V- SUMMARY & CONCLUSIONS

This study was carried out in the Tissue Culture Lab, Hor. Dept, Faculty of Agric., Mosthtohor during the period 1990-1992. The objective of this study was to find out the best microprogagation techniques for overcoming the problem of high demand for healthy highly productive coffee plants of known cultivars.

The retarding factors for further development of the used explants were studied either by the determination of phenolic compounds in both explant and medium or through histological studies for developmental stages of somatic embryogenesis.

Furthermore, multiplication of the resulted plantlets was concerned. The results can be summarized as follows :

I- Inhibitor materials :

1- The leaf disc explant had the highest level of phenolic compounds either before or after pretreatment with antioxidant solution while internode segment was the least in this concern.

2- The rate of reduction in phenolic compounds was very high when leaf disc explants were used while an opposite trend was shown in the case of internode segments.

3- The rate of accumulation of phenolic compounds in the culture medium was almost similar at the end of establishing period for all used explants.

4- Callus contained very low content of phenolic compounds which decreased gradually by the time up to the lowest content after 8 weeks from culturing time.

5- Accumulation of phenolic compounds in the callus culture medium was very low.

6- Antioxidant treatments is critical for micropropagation of C. arabica explants.

7- There were a negative relationship between necrosis and further development of the cultured explant.

8- The combination of antioxidant solution, 3 gm/L activated charcoal and 40 mg/L L-cysteine treatment, was effective in decreasing necrosis and encouraging both callus production when leaf disc or internode segment explants were used and enhanced plantlets regeneration when apical meristem or single node cuttings were used.

9- Charcoal is valuable either alone or combined with any other antioxidants in reducing necrosis and encouraging either callus production or plantlets regeneration.

II- Somatic embryogenesis :

1- Leaf discs were the best explants of coffee giving the highest callus production.

2- 2,4-D surpassed any other auxin used in callus production.

3- The highest concentrations of auxin and kinetin are very important for encouraging callus production (1.0 & 2.0 mg/L 2,4-D combined with 1.0 mg/L kinetin).

4- Induction and development are dependent on callus type as friable callus can not develop into somatic embryos while globular callus develops successfully.

5- 200 mg/L casein hydrolysate enhanced induction of the highest number of somatic embryo.

6- Malt and yeast extracts failed completely in encouraging the induction of somatic embryogenesis.

7- Higher kinetin concentration (0.5 mg/L) in combination with casein hydrolysate induced the highest somatic embryogenesis.

8- Developmental stages of somatic embryogenesis are directly related to callus colour and age.

9- The number of somatic embryos were closely related to callus age as the older and dark brown callus gave the highest number of somatic embryos.

10- The number of lobes multiplied every 2 weeks while the number of somatic embryos per lobe is some what constant.

11- The somatic embryo has 3 stages starting after 3-4 weeks from culturing time with globular embryo, forming heart-shaped embryo after about 2 weeks which finally developed into turpedo shaped embryo after 8-10 weeks from culturing time.

12- Proliferation of somatic embryo took place after 6-8 weeks from culturing time.

13- 20 ml/L coconut milk combined with 0.1 mg/L GA3, 0.1 mg/L kinetin and 1 mg/L NAA enhanced plantlets regeneration from well developed somatic embryo when added to Murashige & Skoog medium.

14- The number of well developed embryos were abundant while only few of them developed into plantlets.

III- Microcuttings propagation :

1- IAA is the most effective auxin in inducing large number of regenerated plantlets.

2- Single node cuttings surpassed apical meristem in number of regenerated plantlets

3- Increasing the concentrations of auxin was most effective in increasing number of regenerated plantlets.

4- Combination of auxins and 6-benzylaminopurine was very important in encouraging plantlets regeneration.

IV- Proliferation :

1- The higher concentration of 6-benzylaminopurine up to 8 mg/L encouraged the largest number of plantlets.

2- The combination of 6-benzylaminopurine with 0.5 mg/L IAA produced the best results of proliferation.

3- 6-benzylaminopurine surpassed kinetin in increasing the multiplication of coffee plants.

It could be concluded that leaf disc explant contained the highest level of phenolic compounds while internode segments contained the lowest level as compared with apical meristem and single node cuttings. Phenolic compounds decreased in different explants but the reverse was true as the culture medium was concerned. The best treatment for reducing

the content of phenolic compounds and in turn leads to reducing necrosis was the combination of antioxidant solution pretreatment together with 3 gm/L activated charcoal and 40 mg/L cysteine in the medium.

Leaf disc explant was found to be the most suitable explant for callus production, induction and development of somatic embryogenesis of coffee plants. Also, 2,4-D with higher concentrations (1.0 & 2.0 mg/L) was suitable for large callus production. Casein hydrolysate proved to be excellent organic additive for induction and development of somatic embryos.

Callus coloures changed in relation to development of somatic embryogenesis stage. Globular embryo appeared after 4 weeks from culturing, then developed into heart-shaped and finally to turpedo-shaped somatic embryo after 8-10 weeks which regenerated into plantlets.

The best plantlets regeneration was obtained when single node cutting was used as explant and 1.0 mg/L IAA combined with 1.0 mg/L BAP were added to the media. In the same time, higher concentrations of BAP combined with 0.5 mg/L IAA encouraged the best proliferation of coffee plantlets.

The most important problem in this investigation is that although large number of somatic embryos were induced and developed but most of these embryos failed to regenerate into plantlets which require further study to regenerate a whole plant.