2) reflected that the presence of the given auxin exerted significant effect on the given trait compare to the control treatment. Results of the number of leaflets and nodes formed per propagule, showed that KIN at either 0.0 or 1.0 mg/l, gave the highest number of the above traits. On the other hand, the effect of NAA, declared that there was a proportional relationship with the given traits. Whereas, the NAA at 0.250 mg/l, resulted in the highest mean value. Subsequently, regarding the interaction between both applied growth regulators, the presence of KIN and NAA at 0.0 and 0.250 mg/l, resulted in the highest number of the both traits. With reference to the mean number of roots formed per propagule, the main effect of KIN showed that the presence of KIN in the culture medium at 0.0 and 3.0 mg/l, led to the highest rooting formed per propagule. Regarding the effect of NAA, augmenting MS medium with 0.125 mg/l, brought about the highest value of the given trait. The interaction between KIN and NAA at 0.0 and 0.125 mg/l, respectively, resulted in the formation of highest rhizogenesis.

Results of Table (3) and Figure (3) manifested that the main effect of IBA and NAA and their interaction, divulged that the presence of both growth regulators into MS medium had no significant effects of the mean number of shoots per propagule. Concerns the mean shoot length per propagule, results of IBA demonstrated that the presence of IBA into the culture medium at 0.500 mg/l, resulted a highest shoot length. In total, the main effect of NAA showed similar performance with that has been noticed as the above-mentioned character. The interaction between IBA at 0.000 mg/l and NAA at 0.500 mg/l, gave the highest mean values of the defined character (6.27). Pertaining the mean number of leaflets and nodes formed per propagule, the effect of IBA indicated that providing the MS medium with IBA at either 0.00 or 0.250 mg/l, produced the highest mean values of the given trait. On the other side, the main effect of NAA had no significant effect on the given traits. With reference to the effect of IBA and NAA on the rooting mass per propagule, it is obvious that, fortifying MS-medium with IBA at 0.500 mg/l, resulted in the highest mean value (2.70). On the other hand, the presence of NAA into culture medium at 0.250 mg/l, brought about the highest mean value (3.08). Likewise, the combinations between IBA at 0.00 mg/l, and NAA at 0.500 mg/l, gave the highest mean value (6.11).

Table 2. Effect of different levels of KIN and NAA (mg/l) and their combinations on the multiplication stage of *Mirabilis jalapa* L. explants

Characters	NAA levels (mg/l)	KIN levels (mg/l)				Mean KIN	Significance		KINXNAA
		0.000	1.000	2.000	3.000		KIN	NAA	
(a) Mean number of shoots formed/propagule:									
	0.000	1.44	1.78	2.00	1.78	1.75	*	*	**
	0.125	1.56	2.00	2.00	1.78	1.83			
	0.250	2.00	1.78	1.56	2.67	2.00			
Mean (KIN)		1.67	1.85	1.85	2.07				
L.S.D. (0.05)							0.26	0.23	0.46
(b) Mean shoot length (cm)/propagule:									
	0.000	3.82	3.09	3.16	3.23	3.33	**	**	**
	0.125	6.30	6.30	3.70	3.92	5.06			
	0.250	4.50	4.94	4.00	3.61	4.26			
Mean (KIN)		4.87	4.78	3.62	3.59				
L.S.D. (0.05)							0.58	0.50	1.00
(c) Mean number of leaflets formed/propagule:									
	0.000	6.00	6.44	4.89	5.56	5.72	**	*	*
	0.125	7.11	7.11	5.56	5.11	6.22			
	0.250	7.33	6.44	6.00	6.22	6.50			
Mean (KIN)		6.81	6.67	5.48	5.63				
L.S.D. (0.05)							0.73	0.63	1.27
(d) Mean number of nodes formed/propagule:									
	0.000	3.44	3.44	2.44	2.78	3.03	**	N.S.	*
	0.125	3.56	3.56	2.78	2.56	3.11			
	0.250	3.89	3.22	3.00	3.11	3.31			
Mean (KIN)		3.63	3.41	2.74	2.81				
L.S.D. (0.05)							0.44	0.38	0.76
(e) Mean number of roots formed/propagule:									
	0.000	0.00	1.78	1.22	2.56	1.39	*	**	**
	0.125	4.00	3.00	2.22	2.78	3.00			
	0.250	3.56	2.00	1.78	3.22	2.64			
Mean (KIN)		2.52	2.26	1.74	2.85				
L.S.D. (0.05)							0.68	0.59	1.18

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.

*, **: Significant or highly significant.

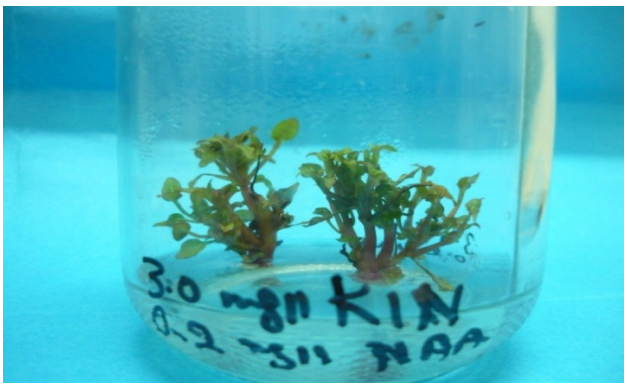

Fig. 2. Multiplication of *Mirabilis jalapa* L. newly formed nodal segments during of initiation stage, upon culturing then on MS medium augmented with KIN and NAA at 3.000 and 0.250 mg/l, consecutively

Table 3. Effect of different levels of IBA and NAA (mg/l) and their combinations on the rooting stage of *Mirabilis jalapa* L. explants

Characters	NAA levels (mg/l)	IBA levels (mg/l)				Mean NAA	Significance		IBAXNAA
		0.000	0.250	0.500	1.000		IBA	NAA	
(a) Mean number of shoots formed/propagule:							N.S.	N.S.	N.S.
	0.000	1.33	1.22	1.33	1.33	1.31			
	0.250	1.44	1.33	1.33	1.33	1.36			
	0.500	1.44	1.44	1.22	1.22	1.33			
Mean (IBA)		1.41	1.33	1.30	1.30				
L.S.D. (0.05)							0.27	0.23	0.46
(b) Mean shoot length (cm)/propagule:							*	*	*
	0.000	3.71	3.82	5.43	5.13	4.53			
	0.250	5.38	3.91	5.18	4.63	4.78			
	0.500	6.27	5.11	5.53	5.12	5.51			
Mean (IBA)		5.12	4.28	5.38	4.96				
L.S.D. (0.05)							0.78	0.67	1.35
(c) Mean number of leaflets formed/propagule:							*	N.S.	*
	0.000	7.78	7.56	7.11	6.44	7.22			
	0.250	7.11	7.56	6.89	7.11	7.17			
	0.500	7.78	7.33	6.67	7.56	7.33			
Mean (IBA)		7.56	7.48	6.89	7.04				
L.S.D. (0.05)							0.50	0.44	0.87
(d) Mean number of nodes formed/propagule:							**	N.S.	*
	0.000	4.22	4.11	3.56	3.22	3.78			
	0.250	3.56	3.89	3.44	3.56	3.61			
	0.500	4.11	3.78	3.33	4.00	3.81			
Mean (IBA)		3.96	3.93	3.44	3.59				
L.S.D. (0.05)							0.32	0.28	0.56
(e) Mean number of roots formed/propagule:							**	**	**
	0.000	0.00	1.22	4.56	1.67	1.86			
	0.250	0.56	1.67	1.00	1.33	1.14			
	0.500	6.11	2.00	2.56	1.67	3.08			
Mean (IBA)		2.22	1.63	2.70	1.56				
L.S.D. (0.05)							0.69	0.60	1.20

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.
*, **: Significant or highly significant.

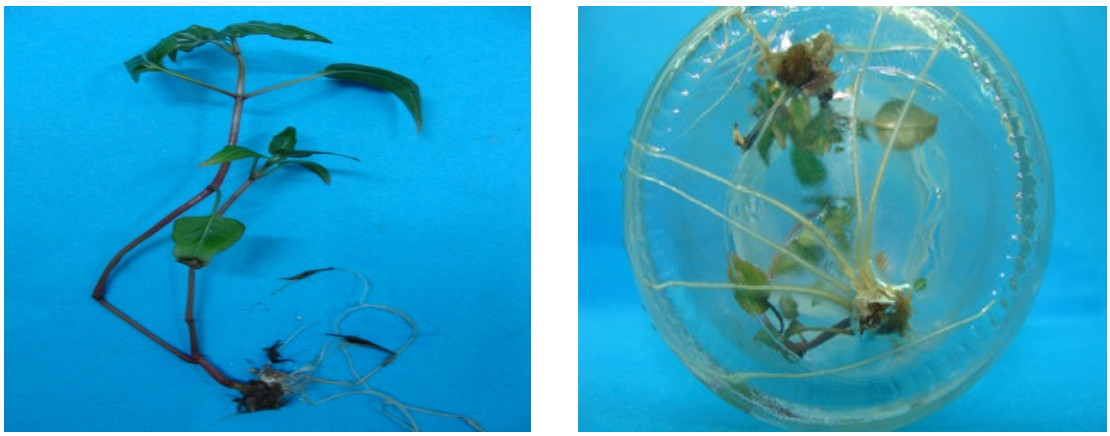

Fig. 3. Rhizogenesis of *Mirabilis jalapa* L. microshoots of multiplication stage, upon culturing then on MS medium fortified with IBA and NAA at 0.0 and 0.5 mg/l, each in turn

Results were consistent with those found by Roy (2008) who found, significantly, higher percentage of root development (50 and 78%) when 0.5 mg/l IBA and either 0.5 mg/l NAA with 0.5 mg/l IBA were combined and supplemented to the medium.

Wesely *et al*. (2010) observed that MS medium supplemented with 0.5 mg/l IBA resulted in 8.3 roots per shoot of *Boerhaavia diffusa*. Ochatt *et al*. (2010) demonstrated that for rooting of *Lathyrus odoratus* L. microshoots, they are explanted onto medium with 0.5 to 1 mg/L NAA. Abido *et al*. (2011) stated that the highest number of roots were obtained when *Mirabilis jalapa* neoformed shoots were treated with NAA at 0.2 mg/l.On the other side, Shah *et al.* (2006) reported that the highest numbers of plants of *Bougainvillea spectabilis* were rooted when MS medium was supplemented with 2.5 mg/l IBA and NAA 2.5 mg/l.

Effect of various levels of both growth regulators (2,4-D and KIN) and their combinations on somatic embryogenesis of four o'clock leaf explants.

The results regarding the effect of both applied growth regulators (2,4-D and KIN) and their interactions on somatic embryogenesis on four o'clock, using leaf explants are presented in Table (4) and shown in Figures (4 a, b, c). Regarding the percentage of explants formed embryonic callus trait, the obtained data revealed that the presence of 2,4-D as a main effect, there was a direct proportionate relationship between the tested levels of 2,4-D and the given trait; whereas, at 4.0 mg/l, brought about the highest percentage of explants that formed embryonic callus, it (4.00 mg/l), exerted highly significant effect on the given trait and resulted in formation vigorous embryonic callus. Respecting KIN levels, it is absence led to the highest perecentage value (80%). Also, the interaction between 2,4-D and KIN at either 4.00 mg/l and at either levels of KIN; resulted in highest mean value (100%). Pertaining the embryonic callus size per propagule, the main effect of 2,4-D declared that the MS medium supplemented with highest concentration of 2,4-D produced, significantly, intensive embryonic callus per propagule. On the other end, the presence of KIN into the culture medium at 0.125 mg/l, led to the formation of high embryonic callus size per propagule. Likewise, the interaction between both applied growth regulators had highly significant effect on the given trait, and the highest callus size was obtained cultured medium was supplemented with 2,4-D at 4.0 mg/l and KIN at 0.125 mg/l.

Table 4. Effect of various levels of both growth regulators (2,4-D and KIN) and their combinations on somatic embryogenesis of *Mirabilis jalapa* L. explants

Characters	KIN levels	2,4-D levels (mg/l)					Mean	Significance		2,4-DXKIN
		0.000	0.500	1.000	2.000	4.000	KIN	2,4-D	KIN	
(a) Percentage of embryogenic callus/propagule:								**	**	**
	0.000	0.00	100.00	100.00	100.00	100.00	80.00			
	0.125	0.00	0.00	0.00	100.00	100.00	40.00			
	0.250	0.00	0.00	0.00	0.00	100.00	20.00			
Mean (2,4-D)		0.00	33.33	33.33	66.67	100.00				
L.S.D. (0.05)								0.00	0.00	0.01
(b) embryogenic callus size/propagule:								**	**	**
	0.000	0.00	1.00	1.00	1.00	2.00	1.00			
	0.125	0.00	0.00	0.00	3.78	4.00	1.56			
	0.250	0.00	0.00	0.00	0.00	3.00	0.60			
Mean (2,4-D)		0.00	0.33	0.33	1.59	3.00				
L.S.D. (0.05)								0.06	0.05	0.11
(e) Mean number of hairy roots formed/propagule:								**	**	**
	0.000	0.00	0.00	0.00	1.00	1.00	0.40			
	0.125	1.00	1.00	2.00	2.00	2.00	1.60			
	0.250	0.44	3.00	4.00	4.00	2.00	2.69			
Mean (2,4-D)		0.48	1.33	2.00	2.33	1.67				
L.S.D. (0.05)								0.07	0.06	0.13

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.

*, **: Significant or highly significant.

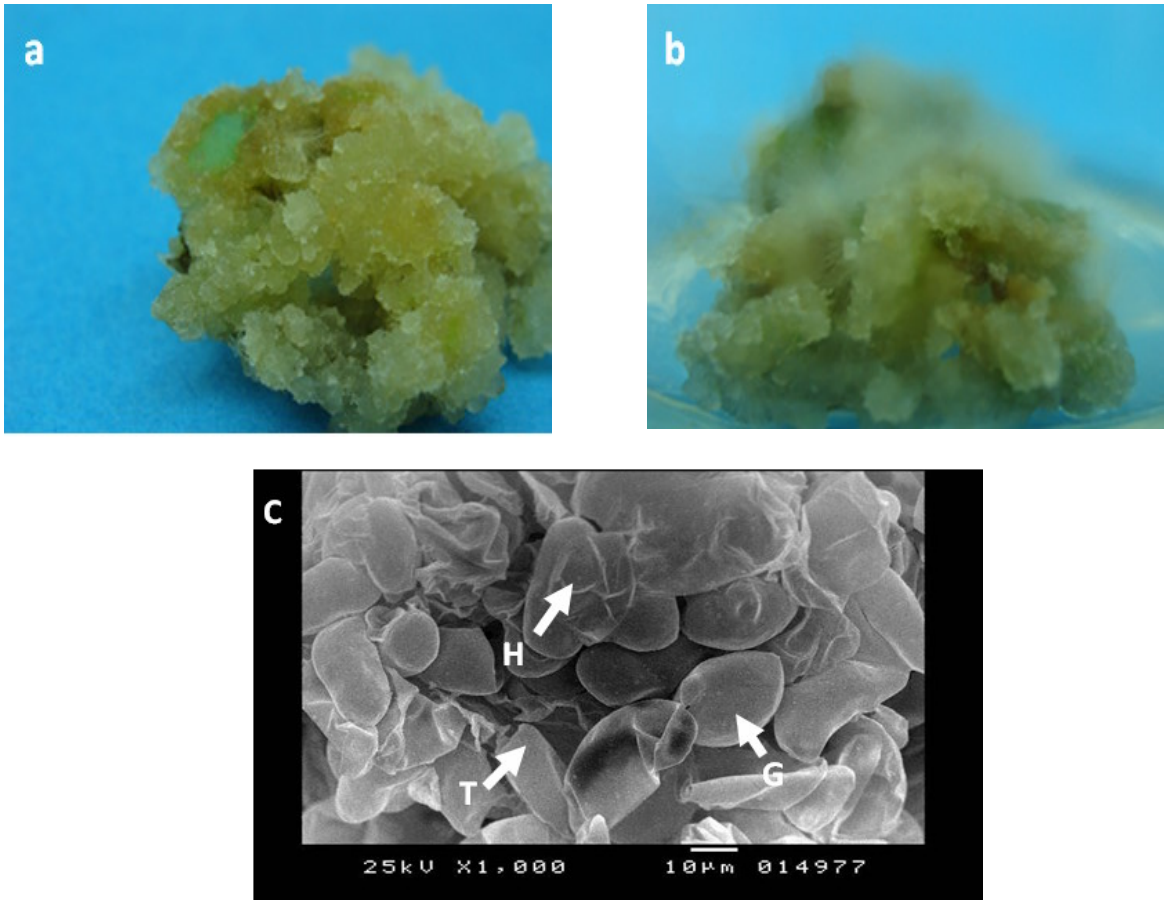

Fig. 4. Embryogenic callus of *Mirabilis jalapa* L. formed due to culture leave explants on MS medium plus 2, 4-D and KIN(a). Hairy roots formed on embryonic callus(b). Different growth stage of embryogenic structures(c); globular(G), Heart (H), Torpedo (T).

Fig. 5. Acclimization of neoformed *Mirabilis jalapa* L. plantlets *ex vitro* (left) and *in vivo* (right)

The most important factors contributing the induction of somatic embryos from the callus; both plant growth regulators and medium formulation. Buyukalaca and Mairrtuna (1996). Among the plant growth regulators, generally, auxin known to be essential for the induction of somatic embryogenesis and 2,4-D is the most commonly used auxin in this respect (Ammirato, 1983). According to Da Silva *et al*. (2005), 2,4-D was the main synthetic auxin used to induce the callogenesis. This is due to one of its main characteristics that capable of simulating the cell division in tissues of several plants to convert somatic cells to embryogenic calluses. In the present study, 2,4-D also managed to induce callus in all the concentrations tested (i.e. 0.5, 1.0, 2.0 and 4.0 mg/l). Results of this study were in harmony with the findings of Ling *et al*. (2009), in which the best embryonic callus response on *M. jalapa* tissuewas established in treatments with 1.0 to 1.5 mg/l of 2,4-D. In addition, Sudarshana *et al*. (2008) observed that the most effective auxin for callus induction from the leaves of *B. diffusa* was 2,4-D followed by KIN and BAP supplemented medium. On the other side, the main effect of KIN indicated that the absence of KIN from the MS culture brought about the highest percentage of the given trait, because of the limited role of cytokinine in this subject.

Likewise, the interaction between both applied growth regulators had a significant effect at different combination on the above-mentioned trait. Rout *et al*. (1999) reported that the KIN supplemented medium, also, induced the callus with varied percentages in *Plumbago zeylanica*; the calli derived-leaves showed greenish in colour and semi-friable and delayed the shoot proliferation. Other than that, Kaul and Sabharwal (2002) reported that Haworthia cultured in MS media with low concentrations of BAP failed to induce any callus. With regard to the number of hairy roots formed per propagule, the main effects of 2,4-D showed that the MS medium fortified with 2,4-D at 2.0 mg/l, resulted in the highest mean value of the given trait. On the other extreme, the effect of KIN declared that there was a proportional relationship between KIN and the given trait. For instance, MS-basal medium supplemented with 0.250 mg/l, gave the highest mean value of the number of hairy roots. The interaction between 2,4-D at either 1.0 or 2.0 mg/l and KIN at 0.250 mg/l, caused the highest mean number of hairy roots formed per propagule. Similar results were reported with Ling *et al*. (2009), who noticed that the hairy adventitious roots in callus induction were induced in the explants cultured with different concentration of 2,4-D. Likewise, in other plant species such as *Morus latifolia* and *Morus alba*, rooting of explants in the presence of 2,4-D was observed (Chitra and Padmaja 1999; Lu 2002).

Ex vitro and in vivo acclimatization

Figure (5) demonstrate the *ex vitro* and *in vivo* successful acclimatization of neoformed plantlets of *Mirabilis jalapa* L. However, the plants expressed true-to-type to the mother plants.

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الملخص العربي

الاكثار المعملى الدقيق وتكوين الأجنة الجسمية لنبات شب الليل باستخدام القطع البرعمية الساقيه والأوراق

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من الأتجاهات الحديثة هذه الأيام، الاهتمام بالإكثار المعملى الدقيق لنبات شب الليل وذلك لأهميتة كنبات زينة يستخدم فى تنسيق الحدائق، بالاضافة إلى إستخدامه كنبات طبى يستخدم فى الوصفات العلاجية فى كثير من دول العالم. لذلك كان الهدف من هذا البحث وجود بروتوكول للإكثار المعملى وتكوين الكالس الجنينى بإستخدام القطع البرعمية الساقية والأوراق. وتم استخدام توليفات من منظمات النمو من حامض نفثالين اسيتك اسيد، الكينيتين، حامض البيوتريك وكذلك 2,4-D باستخدام بيئة موراشيج وسكوج. وأوضحت النتائج ان استخدام توليفة من الكينيتين بتركيز(٣,٠٠ ملجم/لتر) مع حامض نفثالين اسيتك اسيد بتركيز(٠,٢٥٠ ملجم/لتر) أعطت أفضل تضاعف بالنسبة لعدد الافرع المتكونة (٢,٦٧). أما بالنسبة الى مرحلة التجذير فقد أظهرت النتائج أن استخدام حامض نفثالين اسيتك اسيد بتركيز(٠,٥٠٠ ملجم/لتر) أدى الى أفضل عدد للجذور(٦,١١). ومن ناحية اخرى أدى استخدام ال 2,4-D بتركيز(٤,٠٠ ملجم/لتر) الى الحصول على أعلى نسبة لتكوين الكالس الجنينى(١٠٠%) وأفضل حجم بالنسبة للكالس(++++) بإستخدام أوراق النبات. وتمت أقلمة النباتات الناتجة من المعمل تحت ظروف الصوبة بنجاح حتى الوصول الى مرحلة الإزهار.