ENGLISH SUMMARY

5-SUMMARY

This investigation was carried out at the Tissue Culture Unit. Horticulture Department, Faculty of Agriculture. Moshtohor, Benha University during the period 2005 to 2008.

In vitro peach rootstocks (Nemagaurd & Okinawa) and Amar apricot rootstock (Balady) plants were directly prepared by using procedures recommended by Zaied (1997) findings. The plants resulted by using these procedures were employed in different experiments of salinity and drought stresses as well as indirect regeneration.

Different explants (shoot tip, leaf discs and stem segments) from in vitro peach rootstocks (Nemagaurd & Okinawa) and Amar apricot rootstock (Balady) were taken for indirect regeneration purposes. The explants were cultured on a basal modified MS medium supplemented with 2.0 mg/L BAP (6-benzylaminopurine), 0.5 mg/L IBA (Indol-3-butyric acid), 30 g/L sucrose and 7 g/L Difico Bacto agar. The pH of the medium was adjusted to 5.7 and subjected to some treatments to study the treatments which induced indirect regeneration from rootstocks under study (callus production and development and Plantlet regeneration).

5.1. Peach rootstocks

5.1.I. Indirect regeneration:

Different explant types, cooling pretreatments, auxin types, auxin concentrations, storing periods and hormonal balances were tested. The obtained results were as follow:

1- Culturing of leaf discs explant of two peach rootstocks induced a significant reduction in Necrosis. Also, increased Callus production parameters.

2- Callus production parameters were significantly improved when either Nemagaurd or Okinawa rootstock explant was used.

3- Keeping of the explants for 3 days in refrigerator (5°C) improved Necrosis, explant development and Callus production parameters.

4- Adding indole-3-butyric acid (IBA) to the culture medium significantly surpassed both NAA and 2,4-D in increasing callus production parameters and reduced Necrosis.

5- IBA supplementation at 3.0 mg/L level to the culture medium succeeded in reducing Necrosis and maximized explant development and callus production parameters.

6- Culturing of either Nemagaurd or Okinawa rootstock on the medium supplemented with adenine sulphate (80 mg/L) or

yeast extract(300 mg/L) enhanced the highest callus production parameters while reduced Necrosis parameter.

7- Storing of callus for 4 or 6 months showed a significant increase in Necrosis, Smooth callus and plantlets regeneration parameters.

8- Storing callus for 2 months resulted in increasing Globular callus, No. of globules, Callus size and Callus colour parameters.

9- Culturing of either Nemagaurd or Okinawa rootstock on the medium supplemented with 2.0 mg/L BAP and 0.0 , 0.5 or 1.0 mg/L IBA resulted in significant maximizing No. of regenerated plantlets compared with other concentrations.

5.1.II. Stress tolerance:

5.1.II.a. Salinity tolerance:

5.1.II.a.1. Direct regeneration

1- The adverse effect of salinity on tissue culture parameters of peach rootstocks appeared greatly by higher concentrations of NaCl followed with $CaCl_2$ and their combination (NaCl and $CaCl_2$).

2- Increasing NaCl concentration more than 1000 ppm induced a bad effect on all parameters under study up to the lethal concentration caused by 2000 ppm which caused almost

death of the *In vitro* plants. However, the effect of $CaCl_2$ with different levels was less toxic to the plants as compared with the similar levels of NaCl.

3- Increasing ability of rootstocks under study to tolerate 2000 ppm NaCl concentration by gradual increase of NaCl concentration from 1000 ppm in the culture medium up to the lethal concentration by 2500 ppm which caused almost death of the *In vitro* plantlets.

4- Vegetative growth parameters were greatly affected by different salinity concentrations (NaCl & $CaCl_2$) than rooting parameters.

5- Total phenols and proline were maximized by increasing NaCl concentration up to 2000 ppm for peach rootstocks under study. while chlorophyll A&B, total sugars and total indoles decreased in both peach rootstocks.

5.1.II.a.2. Indirect regeneration

1- Increasing NaCl concentration more than 1500 ppm induced a bad effect on all growth parameters of in direct regenerated plants from stored callus.

2- Increasing ability of rootstocks under study to tolerate 2500 ppm NaCl concentration by increasing NaCl concentration from 1500 ppm in culture medium gradually up to the lethal concentration by 3000 ppm which caused

almost death of the *In vitro* regenerated plants from stored callus.

3- The adverse effect of salinity on growth and chemical parameters of *in vitro* regenerated plants from stored callus of both rootstocks under study.

4- Total phenols and proline were maximized by increasing NaCl concentration up to 2000 ppm for peach rootstocks under study. while chlorophyll A&B, total sugars and total indoles were decreased in both peach rootstocks plants from stored callus.

5.1.II.b. Drought tolerance:

5.1.II.b.1. Direct regeneration

1- Increasing mannitol concentrations had an adverse effect on Growth, No. of shoots, Shoot length, Root length and No. of roots and gave the best results with Shoot thickness of peach rootstocks (Nemagaurd & Okinawa).

2- Growth parameters *i.e.* Growth, No. of shoots, Shoot length, Root length and No. of roots were responded positively with the lowest PEG concentration while was Necrosis increased in higher concentrations of PEG.

3- Drought resulted from different concentrations of agar was greatly tolerated as appeared from the reduction of adverse effects on most tissue cultural parameters by most agar concentrations studied.

5.1.II.b.2. Indirect regeneration

1- Increasing ability of rootstocks under study to tolerate up to 60 g/L mannitol concentration and this reflected on most tissue cultural parameters under study.

2- Increasing ability of rootstocks under study to tolerate 6% PEG concentration by increasing PEG concentration from 4% in culture medium gradually up to the lethal concentration by 7% which caused almost death of the *In vitro* regenerated plantlets from stored callus

3- Increasing ability of rootstocks under study to tolerate 10 g/L agar concentration by increasing agar concentration from 8 g/L in culture medium gradually up to the lethal concentration by 11g/L which caused almost death of the *In vitro* regenerated plantlets from stored callus

5.2. Apricot rootstock

5.2.I. Indirect regeneration:

1- leaf discs was showed an increase in callus production of Amar apricot rootstocks (Balady) as compared with both shoot tip and inter nodal segments.

2- Keeping of the explant of Amar apricot rootstocks (Balady) for 3 days in refrigerator (5°C) enhanced improved callus production of the explant and reduced Necrosis and

Browning as well as increased explant development and callus production.

3- Indole-3-butyric acid (IBA) was superior than both NAA and 2,4-D in increasing Explant development and Callus production parameters while reduced Necrosis.

4- Supplementation of the culture medium with 3.0 mg/L IBA caused a decrease of Necrosis and increased Explant development and Callus production parameters.

5- Supplementing culture medium with adenine sulphate (80 mg/L) encouraged a reduction of Necrosis and increased Explant development then adding of yeast extract (300 mg/L) to culture medium which succeeded in maximizing Callus production.

7- Callus production was increased when callus stored up to 6 months.

8- Supplementation of the culture medium with 1.0 mg/L IBA and 3.0 mg/L BAP encouraged Growth, No. of regenerated plantlets and Greening and reduced Necrosis.

5.2.II. Stress tolerance:

5.2.II.a. Salinity tolerance:

5.2.II.a.1. Direct regeneration

1-The adverse effect of salinity on tissue culture parameters of Amar apricot rootstock (Balady) appeared greatly

by higher concentrations of NaCl followed with $CaCl_2$ and their combination (NaCl and $CaCl_2$).

2-Increasing salinity concentrations more than 1000 ppm NaCl had a detrimental effect on most parameters under study up to the lethal concentration 2000 ppm NaCl which caused almost death of *in vitro* plants. However, the effect of $CaCl_2$ with different levels was less toxic to the plantlets compared with the similar levels NaCl. Meanwhile, combination of NaCl + $CaCl_2$ reduced the toxicity of NaCl.

3-Increasing ability of rootstock under study to tolerate 2000 ppm NaCl concentration by increasing NaCl concentration from 1000 ppm in culture medium gradually up to the lethal concentration by 2500 ppm which caused almost death of the *In vitro* plantlets.

4-Vegetative growth parameters were greatly affected by different salinity concentrations (NaCl & $CaCl_2$) than rooting parameters.

5- Total phenols and proline were maximized by increasing NaCl concentration up to 2000 ppm for peach rootstocks under study. while chlorophyll A&B, total sugars and total indoles was decreased in both peach rootstocks.

5.2.II.a.2. Indirect regeneration

1- Increasing NaCl concentrations more than 1500 ppm induced a bad effect on all growth parameters of in direct regenerated

plants from stored callus of the rootstock under study up to the lethal concentration caused by 2000 ppm which caused almost death of the *In vitro* plantlets.

2- Increasing ability of rootstocks under study to tolerate 2000 ppm NaCl concentration by increasing NaCl concentration from 1000 ppm in culture medium gradually up to the lethal concentration by 2500 ppm which caused almost death of the *in vitro* regenerated plants from stored callus.

3- The adverse effect of salinity on growth and chemical parameters of *in vitro* regenerated plants from stored callus of the rootstock under study.

5.2.II.b. Drought tolerance:

5.2.II.b.1. Direct regeneration

1- Increasing mannitol concentrations had an adverse effect on Growth, No. of shoots, Shoot length, Root length and No. of roots while gave the best results with Shoot thickness of apricot rootstock (Balady).

2- Growth parameters *i.e.* Growth, No. of shoots, Shoot length, Root length and No. of roots responded positively with the lowest PEG concentration while Necrosis increased in higher concentration of PEG.

3- Drought resulted from different concentrations of agar was greatly tolerated as appeared from the low adverse effect of most higher agar concentrations on most tissue cultural parameters.

5.2.II.b.2. Indirect regeneration

1- Increasing ability of rootstock under study to tolerate 90 g/L mannitol concentration by increasing mannitol concentration from 80 g/L in culture medium gradually up to the lethal concentration by 100 g/L which caused almost death of the Indirect regenerated plants from stored callus.

2- Increasing ability of rootstocks under study to tolerate 10% PEG concentration by increasing PEG concentration from 8% in culture medium gradually up to the lethal concentration by 11% which caused almost death of the *In vitro* regenerated plantlets from stored callus

3- Increasing ability of rootstock under study to tolerate 14 g/L agar concentration by increasing agar concentration from 12 g/L in culture medium gradually up to the lethal concentration by 15 g/L which caused almost death of the *In vitro* regenerated plantlets from stored callus

5.3. DNA finger print:

RAPD analysis detected genetical differences in peach and apricot rootstocks occurred from callus storage for 6 months (somaclonal variations) treatment.

CONCLUSION

Most tissue culture and chemical parameters were adversely affected by increasing drought and salinity levels in the medium. On the other hand, total phenols and proline contents were increased with increasing salinity. In the meantime, finger print showed a genetical variabilities occurred in Nemagaurd and Okinawa as well as Balady rootstocks as a result of storage of callus specially those stored for 6 months which activated somaclonal variations.