# CONTENTS

# V.SUMMARY AND CONCLUSION

The present study was carried out in Strawberry Tissue Culture Laboratory and Non- Traditional Crops, Improvement Center, Faculty of Agriculture, Ain Shams University during 2000 and 2001 years. Hansen 536 peach rootstock explants of the renewed 7-year old trees grown in the same faculty were the plant material used for this dissertation. Hence, it was hoped to find out an ideal method as true propagation method for providing with the adequate quantities of nursery transplants of this desired rootstock to meet the increasing demand due to its advantages, especially under the new reclaimed area condition so, some treatments dealing with the various stages of direct regeneration through tissue culture technique i.e, establishment; proliferation, rooting and acclimatization were investigated as follows.

## V.1.Establishment stage: -

In this regard a factorial experiment was devoted for studying the effect of explant type (shoot tip and stem node cutting) combined with surface sterilization of these explants by soaking in diluted solution (10 or 20%) of sodium hypochlorite (commercial bleach "Clorox") for either 10, 15, or 20 min. were investigated regarding their influence on contamination; browning and survival % during this stage.

Data were recorded after 4 weeks from culturing on MS base medium supplemented with 3% sucrose and 0.7% agar with adjusted pH at 5.6 - 5.8.

## V.2.Proliferation "shoot multiplication" stage:-

In this stage four weeks old aseptically growing explants obtained from the establishment stage were used as source for the multiplication stage. The excised shoot tips (5-7mm.length) were cultured on full strength solid MS medium to investigate

the effect of some growth regulators added to culturing media. Hence, cytokinin (BA) at 3 concentrations (0.5; 1.0 and 1.5 mg/l) combined with IBA with or without using gibberllin at 0.1 mg/l for each were the investigated treatments through the successive four subcultures included in this stage as follows:

1-Full strength MS salts free hormone medium +vitamins & myo insitol.

2-Full strength MS medium supplemented with BA at 0.5 mg/l.

3-Full strength MS medium supplemented with 0.5mg BA+0.1 mg IBA/l.

4-Full strength MS medium supplemented with 0.5mg BA+0.1 mg IBA+0.1 mg $GA_3$/l.

5-Full strength MS medium supplemented with 1.0 mg BA/l.

6-Full strength MS medium supplemented with 1.0mg BA+0.1 mg IBA/l.

7-Full strength MS medium supplemented with 1.0mg BA+0.1mg IBA+0.1mg $GA_3$/l.

8-Full strength MS medium supplemented with 1.5 mg BA/l.

9-Full strength MS medium supplemented with 1.5mg BA+0.1 mg IBA/l.

10-Full strength MS medium supplemented with 1.5mg BA+0.1mg IBA+0.1 mg $GA_3$/l.

At the end of each subculture of the four included ones in this stage (4 weeks interval) the response to the various investigated treatments was determined through the changes in average number of proliferation shootlets per original cultured shoot; average shoot length and number of leaflets included per each.

## V.3. Elongation stage: -

More elongated proliferated shootlets (<4cm.) obtained from multiplication stage are preferable to achieve higher rooting and survival% throughout both rooting and acclimatization stages, respectively. So, three levels of either $GA_3$ (0.1; 0.2 and

0.4 mg/l) or activated charcoal (1.0; 2.0 and 3.0 g/l) added to the full strength MS medium through this stage were investigated at its end pertaining the response of shoot length and number of leaflets per each.

The investigated treatments were as follows:

1-Full strength basal MS medium +0.1 mg$GA_3$/l.

2-Full strength basal MS medium +0.2 mg$GA_3$/l.

3-Full strength basal MS medium +0.4 mg$GA_3$/l.

4-Full strength basal MS medium +1.0 g. activated charcoal /l.

5-Full strength basal MS medium +2.0 g. activated charcoal /l.

6-Full strength basal MS medium +3.0 g. activated charcoal /l.

## V.4. Rooting stage:

In this respect some rooting measurements (rooting %; number of roots /plantlet and average root length) in response to two auxins i.e. IBA and NAA each added at 3 levels (0.5; 1.0 and 2.0 mg/l) to one half strength of MS medium (either supplemented with 1.0g/l activated charcoal or not) were investigated.

Thus, the twelve (12) investigated culturing media were as follows:

1-Half strength MS supplemented with 1.0 g/l activated charcoal +0.5 mg/l IBA.

2-Half strength MS medium +1.0g /l activated charcoal +1.0 mg/l IBA.

3-Half strength MS medium +2.0g /l activated charcoal +2.0 mg/l IBA.

4-Half strength MS medium +0.5g /l activated charcoal +0.5 mg/l NAA.

5-Half strength MS medium +1.0g /l activated charcoal +1.0 mg/l NAA.

6-Half strength MS medium +2.0g /l activated charcoal +2.0 mg/l NAA.

7-Half strength MS +0.5 mg/l IBA.

8-Half strength MS +1.0 mg/l IBA.

9-Half strength MS +2.0 mg/l IBA.

10-Half strength MS +0.5 mg/l NAA.

11-Half strength MS +1.0 mg/l NAA.

12-Half strength MS +2.0 mg/l NAA.

After explants had been cultured (3 explants per each jar) they were incubated along the rooting stage (4weeks) under two light conditions i.e. either the whole duration in usual light (16 h. light + 8 h. dark) or for one week in dark followed by three weeks in light.

The complete randomized design with three replications was employed for arranged the investigated 24 treatments (2 light conditions of incubation x 12 culture media), whereas every replicate was represented by four jars each cultured with 3 explants.

## V.5. Acclimatization stage:

This stage was carried out under the green house condition which considered as an attempt to acclimate the sensitive succulent plantlets obtained from the in vitro rooting stage and consequently can grow and develop successfully after transplanting in the *Invivo* condition. Accordingly, four autoclaved mixtures were evaluated pertaining their suitability for being used as transplanting media through investigating their effect on survival% and other growth measurements (plant height and number of leaves per plant) during this stage.

The complete randomized design with three replications was used for arranging the following media: -

1-Peat moss+sand (1:1 by volume).

2-Vermiculite + sand (1:1 by volume).

3-Vermiculite + peat moss (1:1 by volume).

4-Vermiculite + peat moss +sand (1:1:1 by volume).

The obtained results could be summarized as follows:

## V.1. Establishment stage:

Referring the response of contamination; browning and survival percentages to specific effect of explant type (shot tip and stem node); surface sterilization treatments (10 and 20% Clorox for 10; 15 and 20 min.), as well as their combinations (interaction effect), obtained data revealed the following:

### A-Specific effect:

Three measurements (contamination, browning and survival %) did,t follow the same trend regarding their response to specific effect of a given investigated factor (explant type or sterilization treatment). Contamination % and survival % followed the same trend regarding their response to specific effect of explant type whereas both measurements exhibited higher values with stem node, while the reverse was true with the browning % as the least browning % was always in concomitant to the stem node.

As for the specific effect of surface sterilization treatment, data obtained revealed that contamination % and survival % were generally decreased with increasing either Clorox concentration or duration of sterilization. Meanwhile, browning % was severely increased by raising both Clorox concentration and duration of explant soaking.

### B-Interaction effect:

Because of the unparalled response of three investigated measurements (contamination; browning and survival %) not only to both studied factors (explant type and sterilization treatment) from one hand but also variance in response of the same measurement (especially survival %) to a given factor from the other, so interaction effect did,t follow firm trend. Anyhow, it could be generally concluded that the highest survival% (85%) accompanied with a considerable accepted percentage of both contamination (about 5%) and browning (about 10%) was

achieved by surface sterilization for 15 min. in 10 and 20 % Clorox with shoot tip and stem node explants, respectively.

## V.2.Multiplication stage:

During the four subcultures included in this stage, specific effect of explant type (shoot tip and stem node) and supplemented growth regulators to full strength MS medium (10 combinations of BA; IBA and $GA_3$), as well as interaction effect of various combinations between two studied factors were investigated during each subculture (each extended for 4 weeks) pertaining the response of number of proliferated shoots; average shoot length and No. of leaflets per each.

### A. Specific effect:

Three growth measurements (No. of shoots; shoot length and No. of leaflets per each) followed the same trend concerning their response to specific effect of explant type. Herein, shoot tip was the superior and its proliferated organs exceeded the analogous ones of stem node. However, differences were significant and more pronounced with average number of both proliferated shoots and leaflets per each during four subcultures. Meanwhile, variances in shoot length in response to explant types was less pronounced, especially during $1^{st}$ and $3^{rd}$ subcultures, whereas both explants were the same from statistical point of view.

As for the specific effect of growth regulators supplemented to full strength Ms medium, it was quite clear that both No. of proliferated shoots and average shoot length followed the same trend, whereas adding BA at either 1.0 or 1.5 mg/l plus IBA $+GA_3$ (each at 0.1 mg/l) were the superior. However, the greatest number of leaflets was coupled with MS medium supplemented with either (0.5 mg/l BA + 0.1 mg/l IBA) or (1.5 mg/l BA + 0.1 mg/l IBA + 0.1 mg/l $GA_3$).

### B. Interaction effect:

Data obtained displayed that the interaction effect was representative of the direct reflection of specific effect of both

investigated factors response to various combinations between explant type and supplemented growth regulators was more pronounced with both No. of proliferated shoots and shoot length as compared to that exhibited with the No. of leaflets/ shoot.

- Herein, with both No. of proliferated shoots and average shoot length MS medium supplemented with (BA at either1.0 or1.5 mg/l + IBA & $GA_3$ each at 0.1 mg/l) from one hand combined with shoot tip or stem node from the other were statistically the most effective as the response of each explant type was individually concerned. Such superior combinations resulted in increasing number of proliferated shoots over control (hormone free MS medium) to approximately 7.0 &5.0 folds as the responses of shoot tip and stem node, respectively were concerned. Moreover, the increase in average shoot length exhibited by the aforesaid two superior treatments (adding 1.0 or 1.5 mg/l BA combined with 0.1 mg/l of both IBA &$GA_3$ to MS medium) reached to about 3.0 times much higher than control with both explant types.

Nevertheless, with the average number of proliferated leaflets per shoot the highest values were in closed relationship to MS medium supplemented with either (0.5 mg/l BA + 0.1 mg/l IBA) or (1.5 mg/l BA + IBA & $GA_3$ each at 0.1 mg/l). Such trend was true as the response of each explant type was individually concerned. The increase in No. of leaflets/ shoot over the control reached about 20-30% as an average of four subcultures was taken into consideration.

### V.3. Elongation stage:

The specific and interaction effect of two studied factors i.e. explant type (shoot tip & stem node) and some addenda ($GA_3$ & activated charcoal each at 3 rates), as well as their possible combinations were investigated regarding the response of shoot length and number of leaflets per each during elongation stage.

**A. Specific effect:**

Data obtained displayed that both parameters respond specifically the explant type. Hence, shoot tip exceeded stem node, however variance in number of leaves was more pronounced than that with shoot length, which did not reach the level of significance in later measurement.

As for the specific effect of addenda supplied to MS medium, data obtained displayed that adding $GA_3$ at the rate of 0.4 mg/l to full strength MS medium was statistically the superior. However, adding of either $GA_3$ (0.2 mg/l) or activated charcoal (3.0g/l) to one liter of full strength MS medium ranked statistically second. Such trend was true with both shoot length and number of leaflets. On the contrary, the least values of both measurements were closely coupled with the MS supplemented with activated charcoal at 1.0 g/l.

**B.Interaction effect:**

The tallest shoots with the greatest number of leaflets per each was significantly coupled to the shoot tip cultured on MS medium supplemented with 0.4 mg/l $GA_3$. Moreover, the stem node cultured on the aforesaid superior medium (MS + 0.4 mg/l $GA_3$) reflected also the maximum favorable response as both parameters were concerned in comparison to the analogous ones on other media.

## V.4. Rooting stage:

In this respect specific effect of light / darkness condition during 1$^{st}$ week of incubation; auxin/ charcoal added to one half strength MS rooting medium and interaction effect of various combinations were investigated during rooting stage pertaining the response of rooting %, number of roots per plantlet and average root length.

**A. Specific effect:**

With regard to specific effect of light / darkness condition of incubation through 4 weeks of rooting stage (the cultured explants subjected through the whole duration to either usual

light or one week dark followed by 3 weeks normal light), it is quite clear that the response was relatively slight for 3 rooting measurements. However, rooting % from one hand and both (number of roots/ plantlet & average root length) from the other followed two opposite trends. Herein, incubated explants under darkness for 1st week increased slightly rooting % but the reverse was true with both number of roots and their average length whereas both later parameters were increased under usual light through the whole duration of incubation (4 weeks of rooting stage). Differences, in 3 rooting parameter were so few to reach level of significance.

Referring the specific effect of auxin (IBA &NAA each at 3 levels) combined with activated charcoal, data obtained displayed that the trends of response of 3 rooting measurements were not entirely coincident. Hence, with rooting % the charcoal omitted MS ($^1/_2$ strength) supplemented with NAA at 0.5 mg/l was the superior (70.83 rooting %), followed by those supplemented with IBA at either 0.5 mg/l in the presence of activated charcoal (treatment, 1) or IBA at 1.0 mg/l with or without charcoal (treatments 2 &8, respectively) which showed (66.67 rooting %).

Nevertheless, with the number of rootlets per each plantlet, the most abundant rootlets were significantly achieved by the half strength MS medium supplemented with IBA at 1.0 mg/l either with omission or presence of activated charcoal (treatments 8 and 2, respectively) which exhibited 3.83 and 3.33 rootlets per plantlet, respectively.

Referring the root length, the tallest one (5.17 cm.) was in closed relationship to the half strength charcoal omitted MS medium supplemented with 0.5 mg/l NAA, followed by those supplemented with 1.0 mg/l IBA regardless of charcoal was added or not (treatments 2 &8, respect.) which showed 5.00 and 4.43 root length, respectively.

From the aggregated/ collected results previously mentioned about the 3 rooting measurements (rooting %; number

of rootlets and length) it could be safely concluded that one half strength MS rooting media supplemented with 1.0 mg/l IBA either with presence or omission of charcoal i.e, treatments (2) and (8) respectively were generally the most favorable ones. Herein, the greatest number and tallest rootlets associated with a significant higher rooting % were always in concomitant to the aforesaid superior rooting media.

On the contrary, adding NAA at the highest level (2.0 mg/l) to the MS rooting medium (one half strength), regardless of activated charcoal was added or not were the worst as the response of rooting %; number of rootlets and average root length was individually concerned.

**B. Interaction effect:**

Data obtained regarding the interaction effect of various combinations between two studied factors (dark / light condition x auxin / activated charcoal on 3 rooting measurements (rooting %, No of roots and average length per each) through rooting stage the following:

1-The highest rooting %(91.67 %) was achieved by culturing on one half strength MS rooting medium supplemented with 1.0 mg/l NAA + 1.0 g/ charcoal under dark condition during the 1$^{st}$ week of incubation, followed by those on 3 rooting media of one half strength MS supplemented by IBA at either 0.5 or 1.0 mg/l (in presence of charcoal without dark or without charcoal with dark, respectively), beside charcoal omitted MS rooting medium + 0.5 mg/l NAA, whereas all exhibited 83.3% rooted plantlets of peach Hansen 536 .

2-The greatest No of roots / plantlet (about 4 rootlets) was in closed relationship to either (adding 1.0 mg/l IBA + 1.0 g/l charcoal) without dark or (0.5 mg/l of NAA / IBA to one liter half strength Ms medium) with dark

3-Adding IBA at 1.0 mg/l to either charcoal omitted or supplemented MS medium with or without dark, respectively; as well as adding 0.5 mg/l NAA to one half strength MS rooting

medium (with neither charcoal nor dark) both resulting in increasing root length as compared to other combinations of rooting media addenda and incubation condition.

4- Adding NAA at the highest rate (2.0 mg/l) regardless of (presence of charcoal and incubation condition) was the worst for 3 rooting measurements, especially when charcoal associated with dark were applied.

Consequently from the aforesaid 1, 2, 3 and 4$^{th}$ topics it could be safely concluded that one half charcoal omitted MS rooting medium supplemented with IBA at 1.0 mg/l when subjected to dark through the 1$^{st}$ week of incubation considered as the most favorable could be recommended whereas the highest rooting %; greatest No of rootlets and relative longer root were achieved. In addition, adding NAA at 0.5 mg/l with dark application to the charcoal omitted MS medium could be also recommended for improving both rooting % and No of roots to the highest level and accepted increase in root length.

## V.5. Acclimatization stage:

Through the acclimatization stage in the green house, some transplanting media consisting of vermiculite + peatmoss + sand mixtures, were investigated regarding their influence on survival % plantlet height and No. of leaflets per each Hansen 536 peach rootstock plantlet.

Data obtained displayed that the vermiculite; peatmoss and sand mixture (1:1:1 by volume) through the acclimatization stage. Such superior medium showed the highest values of survival % (75.00); tallest plantlet (13.67 cm.) and greatest number of leaflets (17.00), while the (sand + peatmoss) was the inferior (33.00%; 7.7 cm. and 10.00 leaves/ plant).

Conclusively, the mixture of (vermiculite + peatmoss + sand) was the superior transplanting medium that could be safely recommended for using as the most favorable medium through acclimatization stage of Hansen 536 peach rootstock plantlets.