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In vitro PROPAGATION OF HANSEN-536 PEACH AND MAC-9 APPLE ROOTSTOCKS

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ABSTRACT

The peach x almond hybrid (Hansen-536) and MAC-9 apple rootstocks were micropropagated through tissue culture technique (using shoot tip and one node cutting explants). Data obtained from different experiments conducted during four stages of tissue culture technique could be summarized as follows:

1- Using the MS medium supplemented with the intermediate rate of both BAP+2ip combination (each at 2.0 mg/L) was the superior for two eaxplants types of Hansen 536, while with MAC9 apple rootstock the higher rate (2.5 ml/L) of two cytokinins kinds was the most effective. Hence, the higher survival %, elongation and growth, as well as least browning rate of cultured explants were obtained.

2- During proliferation/multiplication stage level of two cytokinins play a great role, where the superior for Hansen 536 and MAC9 apple rootstocks, respectively, where the best measurements of (average number and length of proliferated shootlets). Besides multiplication rate during two seasons reached (3.2 and 5.6) and (3.6 and 3.8) folds for Hansen 536 peach and MAC 9 apple rootstocks multiphied on former and later 2ip+BAP combinations, respectively. On the other hand, multiplication rate was progressively increased as the number of subculture advanced, where 4th one resulted in (8.0) and (7.4) folds for Hansen 536 and MAC9 apple rootstocks multiplied on the BAP+2ip supplemented MS medium each at 1.0 or 1.5 mg for 1st and 2nd rootstocks, respectively.

3- During rooting stage, the one half strength MS medium supplemented with two IBA and NAA auxins (each at 1.5 mg/L) improved all rooting parameters especially rooting rate which reached 80% for two rootstocks during both seasons. Besides, one half strength MS medium supplemented with 100 mg/L PG+IBA at either 1.0 mg/L or 2.0 mg/L resulted in 100% rooting for peach and Apple rootstocks, respectively.

4- During acclimatization stage, planting/growing medium (sand + peatmos + vermiculite mixture at equal proportion by volume) proved its superiority over other in investigated mixtures (growing media), where the highest survival rate (80%) for plantlets of both rootstocks with benefits on stem height was recorded under greenhouse condition.

INTRODUCTION

Traditionally, peach seedlings have been used as rootstocks for peach and propagators used their own local source of seed for seedling productions. The most commonly used rootstocks are Nemaguard, Flordaguard and three peach-almond hybrid rootstocks developed in California. Two of the peach-almond hybrids, the other is Hansen.

Hansen 536 cultivar was related from a seedling population of the cross Almond B x peach selection 1-8-2 mad by the late carl Hansen, 1964. Hansen 536 was selected as the most, tolerant to phytophthora syrugae, drought tolerant, more tolerant to lime-induced chlorosis and show less sodium and chloride uptake than peach (Hansen *et al.*, 1981 and Kester and Asay, 1986). This cultivar most

useful in calcareous soils and under marginal or stressful conditions as well as is grafcomatible to almond, peach and Japanese plum cultivars. Hansen 536 cultivar, is too difficult to propagate through cutting or layering (Ismail, 1998).

Apple is considered as the most important economic fruit among the other fruit trees. The recently introduced apple rootstocks encouraged the horizontal extension of apple trees in the new reclaimed soils. The net return is very high as compared with the other fruit trees.

Conventional propagation of either Hansen 536 and MAC9 apple rootstocks failed to overcome the gab between the large number demand and the actual number produced. Moreover, occurrence of the undesired segregations usually associated to the sexual propagation by seeds was hopped to be entirely avoided. Micropropagation is a true to type propagation and most often associated with mass production.

So, in order to meet the increasing demand for a homogenous nursery transplants of such important two rootstocks, this investtigation was initiationed to investigate the possibility of using micro-propagation technique as an ideal vegetative propagation method in this concern.

MATERIALS AND METHODS

This investigation was conducted at the tissue culture Laboratory, Desert Research center during 2006 and 2007 seasons.

A protochol for *in vitro* propagation using tissue culture technique as a means of rapid clonal propagation of two imported rootstocks Hansen 536 peach and MAC9 apple rootstocks was established.

Shoot tips and one stem node cutting were the plant material used for this investigation. Both plant materials of two rootstocks were collected from trees located at the Farm of the Faculty of Agriculture Ain Shams Univ. Shoot tips and stem nodal cuttings of two rootstock were prepared from active shoots in April from seven and 3 year-old trees, for Hansen 536 peach (Almond hybrid) and MAC9 apple rootstocks, respectively.

The collected active developing shoots (4-5 cm in length) for preparing both explant types (shoot tip and one node cutting) put in polyethelen bags and directly transferred to the Laboratory, where they were immediately soaked in running water for ½ hour to get rid of dirts, dust and any residues. In order to get rid of phenolic compounds from explants and overcome culture browning, explants were soaked in antioxidant of 150 mg/L citric acid and 100 mg/L ascorbic for 2 hrs before culturing. The most important natural inhibitor of diphenol oxidase is ascorbic acid (Vigyaza and Mihalyi, 1976). Ascorbic acid may also act directly on the enzyme by chelating with prosthetic group (Vigazo. 1981).

Shoot tips of both rootstocks (MAC9 and Hansen 536) were excised from the terminal part of the shoots with 5-10 mm long containing the apical meristem, where one node cuttings explants were 1-2 cm. Explants of shoot tips and stem node sections of both rootstocks (MAC9, Hansen 536) were cultured on Murashige and Skoog (1962) basal medium (MS medium) supplemented with 7.0 g/L Difico-Bacto agar and 30g/L sucrose. Different types and concentration of additive substances were also added to MS medium according to the investigated stage as will be mentioned later. The pH of the media was adjusted to 5.7-5.8 and autoclaved at 121°C and 1.5 kg/cm^2 steam pressure for 20 minutes.

The complete randomized block design with five replications was used for arranging the differential investigated treatments including in each experiment during various developmental stages. So the following experiments were carried out during both experimental seasons:

I- Establishment stage:

In this stage two experiments were conducted each was devoted for two explant type (shoot tip and one node cutting) of each rootstock to investigate effect of adding two cytokinin types namely G-benzyl amino purine (BAP) and 2-isopentznyl-adenine (2ip) combined together (each at 3 rates 1.5; 2.0 and 2.5 mg/L), beside cytokinin omission as control to the establishment MS medium in order to find out the most effective cytokinin treatments by which the highest (survival, growth and shoot elongation) percentages, as well as the least % or completely absent of browning could be achieved for both cultured explant types of two rootstocks. Thus, the following four cytokinins treatments investtigated in each experiment were as follows:

1- Cytokinin omitted MS medium (control).
2- BAP + 2ip each at 1.5 mg/L were supplemented to MS medium.
3- BAP + 2ip each at 2.0 mg/L were supplemented to MS medium.
4- BAP + 2ip each at 2.5 mg/L were supplemented to MS medium.

Each treatment replicated five times, each replicate represented by twenty explants. In each experiment, shoot tip and one node cutting explants of both studied rootstocks were surface sterilized using sodium hybochlorite (NaOCl-clorox 10% for 20 min.) and cultured individually in the tubes of the corresponding treatment. Cultured explants were incubated under 16 hours of artificial high (with flurocent tubes of 1500-2000 lux intensity) and 8 hours dark at temperature of 28±2°C. This stage was extended to four weeks. Data represented browning, survival, developing growth percentages and shoot elongation were recorded three weeks after culturing during both seasons of study.

2- Shoot proliferation stage:

Plant materials (shoot tips) needed for two-rootstocks during this stage (shoot proliferation), were already prepared from newly emerged shoots had been developed from cultured shoot tips on MS medium supplemented with BAP + 2ip each at 2.5 mg/L throughout the previous stage (establishment).

In this stage the following treatments of three BAP and 2ip combinations besides BAP and 2ip omitted medium used as control were investigated:

1- BAP and 2ip omitted MS medium (control).
2- BAP at 1.0 mg/L + 2ip at 0.5 mg/L supplemented to MS medium.
3- BAP at 1.0 mg/L + 2ip at 1.0 mg/L supplemented to MS medium.
4- BAP at 1.5 mg/L + 2ip at 1.5 mg/L supplemented to MS medium.

Each treatment replicated five times, where every replicate was represented by 20 cultured explants (shootlets) during two seasons of study. Data concerned the response to different treatments were recorded after 4 weeks of culturing as an average of both measurements (number and length) and increment % of proliferated shootlets.

An additional experiment was carried out in order to study the effect of subculture number on some measurements i.e., average (number and length) of proliferated shootlets and proliferation percentage. The average of two seasons, was recorded. Taking into consideration that the required plant material (proliferated shootlets) for this experiment were carefully selected from such shootlets proliferated on the culture media capable to induce the greatest proliferation percentage throuhout the aforesaid step of this stage. In this respect, MS basal medium supplemented with 1.0 mg/L BAP + 1.0 mg/L 2ip was choosen for evaluating the effect of subculture number on proliferation measurements of Hansen 536 peach rootstock, while for MAC9 apple rosstock MS medium supplied with 1.5 mg/L BAP + 1.5 mg/L 2ip was used for this purpose due to its superiority in this concern.

3- Rooting stage:

The proliferated (regenerated) shoots 4 cm in length which originated from shoot cultures during (shoot proliferation stage) of both rootstocks (MAC9 and Hansen 536) were used as plant materials for rooting stage. Shoots which were cultured on MS basal medium supplemented with either 1.0 mg/L BAP + 1.0 mg/L 2ip for Hansen 536 rootstock

or 1.5 mg/L BAP + 1.5 mg/L 2ip for MAC9, apple rootstock were used for this stage.

To select the best auxin combinations and their concentrations capable for enhancing and maximizing rooting parameters and rooting %, half strength MS basal medium supplemented with IBA (Indole-3-butyric acid) at 4 concentrations (0.5, 1.0, 1.5 and 2.0 mg/L) combined with either NAA (Naphthalene acetic-acid) at 4 concentrations (0.5, 1.0, 1.5 and 2.0 mg/L) or PG (poly ethylene glycol at 100 mg/L) were used as rooting media.

Shoots, 4-5 cm in length regenerated from shoot tips were transferred individually in culture tube containing the corresponding investigated rooting media. Cultured shoots were kept and incubated at dark and 20°C for one week then transferred to light regime 16 hours light and 8 hours darkness per day.

Rooting percentage, average number and length of new developed roots were recorded after four weeks later from culturing in the different investigated rooting media.

4- Acclimatization stage:

This stage considered as the most important limiting factor that determines to great extend the successful or failure of using the tissue culture technique as an applicable mean for peach and apple micropropagation.

Some anatomical characteristics of the in vitro regenerated plantlets like as complete/partial lacking of both upper and lower cuticle layers of leaf surface is the real reason for this problem.

The healthy plantlets (rooted shoots) which were developed from rooting stage for both investigated species were rinsed thoroughly with tap water to remove any medium residues then immersed for 5 minutes in 0.5% benelate fungicide solution and finally soaked in IBA (2000 ppm) rooting solution. Plantlets were transferred individually (each in 15 x 15 cm dimension black plastic pot) filled with one of the following growing media:

1. Sand + peat moss (at 1 : 1 ratio v: v)
2. Sand + vermiculite (at 1 : 1 ratio v: v)
3. Peat moss+ vermiculite (at 1 : 1 ratio v: v)
4. Sand + peat moss + vermiculite (at 1: 1: 1 ratio by volume)

The higher survival % and vigour growth of the newly transferred plantlets to the greenhouse mainly depending on reducing transpiration rate, persisting water supply and mineral elements. Each plantlet was covered with white transparent poly ethylene bag to keep the relative humidity around plantlets ranged from 90 – 95% during the first three weeks. One week later from planting, punching holes in poly ethylene bags were carried out periodically for two weeks, where the cover was completely removed and plantlets allowed to grow under free greenhouse conditions.

In addition, plants were watered with quarter MS inorganic salts once a week and sprayed with fungicide along the acclimatization stage which extend for eight weeks. Survival percentage and average stem length for both two rootstocks at the end of this stage for each experiment season were recorded. Presented data were an average of two experimental seasons

Each treatment (investigated growing medium) was represented by four replicates each with five individual plantlets.

5- Statistical analysis:

All data were subjected to analysis of variance and significant difference among means were determined according to (Snedecor and Cochran, 1972). In addition significant difference among means were distinguished according to the Duncans, multiple test range (Duncan, 1955).

RESULTS AND DISCUSSION

1. Establishment stage:

1.1. Effect of different Combinations between BAP and 2ip

1.1.1. Hansen 536 peach rootstock :

1.1.1.a. Shoot tip explants:

Different combinations between BAP and 2ip were investigated concerning shoot tip parameters.

Data in Table (1) indicate that all combinations between BAP and 2ip succeeded in increasing significantly survival % and growth percentages as compared with cytoinin omitted medium (control). The maximum increament of both parameters was achieved with the 3rd treatment (2.0 mg/L of both BAP and during 2ip) for both seasons of study.

On the other hand, all combinations succeeded in reducing browning parameter significantly as compared with control. The third treatment was superior in this respect.

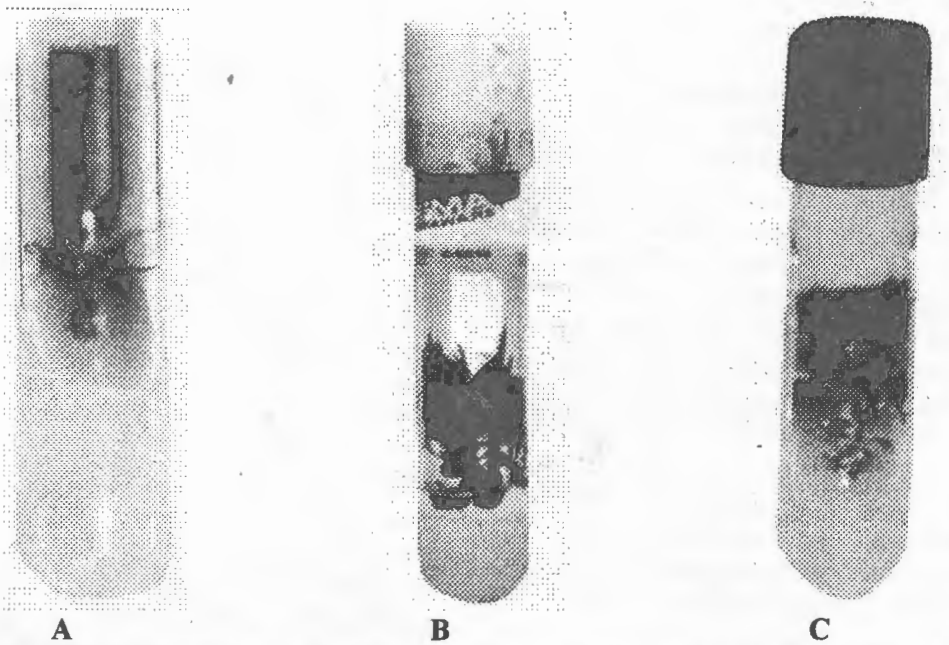
Dealing with average shoot elongation parameter as affected by BAP and 2ip combinations. Table (1) shows that statistical differences between BAP and 2ip combinations were nil as shoot elmgation parameter was concerned during two seasons.

However, the 4th treatment (2-5 mg/L BAP + 2.5 mg/L 2ip) was effective in improing shoot elongation (Photo 1A).

1.1.1.b. One node cutting:

Dealing with the effect of different BAP and 2ip combinations one node cutting parameters at establishment stage. Table (1) clear that the response was pronounced than those of shoot tip parameters.

However, the 3rd treatment (adding both BAP and 2ip each at 2.0 mg/L) was more effective for all evaluated parameters, but differences were significantly absent as comared to two other cytokinins combinations except with survival %, where different-ces were significant during both seasons.



A B C

Photo (1): Shoot elongation of Hansen 536 peach rootstock and MAC9 apple rootstocks during establishment stage.

A- Shoot tip of Hansen 536 cultured in: 2.5 mg/L BAP + 2.5 mg/L 2ip

B- Stem tip section of Hansen 536 cultured in: 2.0 mg/L BAP + 2.0 mg/L 2ip

C- Shoot tip of MAC9 cultured in: 2.5 mg/L BAP + 2.0 mg/L 2ip

Table (1): Effect of different BA +2ip combinations on some measurements of cultured shoot tip and one node cutting explants of Hansen 536 peach rootstock (recorded after 4 weeks) through establishment stage during 2006 and 2007.

Explants	Shoot tip			
Parameters / BA + 2ip concentration (mg/L)	Browning (%)	Survival (%)	Growth (%)	Average of shoot ealongation (cm)
2006				
0.0 + 0.0	65.2 A	30.0 C	34.8 B	0.8 B
1.5 + 1.5	50.0 A	50.0 B	37.0 B	1.3 A
2.0 + 2.0	11.4 B	85.0 A	88.6 A	1.4 A
2.5 + 2.5	24.5 AB	60.0 A	75.5 A	1.6 A
2007				
0.0 + 0.0	42.2 A	25.0 C	57.8 B	0.8 B
1.5 + 1.5	36.2 A	55.0 B	63.8 B	1.3 A
2.0 + 2.0	5.6 B	90.0 A	94.4 A	1.3 A
2.5 + 2.5	23.4 AB	65.0 B	76.6 AB	1.5 A
	Stem node section			
2006				
0.0 + 0.0	66.7 A	15.0 C	33.3 B	0.9 A
1.5 + 1.5	33.2 B	30.0 B	66.8 A	1.1 A
2.0 + 2.0	19.2 B	50.0 A	80.8 A	1.3 A
2.5 + 2.5	24.8 B	40.0 AB	75.3 A	1.2 A
2007				
0.0 + 0.0	66.7 A	15.0 D	33.3 B	1.0 A
1.5 + 1.5	35.9 B	30.0 C	64.1 AB	1.2 A
2.0 + 2.0	17.5 B	60.0 A	82.5 A	1.4 A
2.5 + 2.5	22.9 B	40.0 B	77.1 A	1.3 A

♦ Means followed by the same letters within each column for each measured characteristic are not significantly different from each other at 1% level.

1.1.2. MAC 9 apple rootstock

Table (2) shows that all BA + 2ip combinations improved significantly all investigated parameters for both explants of MAC9 apple rootstock during establishment stage (especially those of two higher concentrations) as compared with control in both seasons. Whereas they maximized growth and survival percentages but reduced significantly the browning percentage. It was also noticed that response of shoot elongation to BAP and 2ip combinations was not significant as compared each other with shoot tip explant, however with one node cutting the differences were completely absent even with comparing to control during two seasons.

These results are in general agreement with those of Dantas *et al.* (2002) and El-Hammady *et al.* (2005), who found that the longest shoots during the establishment stage were recorded for Hansen cultured in MS medium with 2.0 mg/L BAP.

2. Proliferation stage:

2.1. Effect of BAP + 2ip combinations:

An experiment was carried out for each investigated rootstock (Hansen 536, peach rootstock, and MAC9 apple rootstock), whereas three combinations between two concentrations of BAP (1.0 and 1.5 mg/L) and three concentrations of 2ip (0.5, 1.0 and 1.5 mg/L) were added to MS basal medium as well as MS cytoknin omitted medium (control) were used. The effect of the previous combinations on some multiplication parameters during proliferation stage was investigated. Obtained data were recorded in Tables (3 and 4).

Table (2): Effect of BA + 2ip concentrations on some measurements of cultured shoot tip and one node cutting explants of MAC9 apple rootstock (recorded after 4 weeks) through establishment stage during 2006 and 2007 experimental seasons.

Explants	Shoot tip			
Parameters / BA + 2ip concentration (mg/L)	Browning (%)	Survival (%)	Growth (%)	Average of shoot elongation (cm)
2006				
0.0 + 0.0	50.0 A	20 C	50.0 B	0.5 B
1.5 + 1.5	51.0 A	50 B	49.0 B	0.9 A
2.0 + 2.0	26.1 B	60 B	73.9 A	1.1 A
2.5 + 2.5	6.3 C	80 A	93.7 A	1.3 A
2007				
0.0 + 0.0	36.1 A	15 D	63.9 C	0.7 B
1.5 + 1.5	46.0 A	55 C	54.0 C	1.1 A
2.0 + 2.0	21.6 B	70 B	78.4 B	1.3 A
2.5 + 2.5	5.9 C	85 A	94.1 A	1.4 A
	Stem node section			
2006				
0.0 + 0.0	36.1 A	15.0 B	63. 9 B	1.0 A
1.5 + 1.5	40.0 A	25.0 B	60.0 B	1.1 A
2.0 + 2.0	30.6 B	40.0 A	61.5 B	1. 1 A
2.5 + 2.5	38.5 A	50.0 A	69.4 A	1.4 A
2007				
0.0 + 0.0	47.8 A	20.0 B	52.2 B	0.7 B
1.5 + 1.5	42.6 A	35.0 A	57.4 B	1.4 A
2.0 + 2.0	22.3 B	45.0 A	77.7 A	1.5 A
2.5 + 2.5	28.5 B	55.0 A	81.8 A	1.6 A

♦ Means followed by the same letters within each column for each measured parameter are not significantly different from each other at 1% level.

2.1.1. Hansen 536, Peach rootstock :

Data presented in Table (2) revealed clearly that MS basal medium supplemented with 1.0 mg/L BAP and 1.0 mg/L 2ip (treatment C) maximized statistically both average number and increament % of prolixferated shoots. However, average length of proliferated shoots reached its peak by culturing on MS medium supplemented with the higher rate i.e. 1.5 mg/L BAP and 2ip (Photo 2A).

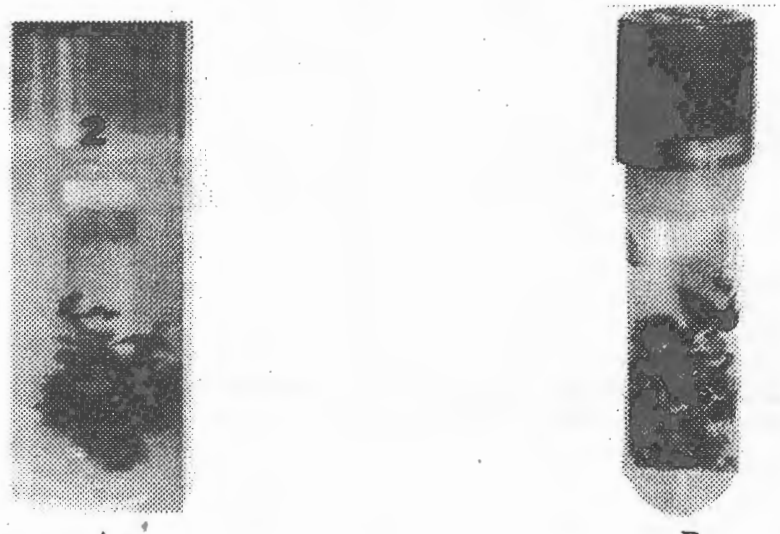
Moreover, increasing cytokinin rate to 1.5 mg/L of each BAP and 2ip (treatment D) or decreasing it to 1.0 mg/L BAP + 0.5 mg/L 2ip (Table 3) resulted significantly in an obvious reduction of proliferated measurements as compared to treatment D during two seasons of study. Data presented in Table (3) and Photo (2A) revealed clearly that MS basal medium supplemented with 1.0 mg/L from each cytokinin type (BAP and 2ip) i.e. treatent C maximized statically both average number and increament % of proliferated shootlets. Moreover, increasing cytokinin rate to 1.5 mg/L of each (BAP and 2ip) i.e. treatment D or reducing it to 1.5 mg/L BAP + 0.5 mg/L 2ip (treatment B) reduced significantly both parameters.

However, the average length of proliferated shoots reached significantly its peak after culturing on MS medium supplemented with the highest rate of cytokinins (BAP and 2ip), each at 1.5 mg/L. The same trend was true during two seasons of study. Cytokinin omitted medium (control) revealed the lowest values of all investigated parameters during both seasons of study. These results are in harmony with Bayomi (1998) who recommended BAP than kinetin for the best proliferation of peach.

2.2. MAC9 apple rootstock:

Regarding the effect of different BAP and 2ip combinations added to MS basal culture medium on some multiplication parameters during proliferation stage, data presented in Table (2) reflect that adding BAP and 2ip to MS basal medium each at the higher level (1-5 mg/L) was the most effective treatment in enhancing and maximizing the values of all investigated MAC9 apple parameters as compared with the other treatments. Such trend was true during both seasons of study. Whereas, treatment (C) came statistically 2nd followed in descending order by treatment (B) and control during two seasons of study.

It could be concluded from the previous results, that all investigated proliferation parameters of cultured shoots during multiplication stage was greatly responded to the addition of both cytokinins (BAP and 2ip) to shoot culture medium. However, each rootstock followed its own trend, but it could be emphasized that the combination between BAP and 2ip each at 1.0 mg/L was the superior treatment with Hansen 536 peach rootstock. In addition the combination between the higher level (1-5 mg/L) of both cyloknins (BAP and 2ip) was the most prerable treatment for MAC9 apple rootstock (Photo 2B) .



A B

Photo (2): Shoot proliferation of shoot tip culture of Hansen 536 and MAC9 rootstock

A- Hansen 536 MS + 1.0 mg/L BAP + 1.0 mg/L 2ip

B- MAC9 MS + 1.5 mg/L BAP + 1.5 mg/L 2ip

These results emphasized that shoot tip cultured of both studied rootstocks (Hansen 536 and MAC9) in medium containing BAP and 2ip enhanced shoot prolixferation since both cytokinins maximized prolieration parameters. Proliferation rates were 4.9 and 5.0 fold increase for Hansen 536 and MAC9 rootstocks respectively, depending on cytokinin concentrations. These results are in agreement with the findings of James and Thurbon (1981), who reported that multiplication rate of apple M9 shoot culture varied between 2 and 4.5 fold depending on auxin: cytokinin levels. 1-2 mg/L BAP and 0.1-0.5 mg/L IBA were most favourable although the auxin could be omitted without adverse affect the multiplication rate.

Baruah *et al.* (1996) and Cos *et al.* (2004), recommended 2.0 mg/L BAP for the best proliferation of apple and peach rootstocks.

2.3. Effect of subculture number on shoot proliferation:

Shoots needed for both rootstocks which were used from those developed by shoot tip cultures through establishment were exiced, transferred and cultured on the best medium for each rootstock which improved all shoot parameters during proliferation stage.

Whereas shoots of Hansen 536 peach rootstock were cultured on MS medium supplemented with BAP and 2ip each at 1.0 mg/L, while shoots of MAC9 apple rootstock were cultured on MS medium supplemented with BAP and 2ip each at 1.5 mg/L. Subculturing was continued four times on the same starting culture media of each rootstock at 4 weeks intervals. The response of the average number, and length of proliferated shoots and percent of proliferated shoots during four subcultures were recorded as an average of two seasons in Tables (5 & 6).

Data obtained as shown from Tables (5 & 6) and Photo (3A&B) revealed that all the investigated parameters increased as the number of subculture was advanced. The fourth subculture was the superior in this respect whereas it maximized all studied parameters for both rootstocks.

These results are in line with those reported by Wanas (1999), Sun *et al.* (2000), Martins and Pedrotti (2001) and Cos *et al.* (2004) who found that regeneration rate of apple M.7, M.9 and peach- almond hybrid could reach 5 fold in MS medium supplemented with 1.5 mg/L BAP.

3. Rooting stage:

3.1. Hansen 536 peach rootstock:

3.1.a. Effect of IBA and NAA combination:

Four combinations between two kinds of auxins (IBA and NAA) each was added to half strength MS medium at four levels (0.5, 1.0, 1.5 and 2.0 mg/L) as well as auxnin omitted half strength MS medium as control were investigated regarding their effect on some rooting parameters (rooting %, average root number and average root length) of Hansen 536 cultured shoots.

The obtained data during both 2006 and 2007 seasons are presented in Table (7).

Data revealed that the three investtigated rooting parameters were in positive relation ship. All investigated rooting media which supplied with different levels of IBA + NAA enhanced clearly all the rooting parameters than the control during both seasons of study. The combination between IBA + NAA each at 1.5 mg/L treatment (D) was the superior one in this respect, as it increased significantly the all rooting parameters Photo (4A). Whereas, the lower concentration of both auxins (1.0 mg/L) came in the second rank. On the contrary the higher level of both auxins (2.0 mg/L) was less effective.

3.1.b. Effect of IBA and PG combination.

Four treatments represented different combinations between IBA which added to rooting media at four levels (0.5, 1.0, 1.5 and 2.0 mg/L) and PG at one level 100 mg/L as well as auxin omitted half strength MS medium as control, were investigated in response to rotting parameters. Data tabulated in Table (8) and illustrated by Photo (4B) clear that culturing shoots of Hansen 536 peach rootstock on half strength MS medium supplemented with IBA and PG improved all the rooting parameters over the control, during both reasons of study. Moreover, ½ slenght MS medium + 1.0 mg/L IBA + 100 mg/LPG gave the highest values of all investigated rooting parameters followed by treatment (D) (½ MS + 1.5 mg/L + 100 mg/L PG). On the other hand, auxin omitted medium (control) had the lowest values.

3.2. MAC9 apple rootstock:

3.2.a. Effect of IBA and NAA combination:

It is quite evident from Table (9) that IBA + NAA combinations were significantly effective data recorded in Table (9) that, the most promoting response of both rooting % and average root number was achieved by rooting medium contained 1.5 mg/L of each IBA and NAA. (treatment D) for both seasons of study (Photo 4C). On the contrary, treatment (D) gave the lowest value of average root length. Meanwhile average root length was maximized with reducing auxin level in rooting media to 1.0 mg/L for both IBA and NAA. Such trend was true during both seasons of study. The rooting media supplemented with the lowest level (0.5 mg/L) highest level (2.0 mg/L) of IBA + NAA came in the second rank regarding the improvement of rooting parameters. Auxin omitted medium (control) was the least effective one in response to rooting parameters.

Table (3): Effect of different combination between BAP and 2ip on shoot tips proliferation parameters of Hansen 536, peach rootstock during 2006 and 2007 seasons.

Parameters / Treatments	2006			2007		
	Average No. of proliferated shoots *	Proliferated shoot (%)	Average length of proliferated shoots (mm) *	Average No. of proliferated shoots *	Proliferated shoot (%)	Average length of proliferated shoots (mm) *
A	2.0 D	100 D	9.0 C	2.2 D	120 D	7.2 D
B	3.0 C	200 C	8.7 D	3.0 C	200 C	9.3 C
C	5.2 A	420 A	10.7 B	6.6 A	560 A	10.6 B
D	4.0 B	300 B	12.0 A	4.6 B	360 B	12.6 A

Table (4): Effect of different combination between BAP and 2ip on shoot tips proliferation parameters of Apple MAC9 rootstock during 2006 and 2007 seasons.

Parameters / Treatments	2006			2007		
	Average No. of proliferated shoots *	Proliferated shoot (%)	Average length of proliferated shoots (mm) *	Average No. of proliferated shoots *	Proliferated shoot (%)	Average length of proliferated shoots (mm) *
A	1.4 D	40 D	11.4 B	1.4 D	40 D	13.4 B
B	3.6 C	260 C	11.0 C	4.0 C	300 C	11.9 C
C	4.6 B	360 B	9.2 D	4.8 B	380 B	11.4 D
D	5.8 A	480 A	13.2 A	6.2 A	520 A	16.0 A

A MS cytokinion omitted medium (Control) MS + 1.0 mg/L BAP + 0.5 mg/L 2ip
C MS + 1.0 mg/L BAP + 1.0 mg/L 2ip MS + 1.5 mg/L BAP + 1.5 mg/L 2ip
MS Murashige and skoog nedium supplemented with 30g/L sucrose and 7.0 g/L agar
* An average of five culture tubes.

Table (5): Some proliferation measurements of Hansen 536 peach rootstock as influenced by number of subcultures (4 weeks intervals)..

Subculture no.	Average No. of proliferated shoots *	Proliferated shoots (%) *	Average length of proliferated shoots (mm) *
1st	6.2 B	520 D	17.9 D
2nd	6.8 B	580 C	22.0 C
3rd	9.0 A	800 B	24.4 B
4th	9.4 A	850 A	25.5 A

Table (6): Some proliferation measurements of MAC9 apple rootstock as influenced by number of subcultures (4 weeks intervals)..

Subculture no.	Average No. of proliferated shoots *	Proliferated shoots (%) *	Average length of Proliferated shoots (mm) *
1st	6.8 C	580.0 D	17.6 D
2nd	7.4 BC	640.0 C	18.2 C
3rd	8.0 AB	700.0 B	19.8 B
4th	8.4 A	740.0 A	23.3 A

- Initial shoot number was 5 shoots.
- Sub-culture was carried out 4 weeks intervals.
- MS medium +1.5 mg/L BAP + 1.5 mg/L 2ip was used for all subcultures.

* An average of two seasons.

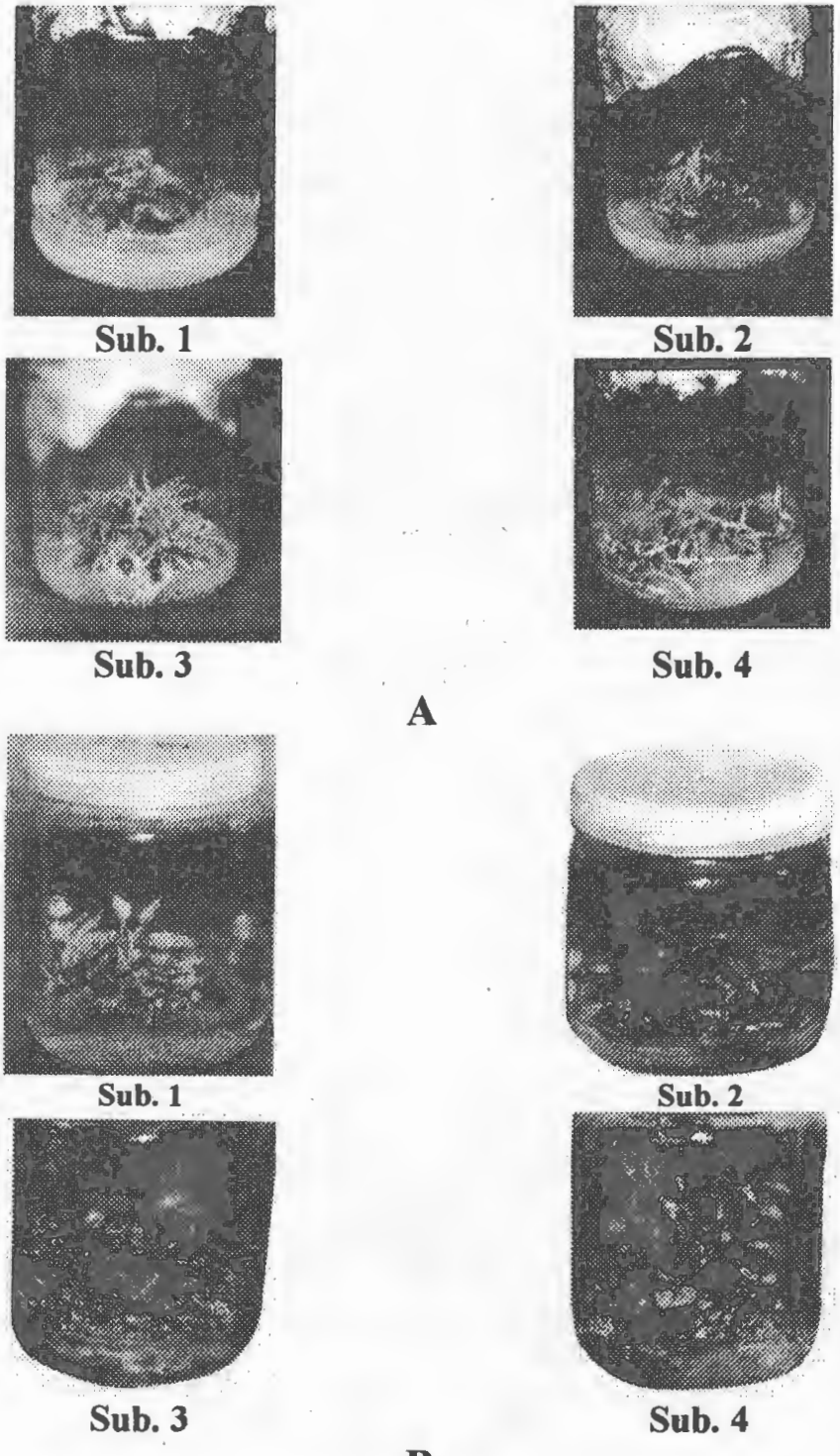


Photo (3): Effect of number of subcultures on shoot culture proliferation of Hansen 536 and MAC9 rootstock

(A): Shoot proliferation of Hansen 536 peach rootstock, subcultured in: 1.0 mg/L BAP + 1.0 mg/L 2ip .

(B): Shoot proliferation of MAC9 a apple rootstock subcutlured in: 1.5 mg/L BAP + 1.5 mg/L 2ip.

Table (7): Effect of IBA and NAA combinations on rooting parameters after four weeks from culturing multiplied shoots of Hansen 536 peach rootstock during 2006 and 2007 seasons.

Parameters / Treatments	2006			2007		
	Rooting %	Average root number	Average root length (mm)	Rooting %	Average root number	Average root length (mm)
A	30.0 D	1.2 D	80.0 D	25.0 D	1.3 D	80.0 D
B	60.0 B	2.2 C	126.0 B	60.0 B	2.5 C	120.0 C
C	60.0 B	3.0 B	136.0 A	60.0 B	3.0 B	122.0 C
D	80.0 A	3.3 A	138.0 A	80.0 A	3.3 A	130.0 A
E	40.0 C	2.5 C	120.0 C	40.0 C	2.7 C	125.0 B

One helf strength auxin omitted MS medium. A ½ MS + auxin omitted medium (Control)
B ½ MS + 0.5 mg/L IBA + 0.5 mg/L NAA C ½ MS + 1.0 mg/L IBA + 1.0 mg/L NAA
D ½ MS + 1.5 mg/L IBA + 1.5 mg/L NAA E ½MS + 2.0 mg/L IBA + 2.0 mg/L NAA

Table (8): Effect of IBA and PG combinations on rooting parameters of cultured shoots of Hansen 536 peach rootstock after four weeks from culturing during 2006 and 2007.

Parameters / Treatments	2006			2007		
	Rooting %	Average root number	Average root length (mm)	Rooting %	Average root number	Average root length (mm)
A	40.0 C	1.9 D	70.0 D	40.0 D	1.8 D	83.0 D
B	60.0 B	3.6 C	100.0 C	80.0 B	3.6 C	100.0 C
C	80.0 A	6.5 A	149.0 A	100.0 A	6.4 A	148.0 A
D	60.0 B	4.0 B	122.0 B	100.0 A	4.5 C	125.0 B
E	60.0 B	4.0 C	109.0 C	60.0 C	4.6 B	110.0 C

One helf strength auxin omitted MS medium. A ½ MS + auxin omitted medium (Control)
B ½ MS + 0.5 mg/L IBA + 100 mg/L PG C ½ MS + 1.0 mg/L IBA + 100 mg/L PG
D ½ MS + 1.5 mg/L IBA + 100 mg/L PG E ½MS + 2.0 mg/L IBA + 100 mg/L PG

Table (9): Effect of IBA and NAA combinations on rooting parameters after four weeks from culturing multiplied shoots of MAC9 apple rootstock during 2006 and 2007 seasons..

Para-meters / Treat-ments	2006			2007		
	Rooting %	Average root number	Average root length (mm)	Rooting %	Average root number	Average root length (mm)
A	20.0 C	1.3 D	100.0 D	25.0 D	1.6 D	100.0 D
B	40.0 B	2.0 C	145.0 B	40.0 C	2.0 C	150.0 B
C	40.0 B	2.5 B	196.0 A	40.0 C	2.0 C	210.0 A
D	60.0 A	2.3 A	114.0 D	80.0 A	3.8 A	105.0 D
E	60.0 A	2.3 B	130.0 C	60.0 B	2.8 B	129.0 C

One helf strength auxin omitted MS medium. A ½ MS + auxin omitted medium (Control)
B ½ MS + 0.5 mg/L IBA + 0.5 mg/L NAA C ½ MS + 1.0 mg/L IBA + 1.0 mg/L NAA
D ½ MS + 1.5 mg/L IBA + 1.5 mg/L NAA E ½MS + 2.0 mg/L IBA + 2.0 mg/L NAA

3.2.b. Effect of IBA and PG combination:

Four IBA rates (0.5, 1.0, 1.5 and 2.0 mg/L) with 100 mg/L PG added to ½ strength MS medium were investigated regarding their effect on some rooting parameters. Table (10) shows that the highest level of IBA (2.0 mg/L) associated with 100 mg/L PG (treatment E) statistically maximized all investigated rooting parameters during both seasons of study (Photo 4D). Meanwhile the lowest IBA level (0.5 mg/L) combined with 100 mg/L PG resulted statistically in the least increase for all rooting parameters as compared to the auxin omitted medium (control). The above results verify that the addition of 100 mg/L PG to 1.0 or 1.5 mg/L IBA in rooting medium for Hansen 536 and MAC9, respectively, could be safely recommended to improve rooting percentages (100 and 80%) for former and later rootstock.

These results are in general agreement with the findings of Wanas (1999), Gamage *et al.* (2000) and Jun-Jihae *et al.* (2001). They recommended half strength MS medium in increasing rooting parameters. Meanwhile, Manta *et al.* (2000) recorded 80% rooting of M.106 and cleared that the addition of PG to rooting medium was not beneficial. On the contrary, a synergistic effect of IBA and PG on M.9 rooting was observed inspective of whether the transfer to hormone-free medium was done (Jame and Thurbon, 1979). In another study, they reported that PG at 162 mg/l had no effect on the shoot multiplication rate of M.9, but shoot cultures grown in the presence of PG gave higher rooting percentages than cultures grown in its absence. PG acted as an auxin synergist in root initiation (James and Thurbon, 1981).

4. Acclimatization stage:

The acclimatization period extended under greenhouse condition for 2 months, where. Hansen 536 peach and MAC9 apple plantlets were transplanted individually, each in plastic pot (25 x 25 cm) filled with one of the four investigated growing media mixtures. Each plantlet was completely covered with while transparent plastic bag for three weeks.

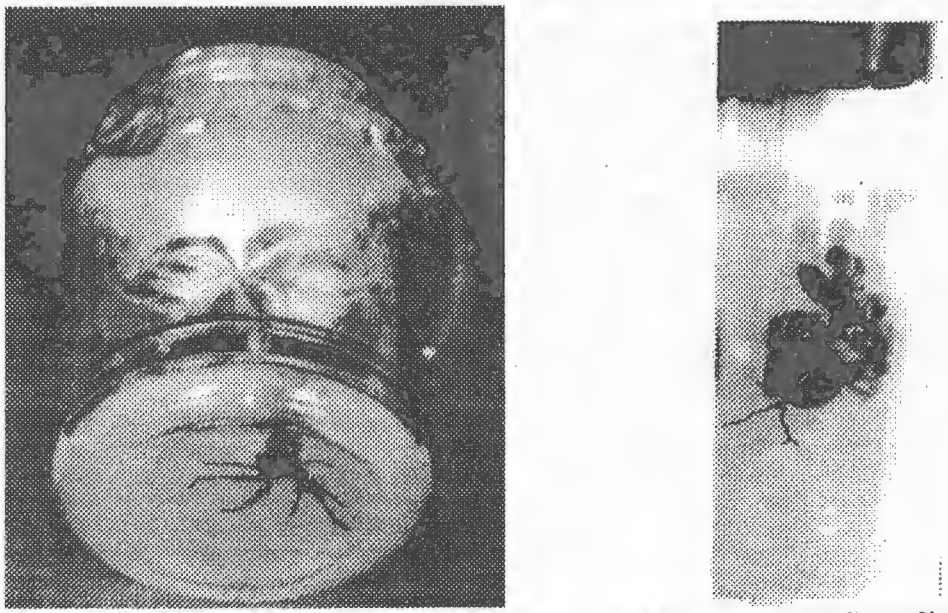
Survival percentage and average stem length of transplanted plantlets for both rootstocks in relation to four investigated growing media (mixtures of sand, peatmoss and vermiculite) were recorded after eight weeks. Data obtained during two successive months of the acclimatization were tabulated in Table (11) and illustrated by Photo (5).

Regarding the influence of sand, peat moss and vermiculite mixture investigated as growing media on survival percentage %, it was quite clear that a significant differences were clearly detected during two months of acclimatization for both rootstock plantlets.

Herein, the growing medium of Sand + peat moss + vermiculite mixtures at 1:1: 1 ratios exhibited statistically the highest survival percentage (80%) for both rootstock plantlets (Photo 5). On the contrary, both mixtures of peat moss + vermiculite (at 1: 1 ratio) and sand + peat moss (at 1 : 1 ratio) resulted significantly in the least survival percentage (40%) for MAC9 apple rootstock and Hansen 536 peach rootstock, respectively. In addition, sand mixture with either peatmoss or vermiculite each at equal proportion were statistically in between for MAC9 apple plantlets (60% survival). However vermiculite + either sand or peat moss at equal proportion as growing media were statistically in between (60% survival) of Hansen 536 peach rootstock plantlets.

Concerning the average of stem length as influenced by different mixtures of growing media, data presented in Table (5) show that planting media comprised of sand + peat moss + vermiculite at equal proportion by volume was the superior, where the tallest of both investigated rootstock plantlets was detected. The reverse was true when sand + peat moss at equal proportion was used as growing medium for both rootstock plantlets.

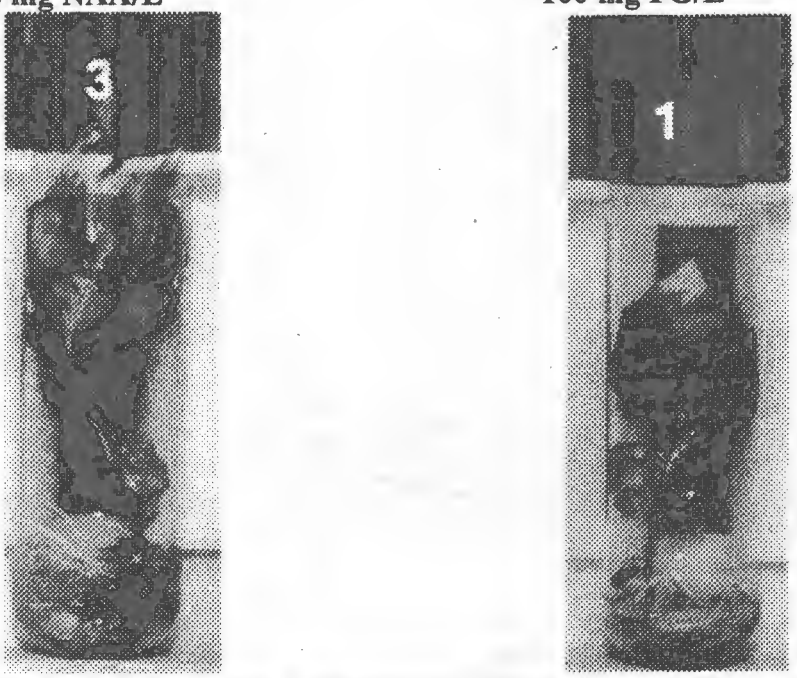
Generally it could be concluded that survival % and average stem length of both investigated rootstock plantlets through the acclimatization stage was obviously influenced by the different mixtures used as growing media. In this respect, mixture of sand + peat moss + vermiculite at equal proportion was statistically the superior growing medium since it exhibited the maximum survival % (80%) of the tallest stem for both rootstocks.



Shoot rooting of Hansen 536 cultured in 1/2 strength MS medium supplemented with:

A- 1.5 mg IBA/L
1.5 mg NAA/L

B- 2.0 mg IBA/L
100 mg PG/L

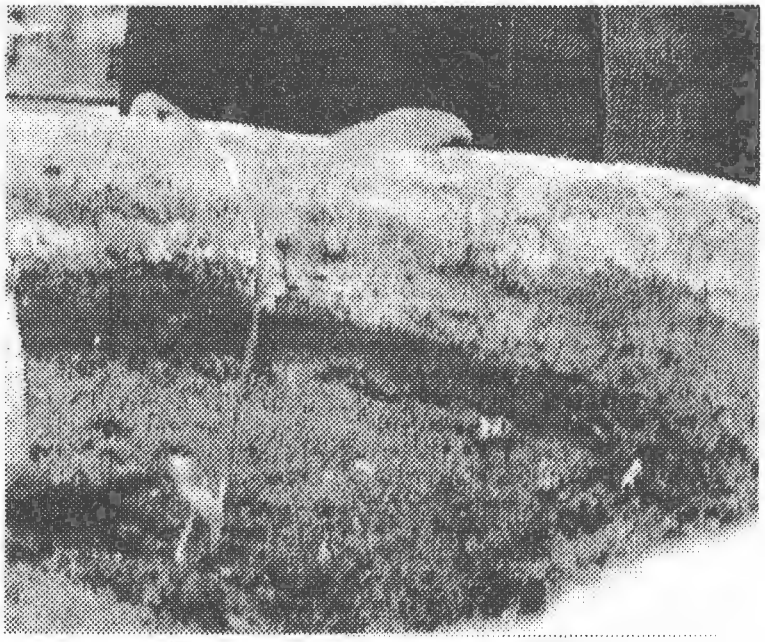


Shoot rooting of MAC9 cultured in 1/2 strength MS medium supplemented with:

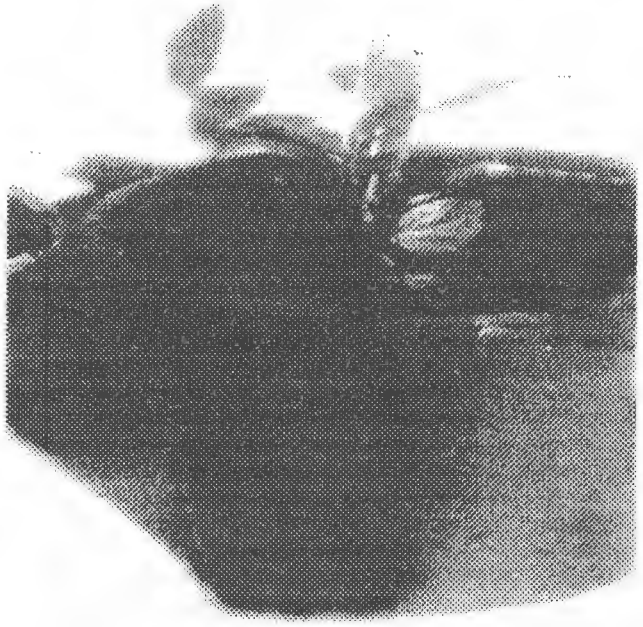
C- 1.5 mg IBA/L
1.5 mg NAA/L

D- 2.0 mg IBA/L
100 mg PG/L

Photo (4): Shoot rooting originated from shoot tip culture of Hansen 536 and MAC9 rootstocks



Hanson 536 peach rootstock



MAC9 apple rootstock

Photo (5): Plantelets of Hansen 536 and MAC9 rootstocks during acclimatization stage.

These results are in general agreement with the findings of Le and Collet (1991) and Nunes *et al.* (1991) on MAC9 and M.26 apple rootstocks. They reported that plantlets of MAC9 and M.26 were transferred to vermiculite or commercial substrate under greenhouse conditions with survival rates of 90 and 70%, respectively, after 30 days.

Table (10): Effect of IBA and PG combinations on rooting parameters of cultured shoots of MAC9 apple rootstock after four weeks from culturing during 2006 and 2007.

Parameters / Treatments	2006			2007		
	Rooting %	Average root number	Average root length (mm)	Rooting %	Average root number	Average root length (mm)
A	40.0 D	1.9 C	100.0 D	45.0 D	2.3 D	110.0 D
B	60.0 C	2.5 B	128.0 C	60.0 C	2.9 D	125.0 D
C	60.0 C	2.8 B	178.0 B	80.0 B	3.0 C	172.0 C
D	80.0 B	3.2 A	178.0 B	80.0 B	3.6 B	194.0 B
E	100.0 A	3.2 A	182.0 A	100.0 A	3.8 A	203.0 A

One helf strength auxin omitted MS medium. A ½ MS + auxin omitted medium (Control)
B ½ MS + 0.5 mg/L IBA + 100 mg/L PG C ½ MS + 1.0 mg/L IBA + 100 mg/L PG
D ½ MS + 1.5 mg/L IBA + 100 mg/L PG E ½MS + 2.0 mg/L IBA + 100 mg/L PG

Table (11): Survival percentage and average of stem length of Hansen 536 peach and MAC9 apple rootstock plantlets (in vitro propagated by shoot tip culture) as influenced by planting media used during acclimatization stage (2 months).

Planting media mixtures ratios by volume	Hansen 536		MAC9	
	Survival (%) *	Average of stem length *	Survival (%) *	Average of stem length *
Sand + Peat-moss 1 : 1	40.00 C	11.15 D	60.00 B	12.30 D
Sand + Vermiculite 1 : 1	60.00 B	11.50 C	60.00 B	14.10 C
Peal-moss + Vermiculite 1 : 1	60.00 B	12.00 B	40.00 C	15.0 B
Sand – Peal + Vermi 1 : 1 : 1	80.00 A	12.80 A	80.00 A	16.15 A

- Means with in column followed by the seam letters are not significantly different at 5% level.
* An average of two seasons.

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الإكثار المعملى لأصلى الخوخ هانسون ٥٣٦ والتفاح ماك ٩

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أجريت هذه الدراسة على أصلى الخوخ هانسون ٥٣٦ والتفاح ماك ٩ بمعمل زراعة الأنسجة – مركز بحوث الصحراء – المطرية – القاهرة خلال عامى ٢٠٠٦، ٢٠٠٧م. بهدف أكثار هذين الأصلين خضريا عن طريق زراعة الأنسجة لتلبية الرغبات المتزايدة لأصحاب المشاتل من هذين الأصلين لتميزهما بالعديد من الصفات التى تلائم ظروف الأراضى المستصلحة مثل تحمل الجفاف ومقاومة النيماتودا وقلة احتياجات البرودة نسبيا لأصل الخوخ هانسون ٥٣٦ وكذلك مقاومة العفن والتوافق مع معظم أصناف التفاح ماك ٩. وقد استخدم منفصلى القمة النامية والعقل الساقية ذات العقدة الواحدة تم تجهيزها من أرخ حديثة لأشجار نامية بمزرعة كلية الزراعة – جامعة عين شمس بأعمار ٧، ٣ سنوات للخوخ والتفاح على التوالى. وعن أهم النتائج المتحصل عليها ما يلى:

أولا: مرحلة الأساس:

تحسنت قياسات جميع القياسات التى درست فى هذه المرحلة (تلوين – نمو – استطالة الفرنيمات للمنفصلات ونسبة بقائها حية) بإضافة BAP + 2ip بأى تركيز لبيئة موارشيج وسكوج. وأن المعاملتين اللتان استخدم فيهما مخلوط نوعى السيتوكينين معا كل تركيز ٢ أو ٢,٥ ملجم/لتر كانتا الأكثر فعالية. ونظرا للفروق الطفيفة بين التركيزين فإنه ينصح باستخدام التركيز الوسط ٢,٠ ملجم/لتر لأسباب اقتصادية.

ثانيا: مرحلة التضاعف:

كان لمستوى السيتوكينين أثره الفعال فى هذا الصدد حيث أن استعمال BAP + 2ip كل بتركيز ١ ملجم/لتر هو الأكثر فعالية بالنسبة لأصل الخوخ هانسون ٥٣٦ فى الموسمين بينما كان التركيز الأعلى وهو ١,٥ ملجم/لتر من كل من نوعى السيتوكينين (BAP& 2ip) هو الأكثر تفوقا بالنسبة للتفاح ماك ٩ حيث حققت هاتان المعاملتان أفضل القياسات (متوسط عدد وأطوال الفرنيمات الناتجة ومعدل أو النسبة المئوية للتضاعف). وكان معدل التضاعف خلال موسمى الدراسة للأصلين خوخ هانسون ٥٣٦، تفاح ماك ٩ هما (٣,٢، ٥,٦)، (٣,٦، ٣,٨) على التوالى.

وعلى الجانب الآخر فقد لوحظ زيادة معدل التضاعف مع تقدم رقم عملية إعادة الزراعة خلال مرحلة التضاعف لكلا الأصلين فقد حققت إعادة الزراعة للمرة الرابعة على بيئة الزراعة المزودة بالسيتوكينين (BAP & 2ip) كل بمعدل ١,٠ ملجم/لتر للخوخ أو ١,٥ ملجم/لتر للتفاح إلى أقصى معدل تضاعف وهو ٨,٠، ٧,٤ مرات على التوالى.

ثالثا: مرحلة التجزير:

أدت إضافة IBA + NAA كل بتركيز ١,٥ ملجم/لتر لبيئة موراشيج وسكوج عند نصف تركيز أملاحها المعدنية إلى زيادة النسبة المئوية لتجزير الأفرخ المتضاعفة لكلا الأصلين لتصل إلى (٨٠%)، (٦٠، ٨٠%) خلال الموسمين الأول والثانى لأصلى الخوخ هانسون ٥٣٦، التفاح ماك ٩ على التوالى. كما أن إضافة IBA عند تركيز ١,٠، ٢,٠ ملجم/لتر لأصلى الخوخ والتفاح على التوالى مع ١٠٠ ملجم/لتر PG أدت إلى زيادة النسبة المئوية للتجزير لتصل (٨٠، ١٠٠ جرام، (١٠٠%) للخوخ والتفاح على التوالى خلال موسمى الدراسة.

رابعا: مرحلة الأقلمة:

كانت بيئة الزراعة المكونة من خليط من الرمل + البيت موس + الفيرميكوليت كل نسبة حجمية واحدة هى الأفضل خلال مرحلة الأقلمة حيث أدت إلى أعلى نسبة تكاثر العينات حية (٨٠% لكلا الأصلين) بالإضافة إلى التأثير الإيجابى الواضح على طول السيقان فى هذا المرحلة تحت ظروف الصوبة.