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V- SUMMARY AND CONCLUSION

The present study was carried out in the tissue culture laboratory, Horticulture Research Institute, Agriculture Research Center during two seasons of 2002 and 2003.

Mulberry (*Morus nigra*) and jojoba (*Simmondsia chinensis*) explants. These trees were selected as various free in Virology Branch Botany Department, Faculty of Agriculture Cairo University. Hence, the essential goal aimed to find out an ideal method as true propagation method for providing with the adequate quantities of nursery transplants from these desired two species. So, some treatments dealing with the various stages of direct regeneration through tissue culture technique i.e., establishment; proliferation “shoot multiplication” rooting and acclimatization stages were investigated as follow:

V- 1- Establishment stage:

In this concern, two factorial experiments were conducted for studying the effect of explants type (shoot tip and nodal cutting) combined with 3 culture media i.e., MS; B₅ and WPM each at either full; one half or one fourth strength whereas an experiment was devoted for every fruit species i.e., mulberry (*Morus nigra*) and jojoba plants. Surface sterilization of these explants was carried out by soaking in diluted solution (20 %) of sodium hypochlorite (commercial bleach “Clorox” for 20 min for Jojoba plant and mercuric chloride at the concentration of 0.1 % for 10 min, for mulberry (*Morus nigra*) and then rinsed three times in sterilized water 10 min for each to remove any residues

of Clorox or mercuric chloride that remained on the sterilized parts.

The prepared explants were cultured on three different nutrient media (**MS**, **B₅** and **WP**) each at 3 strength basal elements (full, half & quarter) and then the cultures were incubated under culture condition and investigated regarding their influence on survival %, shoot length and number of leaves / plantlet. Data recorded after 4 weeks from culturing on the three media base supplemented with 3 % sugars, 0.7 % agar, (0.1 mg/L) IBA, (1.0 mg/L) BA 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 explants were cultured on half strength solid (**B₅**, **MS** and **WP**) media to investigate the effect of some growth regulators added to culturing media i.e., 3 cytokinins kinds (BA; 2IP and kinetin) each at 3 concentrations (2.0, 4.0 and 6.0 mg/L). Thus, the investigated treatments through the successive three subcultures included in this stage were 27 treatments represented the different combinations between (3 culture media x 3 cytokinins kinds x 3 concentrations).

At the end of each subculture of the three 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 induced per each.

V- 3- Rooting stage:

In this respect adding two auxins (IBA or NAA) each solely either at three levels (2, 4, and 6 mg/L for IBA and 1, 2, and 3 for NAA, or combined together (IBA at 4 mg/L + NAA at 2 mg/L) to one half strength of the three **B₅**, **MS** and **WP** media supplemented with 1.0 gm activated charcoal or not in combination were investigated after 4 weeks from incubation through rooting stage regarding the influence on rooting percentage, number of root per plantlet and average root length of mulberry (*Morus nigra*). In this regard, some rooting measurements (rooting %, number of rootlets / plantlet and average rootlets length) in response to 7 auxins treatments (2 auxins i.e., IBA & NAA each added either solely at 2,4,6 mg/L for IBA and 1,2,3 mg/L for NAA or combined together IBA 4mg + NAA2 mg/L) and charcoal added or not to 3 **MS**, **WPM** and **B₅** rooting media (each at one half strength were investigated for mulberry species. However, with jojoba the investigated treatments were represented to two auxin treatments (7mg/LIBA + 1mg/L NAA plus either 1or 1.5mg/L Caffeic acid) x 3rooting media (**MS**; **B₅** and **WPM** each at one half strength) x 2 charcoal treatments (charcoal added or not). These, 42 treatments (3 rooting media x7 auxin treatments x 2 charcoal added) were the investigated combinations for mulberry , while for jojoba 12 treatments (3 culture media x 2 auxin combinations x 2 charcoal added)were involved.

After explants had been cultured (3 explants per each jar) they were incubated along the rooting stage (four weeks) in light except 1st week in dark.

The two experiments were arranged in complete randomized design with, three replicates for each treatment of the 42 and 12 combinations (treatments) investigated for mulberry and jojoba, respectively whereas, every replicate was presented by four jars each cultured with 3 explants.

V- 4- 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 *in vivo* condition. Accordingly, both mulberry and jojoba plantlets were transplanted in (300 ml) plastic pots contain (vermiculite: peat moss: sand mixture) (1:1:1) as growing medium and maintained in green house for four weeks. The experiment was arranged in a complete randomized design with 12 and 42 treatments for jojoba and mulberry transplants.

Data recorded after 4 weeks of transplanting, were dealing with survival %, plant height (cm.) and number of leaves / plant.

The obtained results could be summarized as follows:

V- 1- Establishment stage (1st & 2nd experiments):

Concerning the response of survival % to specific effect of explants type (shoot tip and nodal cutting); strength media (full, half and quarter) and media type (B5, MS and WP) as well as their combinations (interaction effect), obtained data revealed the following:

A- Specific effect:

The three measurements (survival %, number of shootlets and average shoot length) followed the same trend regarding their response to specific effect of a given investigated factor (explants type, media type and concentration of media).

Concerning the specific effect of explants type (shoot tip and nodal cutting) both survival % and number of shoot as well as number of leaflets / shoot followed the same trend regarding their response to specific effect of explants type whereas the three measurements exhibited higher values with shoot tip, while the reverse was true with nodal cutting during the two seasons of study.

As for the specific effect of media type, data obtained revealed that survival %, average shoot length and number of leaflets / shoot were generally increased with MS medium followed in a descending order by B₅ medium and WP medium.

Regarding the specific effect of strength media on survival %, shoot length and number of leaflets/shoot, the obtained data showed that full strength media proved to be the more suitable for the three measurements while quarter strength media was the inferior during two seasons of study.

B- Interaction effect:

A significant difference were exhibited due to interaction effect of different combinations between explants type x media type and strength media. However, shoot tip x MS medium x full strength medium exhibited the highest value of survival %, shoot length (cm.) and number of leaflets/shoot during two seasons of

study. On the other hand, nodal cutting x WP medium x quarter strength medium had the lowest value in this respect.

V- 2- Multiplication stage (1st and 2nd experiments):

During the three subcultures included in this stage, specific effect of media type (**B₅**, **MS** and **WP**), supplemented growth regulators (**BA**, **2IP** and **Kinetin**) full strength media and concentration (2.4 and 6 mg/L), as well as interaction effect of various 27 combinations between 3 studied factors were investigated during each subculture (each extended four weeks) pertaining the response of number of proliferated shoots; average shoot length and number of leaflets per each..

A- Specific effect:

- 1- Three growth measurements (No. of shoots, average shoot length and No. of leaflets / each) followed the same trend concerning their response to specific effect of media type. Herein, **MS** medium was the superior followed in a decreasing order by **B₅** and **WP** media. However, differences were significant during 3 subcultures during the two seasons of study.
- 2- Regarding the specific effect of growth regulators kind (**BA**, **2IP** and **Kinetin**) supplemented to full strength medium, it was quite clear that all three growth measurements (No. of shoots, average shoot length and No. of leaflets / shoot) followed the same trend, whereas adding **BA** to the medium was the superior followed in a decreasing order by **2IP** and **Kinetin** during the study.
- 3- As for the specific effect of concentration of growth regulators, it was quite clear that all 3 growth measurements

followed the same trend, whereas adding (2 mg/L) was the superior followed in a decreasing order by both (4 mg/L) and (6 mg/L) which ranked last during each subculture in both seasons.

B- Interaction effect:

A remarkable interaction was shown between media type, growth regulators kind and concentrations of growth regulators. Meanwhile, adding BA at (2 mg/L) to full strength MS medium was the superior and had the greatest number of shoots, average shoot length (cm.) and number of leaflets / shoot. On the other hand, supplemented Kinetin at (6 mg/L) to full (B₅, MS and WP) media had the lowest value of three measurements during 3 subculture in this respect.

V- 3- Rooting stage:

A- Specific effect:

- 1- Concerning the specific effect of media type on the three growth measurements (rooting percentage, number of roots / plant and average root length / each) followed the same trend concerning their response to specific effect of media type. Hence, MS medium was the superior for *Morus nigra* while WPM was the most suitable for jojoba, during the two seasons of study.
- 2- With regard to specific effect of activated charcoal, it is quite clear that its presence activated charcoal increased significantly rooting %, number of roots / plant and average root length (cm.) for both *Morus nigra* and jojoba during the study.

3- As for the specific effect of auxins treatments, the obtained results revealed that, IBA at (7 mg/L) + NAA at (1 mg/L) + Caffeic acid at (1 mg/L) treatment significantly increased rooting %, number of roots / plant and average root length for jojoba plant. Moreover, IBA at either (6 mg/L), or (4 mg/L) and NAA at 3 mg/L significantly increased both rooting % and number of roots / plant as well as average root length of mulberry *Morus nigra*, respectively during the study.

B- Interaction effect:

Data obtained regarding the interaction effect of various combinations between three studied factors (media type, auxins treatments and activated charcoal on three rooting measurements (rooting %, number of roots and average length per each) through rooting stage showed the following:

- 1- The highest rooting % was achieved by culturing on half strength **WPM** rooting medium supplemented with (7 mg/L) IBA + (1 mg/L) NAA + (1.0 mg/L) Caffeic acid in presence of activated charcoal for jojoba plant while, the reverse was detected by the charcoal omitted **B₅** medium supplemented with IBA at (7 mg/L) + NAA at (1 mg/L) + Caffeic acid at (1.5 mg/L) during the two seasons of study. In addition, other combinations were in between.
- 2- The highest value of rooting % for mulberry (*Morus nigra*) by half strength **MS** medium supplemented with charcoal + 6mg/L IBA. Meanwhile, the least values were coupled to the charcoal omitted **WPM** supplemented with 1mg/L NAA. In addition, other combinations were in between.

- 3- The greatest No. of roots / plantlet in jojoba plants was in closed relationship to half **WPM** medium supplemented with IBA at (7 mg/L) + NAA at (1 mg/L) with or without activated charcoal during two seasons. On the other hand, the least value of number of roots / plantlet was achieved half **B₅** medium supplemented with IBA at (7 mg/L) + NAA at (1 mg/L) + Caffeic acid at (1.5 mg/L) without activated charcoal. In addition, other combinations were in between.
- 4- The charcoal supplemented half strength **MS** rooting medium supplied with IBA at (6 mg/L) showed the highest number of roots / plant for *Morus nigra*. On the other hand, the least number of roots / plant was found by half **WP** medium supplemented with IBA at (2 mg/L) without activated charcoal. In addition, other combinations were in between.
- 5- Adding IBA at (7 mg/L) + NAA at (1 mg/L) + Caffeic acid at (1 mg/L) in presence of activated charcoal to half strength **WP** medium had the tallest root per Jojoba plant. On the other hand, adding IBA at (7 mg/L) + NAA at (1 mg/L) + Caffeic acid at (1.5 mg/L) without activated charcoal to half strength **B₅** medium showed the shortest rootlets. In addition, other combinations were in between.
- 6- Adding 4mg/L IBA to half strength **MS** rooting medium with activated charcoal induced the tallest root / plant of mulberry (*Morus nigra*). The half strength **MS** medium supplemented with IBA at (2 mg/L) without activated charcoal showed the shortest rootlets. In addition, other combinations were in between.

V- 4- Acclimatization stage:

Through the acclimatization stage in the green house, both jojoba and mulberry (*Morus nigra*) were transplanted from the **B₅**, **MS** and **WP** rooting media to acclimatization transplanting media. Plantlets were washed with tap water and then they were dipping in rizolix solution as fungicide material for 10 min.

Both mulberry and jojoba plantlets were transplanted in (300 ml) plastic pots containing autoclaved transplanting media (vermiculite: peat moss: sand) (1:1:1) and maintained in green house for four weeks to investigating their effect on survival %, and other growth measurements (plant height and number of leaves per plant) during this stage.

The obtained results could be summarized as follows:

A- Specific effect:

- 1- Three growth measurements (survival %, plantlet height and number of leaflets per each for both mulberry and jojoba followed the same trend concerning their response to specific effect of media type. Hence, both **WP** and **MS** media were the superior and showed the highest values of survival %, tallest plantlet with greatest number of leaflets for both mulberry and jojoba during the two seasons of study.
- 2- As for the specific effect of adding activated charcoal, data obtained revealed that survival %, plant height and number of leaves / plant followed the same trend regarding their response to the beneficial effect of adding activated charcoal to rooting media for both mulberry and jojoba during the two seasons of study.

3- With respect to the specific effect of auxins treatments, on survival %, plant height and No. of leaves / plant. IBA at (7 mg/L) + NAA at (1 mg/L) + (1 mg/L) Caffeic acid and IBA at (6 mg/L) significantly increased the three measurements for jojoba and mulberry, respectively during the two seasons of study.

B- Interaction effect:

A significant effect was detected as a result of the interaction between one half strength WP medium supplemented with IBA at (7 mg/L) + NAA at (1 mg/L) + (1 mg/L) Caffeic acid with activated charcoal and one half strength MS medium supplemented with IBA at (6 mg/L) with activated charcoal showed the highest value of survival %, tallest plant height and greatest No. of leaves / plant for jojoba and mulberry during the study.

In addition, one half strength WP medium without activated charcoal and supplemented with NAA at 1 mg/L treatment had the lowest value of survival %, plant height and No. of leaves / plant for Mulberry during the two seasons of study.

Moreover, one half strength B₅ medium supplemented with IBA at (7 mg/L) + NAA at (1 mg/L) + Caffeic acid at (1.5 or 1 mg/L) with or without activated charcoal exhibited the lowest values of survival %, plant height and No. of leaves / plant for jojoba during two seasons.

Conclusively, it could be safely recommended the following:

- 1- Through establishment the highest increase in survival % , number of shoots and number of leaf lets/each was found by using shoot tip, one half strength MS media for both mulberry (*Morus nigra*) and jojoba (*Simmondsia chinensis*) during the study.
- 2- Proliferation stage during three subcultures exhibited significantly the highest rate by using one full strength MS medium, BA at 2 mg/L during the two seasons of the study.
- 3- The highest rooting % and survival percentage with tallest plant & higher number of leaves/plant was detected by using half strength MS + activated charcoal + IBA (6 mg/L) for mulberry, and half strength WP medium + activated charcoal + IBA (7mg/L) + NAA (1 mg/L) + Caffeic acid (1 mg/L) for jojoba during both seasons of the study.