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EFFECT OF CYTOKININ TYPES ON MICROPROPAGATION OF NEMAGUARD PEACH

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ABSTRCT: *Peach is one of the most important fruit crops in the world, belongs to family rosaceae."Nemaguard" peach (Prunus persica L. × Prunus davidiana) is used extensively as rootstock for peach cultivars. The rootstock and the grafted cultivar influence the vegetative and generative mass and the profitability of fruit production moreover in the efficiency of pest and disease management programs and fruit yield. This investigation was carried out to optimize invitro technique for micropropagation of Nemaguard rootstock. Effect of sodium hypochlorite concentrations (0.37, 0.50, 0.75, 1.50, 2.25 and 3.0%) and exposure periods (2, 4, 6 min) on success of sterilization of nemagurd peach explants was examined. Shoots which were sterilized with 3% NaOCl for 6 min, recorded the highest number of sterilized explants (9.7). Efect of cytokinin types and concentration on proliferation of Nemaguard peach was studied MS medium which supplemented with 2 mg/l BAP gave the highest shoot number (9.0 shoots/explant). While, MS medium supplemented with 2 mg/l $ADSO_4$ in combination with 0.5 mg/l IAA resulted in shoot number (3.0 Shoots/explant), which clear that it is lower than the shoot proliferation in presence of BAP. The lowest shoot proliferation was obtained when MS medium supplemented with KIN. Concerning shoot length of nemagurd peach, all types of used cytokinin adversely affected shoot length; while IAA was promote shoot length; MS medium supplemented with 5 mg/l $ADSO_4$ in combination with 0.5 mg/l IAA resulted in the highest shoot length (3.7 cm). Leaves number was affected by types and concentrations of cytokinins. The highest leaves number was possessed when MS medium supplemented with either 5 mg/l KIN in combination with 0.25 mg/l IAA (22 leaves / shoot) or 5 mg/l $ADSO_4$ in combination with 0.5 mg/l IAA (22 leaves / shoot). The produced shoots were successfully rooted (64%) when transplanted on half strength MS medium supplemented 0.5 mg/l IAA. The rooted plantlets showed 85% successes in acclimatization. Generally, it could be concluded that cytokinin types and concentration affected shoot proliferation of nemagurd peach. Through tested cytokinin, BAP is the most suitable cytokinin for multiplication of nemagurd peach.*

Key words: *$ADSO_4$ – BAP - KIN – IAA - micropropagation - multiplication - nemaguard - rooting – sterilization.*

INTRODUCTION

Peach (*Prunus persica L*.) is one of most important fruit crops of the world. The total planted area of peach in Egypt is about 67683 Feddans producing about 285194 tons of fruit annually (Ministry of Agric., Statistics, 2013).

The main problem with peach trees in Egypt is the declining of the orchard in a short time due to the infection of root system with root-knot nematodes, especially *Meloidogyne incognita* and *M. javanica*.

Rootstocks play major role in modern orchards. Recently, we recognize the importance of the rootstock, which has an essential value for fruit yield. The rootstock and the grafted cultivar influence the vegetative and generative mass and the profitability of fruit production (Racsko *et al.,* 2004). On other hand, the most important agricultural traits and the tree as a biotic unite such as vigor, blossom initiation, fruit set, fruit size and fruit flavor, etc.; may be, substantially, influenced by the rootstock (Tubs, 1974 and Dozier *et al.,* 1984). Moreover, the rootstock determines the

ecological fitness of the tree. Their effects can be recognized in the health status of critical tree phonological stages, tree kilter and tree sensitivity to pests and diseases (Holb, 2000 and 2002), moreover in the efficiency of pest and disease management programs and fruit yield (Holb *et al.,* 2003)

Nemaguard Peach (*Prunus persica* L. × *Prunus davidiana* Carriere) is used extensively as rootstock for peach cultivars; which is a vigorous grower and extremely disease resistant, proven resistance to root-knot nematodes, more resistant to crown gall than other rootstocks and widely used and preferred for peaches, almonds and plums.

Propagation of Nemaguard by hardwood or softwood cuttings is considering a problem (Alsalihy *et al.,* 2004) moreover, occurrence of the undesired segregation usually associated with the sexual propagation by seeds was also hoped to be entirely avoided. Seeds have double dormancy (external and internal dormancy) “External dormancy occurs when a hard, impervious seed coat acts as a barrier to water, oxygen, and the exchange of other gases, or, when seed coat contains chemical inhibitors; meanwhile internal dormancy occurs when internal conditions within the seed itself act as a barrier to germination by inhibitors (Phenolics and abscisic acid), so the seeds need different pre-germination treatments to germination as endocarp removal, stratification and gibberellic acid treatment (Davies and Duray 2011)

All nurseries use the covenantal propagation of Nemaguard by seeds because of difficult root formation of cutting. Since there are considerable cross pollination in peach, the resulted rootstocks are not uniformity and it affect the characteristics of cultivars which be grafted on it. So, vegetative propagation of rootstocks is critical issue in order to replace seedling rootstocks and avoid its bad effects on produced cultivars. There are outstanding efforts made to improve rootstock rootability (Hartmann and Hansen, 1958; Farmer and Besemann, 1974; Robitaille and Yu, 1980), but these systems have proved to be not useful enough to become standard propagation method especially because it 's production is limited by the available number of stock plant. So, establishment of a protocol for micropropagation is very important.

Whereas, micropropagation by use microshoots from Nemaguard peach mother tree as explants is a true to type propagation and most often associated with mass production (Debergh and Read, 1991). Besides plants obtained through micropropagation is less subject to the risks of nursery field infections because the laboratory procedures are carried out in a sterile environment (Battistini and Depaoli, 2002). Martinez *et al.,* (2005) pointed out the possibility of using micropropagation in the stone fruits by in vitro culture.

Cytokinins comprise a separate class of growth substances and growth regulators. They produce various effects when applied to intact plants. They particularly stimulate protein synthesis and participate in cell cycle control. The effect of cytokinins is most noticeable in tissue cultures where they are used, often together with auxins, to stimulate cell division and control morphogenesis. Added to shoot culture media, these compounds overcome apical dominance and release lateral buds from dormancy (Lindsey 1997).

The objectives of this study were:

Optimize tissue culture technique for micropropagation of Nemaguard peach rootstock through :

a. In vitro control of bacterial and fungal infection of Nemaguard, the most famous peach rootstock in Egypt.
b. Establish a protocol for invitro proliferation of Nemaguard.
c. Rooting and acclimatization of Nemaguard.

MATERIALS AND METHODS

This study was carried out at the tissue culture laboratory of Horticulture Department, faculty of Agriculture, Menoufia University, Egypt, and Laboratory of Plant Biotechnology Department, Genetic

Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City during the period 2010 - 2014.

Plant materials were obtained from vigorous shoots which were excised during April to October from seedlings of Nemaguard'(*Prunus persica*, L.× *Prunus davidiana*); peach rootstock growing at Darwish farm in Sadat City, Stem sections (3 to 5 cm) were defoliated, washed with liquid soap to remove dust, washed with running tap water for 10 minutes. Then, Nemaguard stem sections were soaked in different concentrations of NaOCl (0.37, 0.5, 0.75, 1.5, 2.25 and 3 %). Explants were shacked in disinfection solutions for various exposure periods (2, 4 and 6 minutes), then treatments were washed three times with sterile distilled water before cultured on basal MS nutrient medium (Murashige and Skoog, 1962). Each treatment included three replicates and each replicate included 10 steam sections with two nodes.

Microshoot sections (>1.5 cm in length) from established cultures were cultured in glass jars (320 ml) contained 50 ml MS medium supplemented with different concentrations of Benzyl adenine (BAP), kinetin (KIN) and adenine sulfate ($ADSO_4$) (0.0; 2.0; 3.0; 4.0 and 5.0 mg/L) individual or in combination with various concentrations of indole acetic acid (IAA) (0.0; 0.25 and 0.50 mg/L), 30 gm/L sucrose, 7 gm/L agar. The pH of the media was adjusted to 5.8 before autoclaving at 121°C at 1.2 kg/cm^2 steam pressure for 20 min. Each treatment contained five replicates and one explant per replicate. Physical conditions of culture were as reported in the first part of thesis. Shoots were subculture after 21 days. After two subcultures, shoot number/ explant, shoot length (cm) and the leaves number / shoot were recorded. Shoots (2–3 cm in length) were excised from multiplication stage and placed in 50 ml solid rooting medium in 320-ml culture jars. The rooting medium was cytokinin-free; half strength MS medium supplemented with 0.5 mg/L IAA, 3% (w/v) sucrose and 0.5% agar. Physical conditions of culture were as reported in the first part of thesis. Four weeks to the initial rooting, according to (Hammerschlag *et al.,* 1987). Rooted plantlets were rinsed with tap water to remove adhering medium and then they were transplanted to 2 inch pots filled with a peat–vermiculite 1:1 (v/v) mixture.then kept in the incubator for one week and were covered with transparent plastic bags to maintain the high humadity initially needed and grown under shade cloth (50% light reduction). The plants were watered and ventilated every day by temporarily removing the plastic bags. After 10 days, the plastic bags were punctured to allow greater gas exchange, and after 20 days the tops of the plastic bags were cut open. Then light intensity was gradually increased while relative humidity was reduced (5% per day), survival percentage was recorded after 30 days according to Channuntapipat *et al.,* (2003). Results were statistically analyzed by factorial randomized complete design using SAS (1988) package using computer software MSTAT-C (MSTAT Development Team, 1988). The least significant difference among levels of each treatment was compared using LSD. Test at 5% level according to Steel et *al.,* (1997).

RESULTS AND DISCUSSION

Invitro control of Bacterial and fungal infection: Several factors can affect success of sterilization such as season of year, position of culture, location of explant on mother plant, method of sterilization, both type and concentrations of sterilization chemical materials and finally exposure period to sterilization materials. As shown in Table (1) and Fig. (1) cleared the effect of NaOCl concentrations and exposure period on number of uncontaminated explants of Nemaguard peach. NaOCl concentrations negatively affected number of uncontaminated shoots, while, exposure period to NaOCl slightly positive affected number of uncontaminated explants. Regarding the interaction between NaOCl concentration and exposure period to sterile agent, the explants which were sterilized with 3% NaOCl and all exposure period recorded the highest number and percentage of uncontaminated explants (9.7 and 97% respectively). The same result was obtained when explants were sterilized with

2.25 % NaOCl for 6 min as exposure period. Regarding survival of sterilized explants, survival shoots number was negatively affected by both increasing of NaOCl concentrations and exposure period to sterile agent. Interaction results revealed that the high concentrations of NaOCl (2.25 % and 3.00 %) with all exposure period 2, 4 and 6 min resulted in the lowest number and percentage of survival shoots (2.0 as 20 %) and 1.3 as 13 %). On the other hand, the lowest concentration of NaOCl (0.37%) at all exposure periods possessed the highest number and percentage of survival shoots (9.7 and 97 % respectively).

Finally, it could be concluded that the best treatment should result in the highest number of uncontaminated shoots and maximize the number of survival shoots number, so, 0.75% NaOCl with exposure period 2 and 4 min possessed the wanted aim.

These results find support from Ahmad *et al,* (2003) who reported that 0.25% NaOCl had significantly increased the survival percentage of the cultured shoot tips. At 1% NaOCl, minimum survival percentage was observed. It was observed that peach shoots were sensitive to NaOCl and high concentration could damage the tissues. contamination was highest (40%) when NaOCl at 0.25% concentration was used. Thus it can be concluded that although increased concentration of NaOCl can effectively control contamination, its higher concentration badly damages the explants.

Invitro proliferation of Nemaguard:

- Effect of BAP: Results in Table (2) cleared that MS medium supplemented with 2.0 and 3.0 mg/l BAP were significantly maximized shoots number (7.0 shoots /explant). While, Shoots number were significantly reduced when MS medium supplemented with 0.25 and 0.5mg/l IAA compared with MS medium supplemented with 0.0 mg/l IAA (4.4, 4.8 and 5.2 shoots/explant, respectively). Interaction between BAP and IAA concentrations cleared that MS medium supplemented with 2.0 mg/l BAP and 0.00 mg/l IAA gave the highest shoot number/explant (9.0 shoots/explant) (Fig. 2 and 3).

Table (1): Effect of exposure period of sodium hypochlorite (NaOCl) concentrations on success of sterilization and survival of Nemaguard peach

NaOCl (%)(A)	Uncontaminated explants number				Survived explants number			
	Exposure period (min) (B)				Exposure period (min) (B)			
	2	4	6	Mean (A)	2	4	6	Mean (A)
0.37	4.0	4.7	5.0	4.6	9.7	9.7	9.7	9.7
0.50	5.0	6.0	6.0	5.7	9.7	9.7	9.0	9.4
0.75	7.0	8.0	8.0	7.7	8.0	7.0	6.0	7.0
1.50	8.0	8.0	9.0	8.3	5.0	5.0	4.0	4.7
2.25	9.0	9.0	9.7	9.2	2.0	2.0	2.0	2.0
3.00	9.7	9.7	9.7	9.7	2.0	2.0	1.3	1.8
Mean (B)	7.1	7.6	7.9		6.1	5.9	5.3	
LSD at level 5%	**A**	**B**	**A×B**		**A**	**B**	**A×B**	
	0.2	**0.2**	**0.4**		**0.2**	**0.2**	**0.5**	

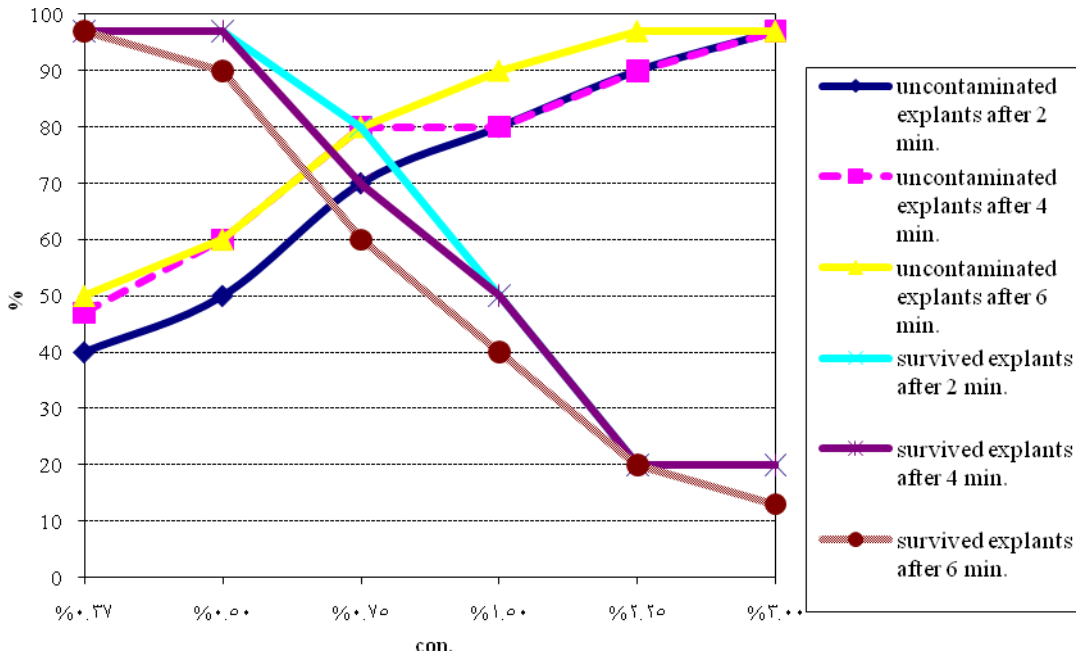

Fig. (1): Effect of exposure period of sodium hypochlorite (NaOCl) concentrations on success of sterilization and survival of Nemaguard peach

Table (2): Effect of Benzyl-Amino-Purine (BAP) and indole acetic acid (IAA) concentrations on shoot multiplication, Shoot length and Leaves number of Nemaguard peach

BAP (mg/l)(A)	Shoots No./explant				Shoot length (cm)				Leaves No./ shoot			
	IAA (mg/l)(B)				IAA (mg/l)(B)				IAA (mg/l)(B)			
	0.0	0.25	0.50	Mean (A)	0.0	0.25	0.50	Mean (A)	0 .0	0.25	0.50	Mean (A)
0	1.0	2.0	2.0	1.6	0.5	0.50	3.5	1.5	5.0	11.0	11.0	9.0
2	9.0	5.0	7.0	7.0	1.5	1.0	0.5	3	12.0	10.0	10.0	10.6
3	8.0	5.0	8.0	7.0	2.0	0.50	0.7	1.1	9.0	7.0	9.0	8.3
4	5.0	5.0	5.0	5.0	0.5	0.50	0.5	0.5	7.0	8.0	9.0	8.0
5	4.0	5.0	2.0	3.6	0.5	0.50	0.3	0.4	10.0	6.0	4.0	6.6
Mean (B)	5.4	4.4	4.8		1.0	0.6	1.1		8.6	8.4	8.6	
LSD at 5%	A	B	AxB		A	B	AxB		A	B	AxB	
	0.4	0.2	0.6		0.2	0.1	0.3		0.8	0.6	1.5	

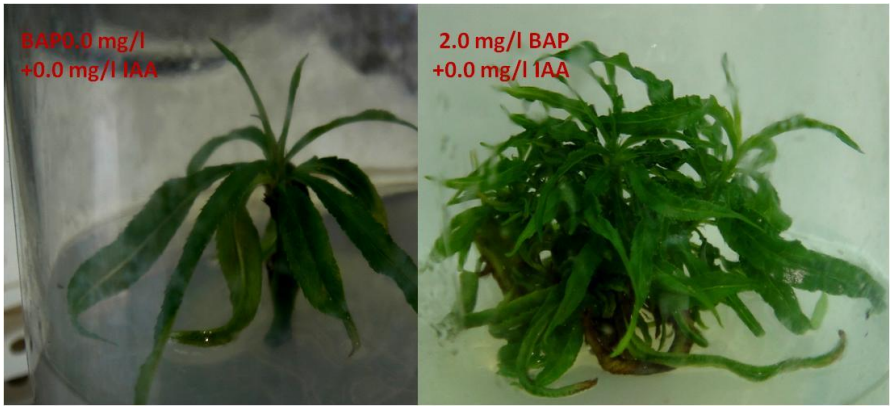

Fig. (2): Effect of both Benzyl aminopurine (BAP) and indole acetic acid (IAA) concentrations on multiplication parameters of Nemaguard peach

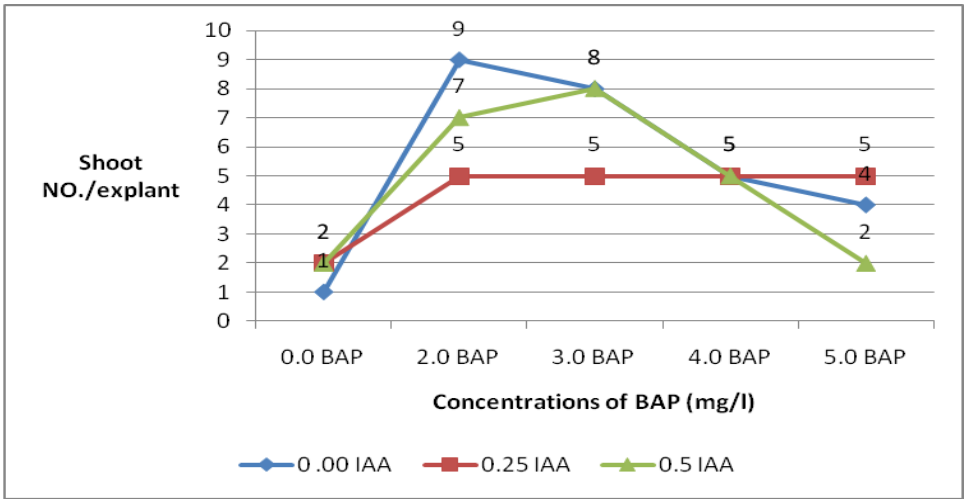

Fig. (3): Effect of Benzyl-Amino-Purine (BAP) and indole acetic acid (IAA) concentrations on shoot multiplication of Nemaguard peach

These results find support from the previous workers like Hammerschlag *et al.,* (1987) who reported that response of shoot induction increased with increasing of hormone level. However shoot induction response was optimum at 1.0 mg/l of BAP, while, shoot induction decreased with increasing of hormone concentration.

Shoot length was adversely related with BAP concentrations. MS medium supplemented with 0.0 mg/l BAP gave the highest shoot length (1.5 cm) while MS medium supplemented with 4.0 and 5.0mg/l BAP resulted in the lowest shoot length (0.5 and 0.4 cm, respectively). The same results were observed in the case of different concentration of IAA. Interaction between concentrations of both BAP and IAA revealed that MS medium supplemented with 0.0 mg/l BAP with 0.5 mg/l IAA gave the highest shoot length (3.5 cm) followed by 3.0 and 2.0mg/l BAP in absence of IAA (2.0 and 1.5 cm, respectively), However 5 mg/l BAP with 0.5 mg/l IAA gave the lowest shoot length (0.3 cm). Generally, it could be concluded that IAA at high concentration enhance shoot length, while BAP at high concentrations enhance shoot proliferation and inhibit shoot length.

These results are similar to those obtained by Leontiev-Orlov *et al.,* (2000a, 2000b) and Pérez- Tornero *et al.,* (2000) who observed that increasing levels of cytokinins inhibited shoot elongation in plum trees.

Increasing BAP concentration resulted in decreasing leaves number, it ranged from 10.6 to 8.0 leaves /explant. While IAA concentrations did not significantly affected number of leaves of Nemaguard peach. Data of the interaction showed that the highest leaves number were observed on MS medium supplemented with 2 mg/l BAP without IAA (12.0 leaves/explant) followed by 0.25 and 0.50 mg/l IAA without BAP (11.0 leaves/explant for the both concentrations).

These results are in accordance with that of Pérez-Tornero and Burgos (2000) who suggested that the effect of nutrient media and BA concentration were strongly genotype dependent, and best results were achieved when BA concentration was between 1.78 and 3.11 µM.

-Effect of KIN: Results in Table (3) clear that Shoots number were significantly maximized when shoots were cultured on MS medium supplemented with 4mg/l KIN (1.8 shoots/explant). Generally, shoots number were significantly adversely affected with increasing KIN concentrations. Interaction between KIN and IAA concentrations cleared that MS medium supplemented with 4 mg/l KIN in combination with 0.25 or 0.50 mg/l IAA significantly enhanced shoots number (2.2 shoots/explant for each treatment) (Fig. 4 and 5)

These results are, to some extent, in agreement with the data obtained by Martinelli (1985) who demonstrated that kinetin promoted only growth of single shoots of peach-almond hybrids "Hansen 2168" and "Hansen 536".

Table (3): Effect of Benzyl-Amino-Purine (KIN) and indole acetic acid (IAA) concentrations on shoot multiplication, Shoot length and Leaves number of Nemaguard peach

KIN (mg/l)(A)	Shoots No./explant				Shoot length (cm)				Leaves No./ shoot			
	IAA (mg/l)(B)				IAA (mg/l)(B)				IAA (mg/l)(B)			
	0.0	**0.25**	**0.50**	Mean (A)	**0.0**	**0.25**	**0.50**	Mean (A)	**0 .0**	**0.25**	**0.50**	Mean (A)
0	1.0	2.0	2.0	1.6	0.5	0.5	3.5	1.5	5.0	11.0	22.0	12.6
2	1.0	1.0	1.0	1.0	2.0	3.0	0.5	1.8	13.0	17.0	7.0	12.3
3	1.0	1.0	1.0	1.0	1.0	1.0	1.5	1.1	10.0	10.0	13.0	11.0
4	1.0	2.2	2.2	1.8	1.0	1.6	1.6	1.4	10.0	17.0	17.0	14.6
5	1.0	1.0	1.0	1.0	1.5	2.4	0.5	1.4	13.0	22.0	8.0	14.3
Mean (B)	1.0	1.4	1.4		1.2	1.7	1.5		9.3	15.4	13.4	
LSD at 5%	**A**	**B**	**AxB**		**A**	**B**	**AxB**		**A**	**B**	**AxB**	
	0.1	**0.1**	**0.1**		**0.3**	**0.2**	**0.6**		**0.8**	**0.6**	**1.5**	

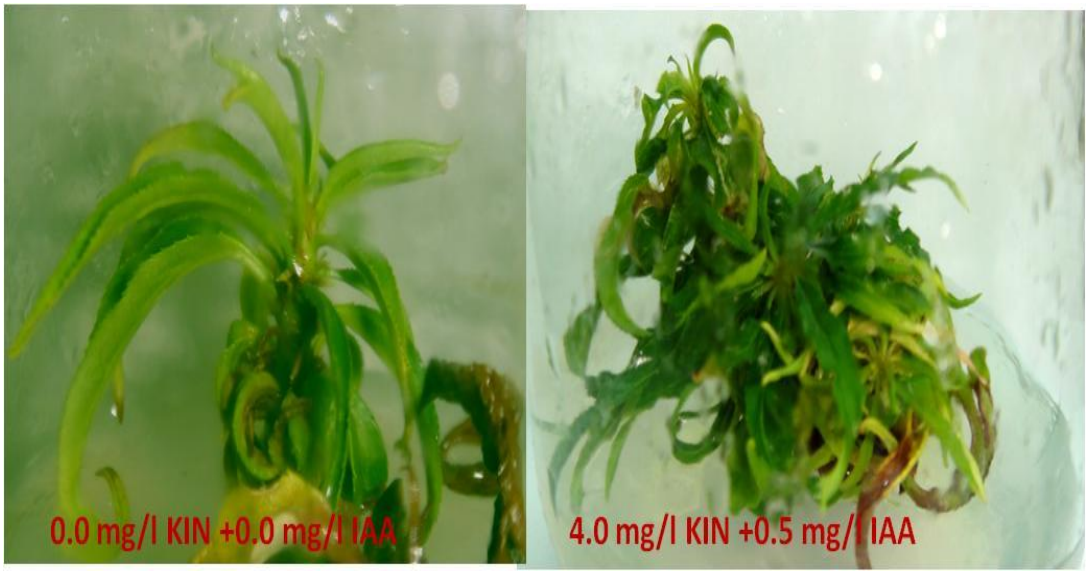

Fig. (4): Effect of different concentrations of Kinetin (KIN) and indole acetic acid (IAA) concentrations on shoot parameters of Nemaguard peach

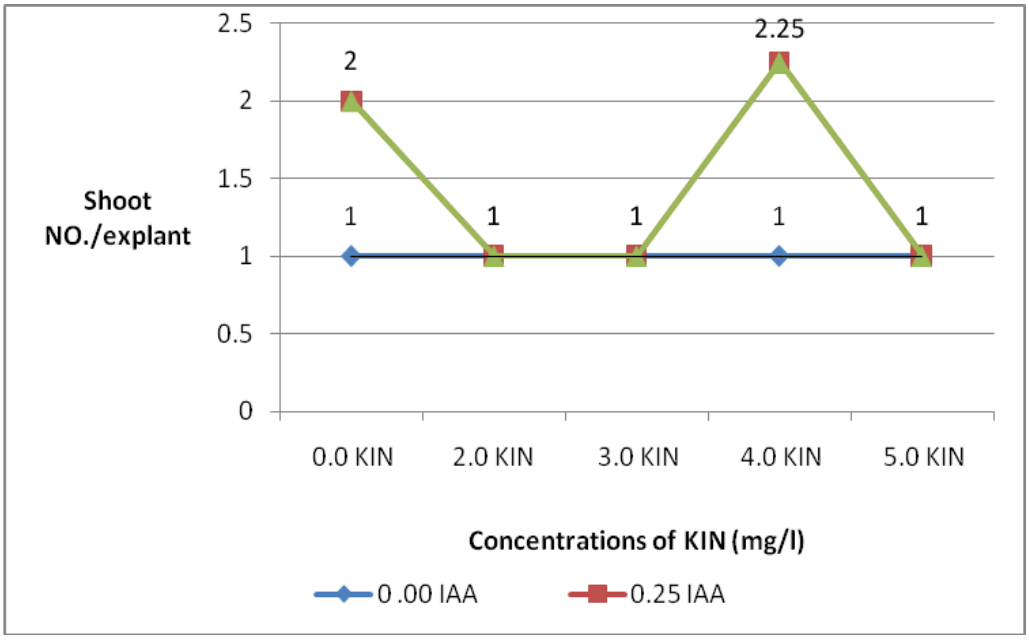

Fig.(5): Effect of different concentrations of Kinetin (KIN) and indole acetic acid (IAA) concentrations on shoot multiplication of Nemaguard peach

Shoot length was negatively related with KIN concentrations, the highest shoot length was obtained when MS medium supplemented with 2 mg/l KIN (1.8 cm) while MS media supplemented with 3, 4 and 5 mg/l KIN resulted in decreased shoot length (1.1, 1.4 and 1.4 cm, respectively) with no significant differences between each other. Adding different concentrations of IAA (0.0, 0.25 and 0.50mg/l) to MS medium resulted in increasing shoot length significantly (1.2, 1.7 and 1.5 cm, respectively). Interaction

between concentrations of both KIN and IAA revealed that MS medium supplemented with 0.50 mg/l IAA individual or MS medium supplemented with 2 mg/l KIN in combination with 0.25 mg/l IAA gave the highest shoot length (3.5 and 3.0 cm, respectively), while MS supplemented with 5 mg/l KINin combination with 0.50 mg/l IAA gave the lowest shoot length (0.5 cm). Comparable results were introduced by Silva *et al.,* (2003) and Al-Qudah *et al.,* (2008) who declared that high KIN concentration inhibited shoot elongation. On the contrary, Obaid (2012) indicated that 4 mg/l increased shoot length from nodes cutting of the peach rootstock baydawi. Leaves number was significantly affected by KIN concentrations, MS media which were supplemented with 4 or 5 mg/l KIN were superior (1.6 and 14.3 leaves/shoot, respectively). Also, leaves number was affected by different concentrations of IAA. MS medium containing 0.25mg/l IAA resulted in the highest number of leaves (15.0 leaves /shoot). Results revealed that interaction between different concentrations of KIN and IAA affected number of leaves of Nemaguard peach. MS media supplemented with 0 mg/l KIN in combination with 0.50 mg/l IAA or 2 mg/l KIN in combination with 0.25 mg/l IAA or 5.0 mg/l KIN in combination with 0.25 mg/l IAA resulted in maximization of leave number of Nemaguard peach (22.0, 17.0 and 22.0 leaves /shoot, respectively).

- Effect of $ADSO_4$: Results in Table (4) cleared that shoots number were significantly maximized with 2 mg/l $ADSO_4$ (1.6 shoots/explant). While, shoots number were significantly minimized with increasing $ADSO_4$ concentrations. Interaction between $ADSO_4$ and IAA concentrations cleared significantly high shoots number (3 shoots/explant) at concentration 2 mg/l $ADSO_4$ 0.50 mg/l IAA (Fig. 6 and 7).

Table (4): Effect of Benzyl-Amino-Purine ($ADSO_4$) and indole acetic acid (IAA) concentrations on shoot multiplication, Shoot length and Leaves number of Nemaguard peach

$ADSO_4$ (mg/l)(A)	Shoots No./explant				Shoot length (cm)				Leaves No./ shoot			
	IAA (mg/l)(B)				IAA (mg/l)(B)				IAA (mg/l)(B)			
	0.0	0.25	0.50	Mean (A)	0.0	0.25	0.50	Mean (A)	0.0	0.25	0.50	Mean (A)
0	1.0	1.0	1.0	1.0	0.5	0.5	3.5	1.5	5.0	11.0	22.0	12.6
2	1.0	1.0	3.0	1.6	1.0	1.3	1.7	1.3	16.0	13.0	13.0	14.0
3	1.0	1.0	1.0	1.0	2.7	3.0	3.5	3.1	16.0	17.0	22.0	18.3
4	1.0	1.0	1.0	1.0	2.0	2.5	2.0	2.1	16.0	19.0	15.0	16.6
5	1.0	1.0	1.0	1.0	2.7	2.0	3.7	2.8	16.0	15.0	22.0	17.6
Mean (B)	1.0	1.0	1.4		1.7	1.8	2.8		13.8	15.0	18.8	
LSD at 5%	A	B	AxB		A	B	AxB		A	B	AxB	
	0.1	0.1	0.2		0.1	0.2	0.3		0.7	0.6	1.3	

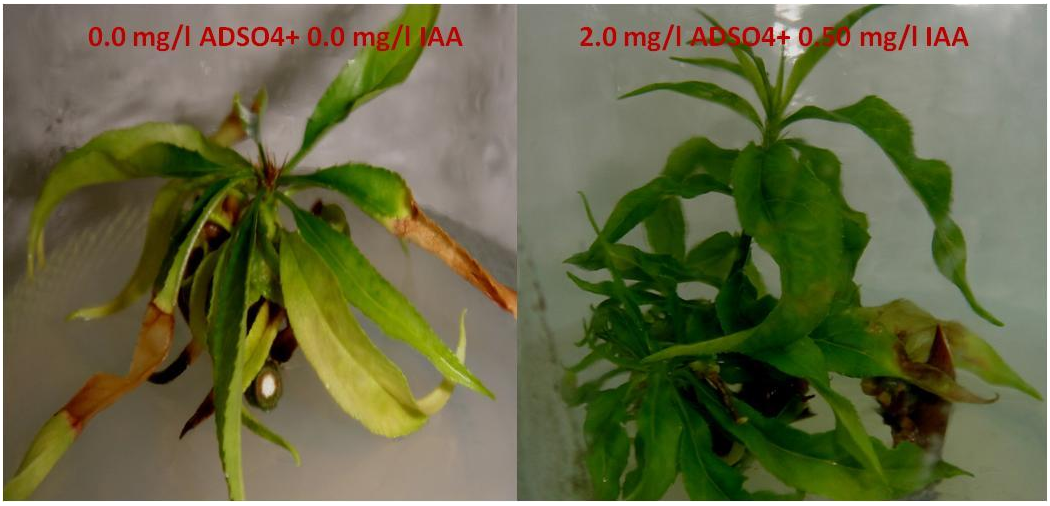

Fig. (6): Effect of Adenine sulfate ($ADSO_4$) and indole acetic acid (IAA) concentrations on growth parameters of Nemaguard peach *in vitro*

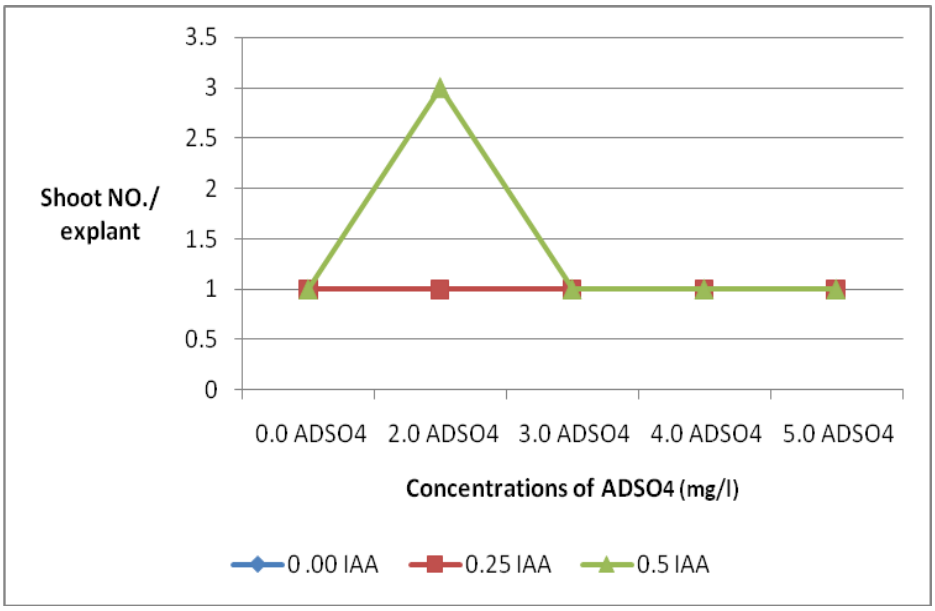

Fig (7): Effect of Adenine sulfate ($ADSO_4$) and indole acetic acid (IAA) concentrations on shoot multiplication of Nemaguard peach rootstock

shoot length was adversely related with $ADSO_4$ concentrations which use 3 mg/l $ADSO_4$ give high shoot length results (3.1 cm) while 4 and 5 mg/l $ADSO_4$ give low shoot length (2.1 and 2.8 cm, respectively). Interaction between concentrations of both $ADSO_4$ and IAA revealed that 3 and 5 mg/l $ADSO_4$ with 0.5 mg/l IAA gave the highest shoot length (3.5 and 3.7 cm respectively), However 0 and 2 mg/l $ADSO_4$ with 0.0 and 0.25 mg/l IAA gave the lowest shoot length (0.5 cm).

MS media which supplemented with 3 or 5 mg/l $ADSO_4$ gave the highest leaves number (18.3 and 17.6 leaf/shoot, respectively). While, MS media which supplemented with 0.0 and 2 mg/l $ADSO_4$

resulted in the lowest number of leaves (12.6 and 14 leaf/shoot, respectively). Interaction between concentrations of both $ADSO_4$ and IAA revealed that MS media which supplemented with 0, 3, and 5 mg/l $ADSO_4$ in combination with 0.5 mg/l IAA resulted in the highest leaves number (22.0 leaf /shoot for all combinations). On other side, MS media which supplemented with 2 mg/l $ADSO_4$ in combination with 0.25 and 0.50 mg/l IAA resulted in the lowest leaves number (13.0 leaf /shoot for both combinations).

Finally, it could analysis the different among the effect of various cytokinin types and concentrations on shoot number and shoot length. Fig. (8) and Fig. (9) Showed that BAP is the best choice for Nemaguard peach multiplication, while $ADSO_4$ is the best choice when shoot length is preferred.

Invitro rooting rate are 64% as well as root number per shoot in rooting stag by using 0.5 mg/L of IAA. The rooted plants showed 85% survival rate in acclimatization stage and plants produced good green color Fig. (10).

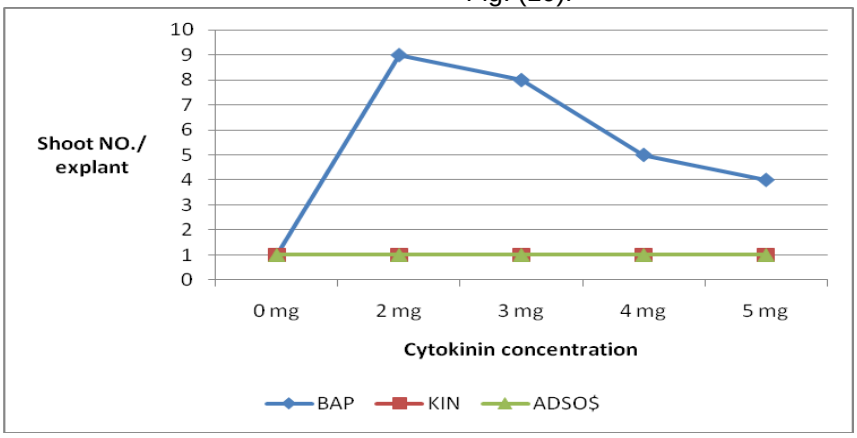

Fig. (8): Comparison between cytokinin types and concentrations on shoot number of Nemaguard peach

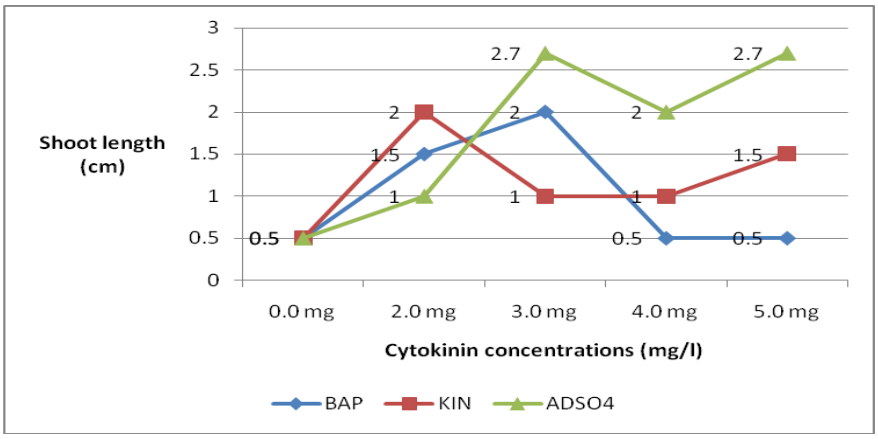

Fig. (9): Comparison between cytokinin types and concentrations on shoot length of Nemaguard peach .

Fig. (10). Rooting and acclimatization of Nemaguard peach

Recommendation:

From the results obtained in our experiments on Nemaguard peach rootstock micropropagation the fowling recondition should be precede:

1. To obtain the highest of uncontaminated explants and survival number of explants in establishment stage, using 3% NaOCl for 2 minutes should be flowed.
2. To obtain the highest shoots number/explant of use 2.0 mg/L BAP MS medium in multiplication stage.
3. To obtain the highest shoot length adding 5 mg/L $ADSO_4$ in combination with 0.5 mg/L IAA to MS medium.
4. To obtain the highest leaves number adding 5 mg/L $ADSO_4$ + 0.5 mg/L IAA or 5 mg/L KIN + 0.25 mg/L IAA in the MS medium.

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تاثير انواع السيتوكينينات المختلفة علي الاكثار الدقيق لخوخ النيماجارد

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الملخص العربي

يعتبر الخوخ واحد من اهم محاصيل الفاكهه علي مستوي العالم وينتمي الي العائله الورديه ويستخدم علي نطاق واسع كأصل للتطعيم في مزارع الخوخ . يؤثر كلا من الاصل والطعم علي النمو الخضري والثمري. تم اجراء هذا البحث لتحديد طريقه للاكثار الدقيق لاصل النيماجارد حيث تم اختبار تاثير تركيزات مختلفه من هيبوكلوريت الصوديوم (٠.٣٧ – ٠.٥ – ٠.٧٥- ١.٥ – ٢.٢٥ و ٣.٠ %) ولفترات تعرض (٢ - ٤ – ٦ دقائق) علي نجاح التعقيم لخوخ النيماجارد حيث ان السيقان التي تم تعقيمها باستخدام ٣% هيبوكلوريت الصوديوم لمده ٦ دقائق سجلت اعلي عدد نبات غير ملوثه. كما تم اختبار تاثير انواع وتركيزات مختلفه من السيتوكينينات علي تضاعف خوخ النيماجارد حيث ان استخدام بيئه زراعه موراشج وسكوج مزود ٢ ملي جرام / لتر بنزيل ادينين اعطت اعلي عدد فروع (٩ افرع) في حين استخدام بيئه زراعه موراشج وسكوج المضاف اليها ٢ ملي جرام / لتر ادينين سلفات مع ٠.٥ ملي جرام / لتر اندول حمض الخليك اعطت عدد فروع (٣ افرع) حيث من الواضح ان ذلك المعدل اقل من معدل التضاعف في وجود البنزيل ادينين بينما استخدام بيئه زراعه موراشج وسكوج المضاف اليها الكينتين اعطي اقل معدل للتضاعف. وبالنسبه لطول الافرع لخوخ النيما جارد كل انواع السيتوكينين المستخدمه كان لها اثر سلبي علي طول الافرع في حين ان استخدام اندول حمض الخليك شجع طول الافرع ، استخدام بيئه زراعه موراشج وسكوج المضاف ٥ ملي جرام/ لتر ادينين سلفات مع ٠.٥ ملي جرام / التر اندول حمض الخليك اعطي اعلي طول للافرع (٣.٧ سم). استخدام انواع وتركيزات مختلفه من السيتوكينين اثر علي عدد الاوراق ، استخدام بيئه زراعه موراشج وسكوج المضاف ٥ ملي جرام / لتر كينتين مع ٠.٢٥ ملي جرام / لتر اندول حامض خليك اعطي اعلي عدد للاوراق (٢٢ ورقه). تم تجذير النباتات الناتجه بنسبه نجاح (٦٤%) في حاله استخدام بيئه زراعه موراشج وسكوج بنصف قوه الاملاح والمضاف اليها ٠.٥ ملي جرام / لتر اندول حمض الخليك. وكان نسبه نجاح النبيتات الناتجه في مرحله الاقلمه (٨٥%). ومن هذا نستنتج ان الانواع والتركيزات المختلفه من السيتوكينينات تؤثر علي تضاعف خوخ النيماجارد. ومن خلال اختبار السيتوكينينات المختلفه كان البنزيل ادينين انسب سيتوكينين لتضاعف خوخ النيماجارد.

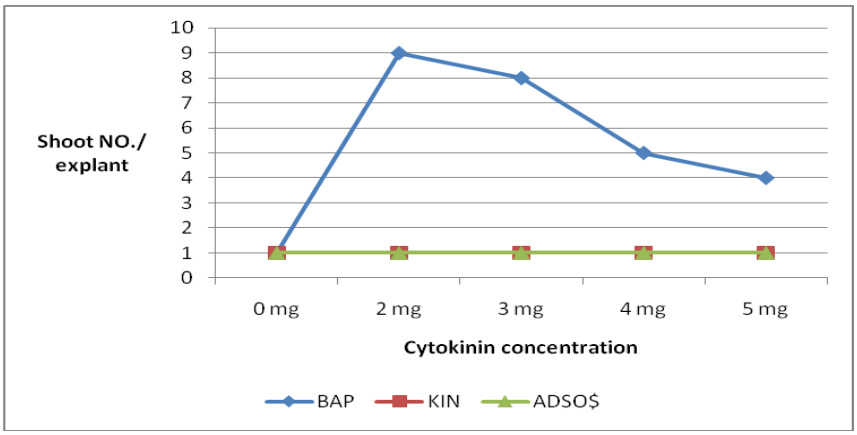

Fig. (8): comparison between cytokinin types and concentrations on shoot number of Nemaguard peach

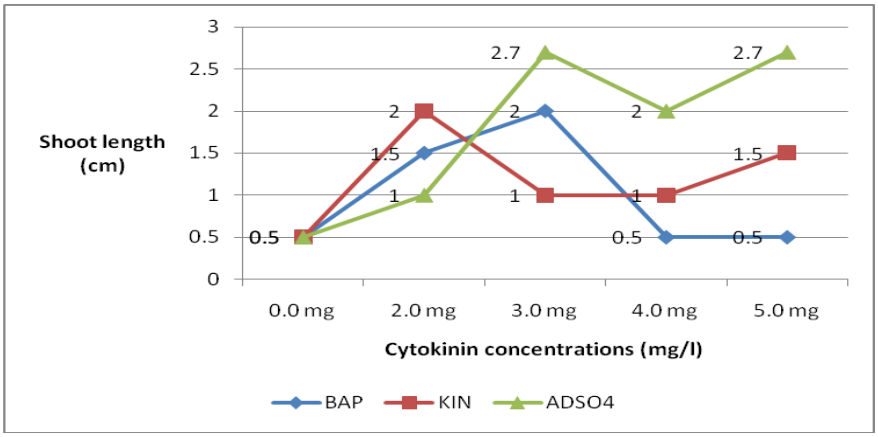

Fig. (9): comparison between cytokinin types and concentrations on shoot length of Nemaguard peach

Invitro rooting rate are 64% as well as root number per shoot in rooting stag by using 0.5 mg/L of IAA. The rooted plants showed 85% survival rate in acclimatization stage and plants produced good green color Fig. (10).

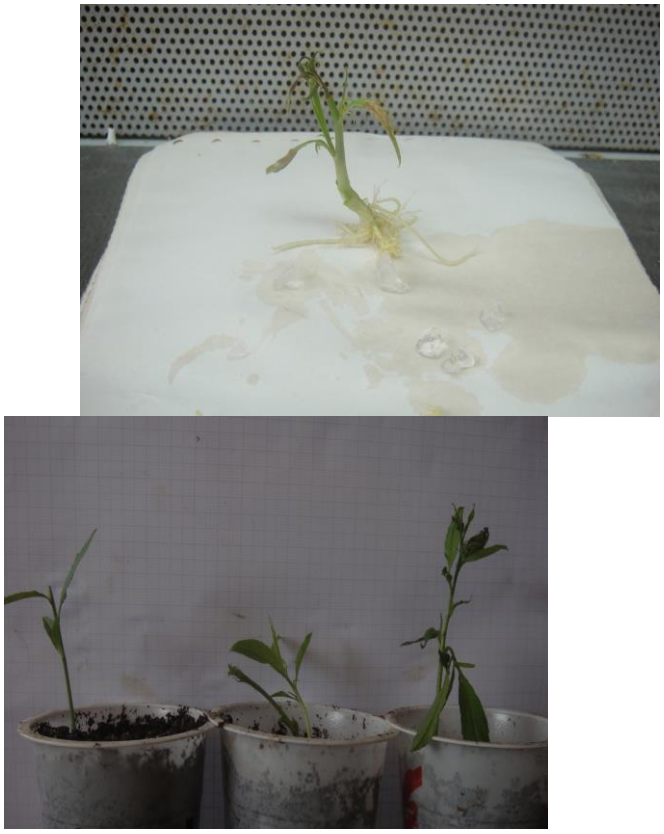

Fig. (10). Rooting and acclimatization of Nemaguard peach

Recommendation:

From the results obtained in our experiments on Nemaguard peach rootstock micropropagation the fowling recondition should be precede:

5. To obtain the highest of uncontaminated explants and survival number of explants in establishment stage, using 3% NaOCl for 2 minutes should be flowed.
6. To obtain the highest shoots number/explant of use 2.0 mg/L BAP MS medium in multiplication stage.
7. To obtain the highest shoot length adding 5 mg/L $ADSO_4$ in combination with 0.5 mg/L IAA to MS medium.

8. To obtain the highest leaves number adding 5 mg/L $ADSO_4$ + 0.5 mg/L IAA or 5 mg/L KIN + 0.25 mg/L IAA in the MS medium.

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