V. SUMMARY

This investigation was carried out at the Tissue Culture Unit. Horticulture Department, Faculty of Agriculture, Moshtohor, Benha University during the period from 2003 to 2006.

New leaves from *in vitro* and sterilized *in vivo* pineapple (*Ananas comnosus* cv. Smoth Cauun) and banana spp. (Musa cv. Grand Naine) were taken and divided into small sections 1-2 mm. These sections of both *in vitro* and *in vivo* explant source were immersed in different enzyme mixtures in small Petri-dish (6 cm in diameter). Also, different shaking periods and speeds were used during protoplast isolation stage. Meanwhile, different pore size of mesh sieves and centrifugation speeds were concerned in filtration stage. Moreover, different medium types, protoplast densities and different concentrations of auxin and cytokinin as well as different antibiotis were added to culture media, during protoplast culture stage.

The obtained results can be summarized as follow:

5.1. Pineapple:

5.1.a. Protoplast isolation:

1- *In vitro* explant source surpassed *in vivo* explant in increasing protoplast isolation.

2- Enzyme mixture consists of 1% cellulase + 0.5% Macerozyme was more superior in protoplast isolation as compared with the other enzyme mixtures used.

3- The highest protoplasts were obtained when *in vitro* explant source was immersed in solution supplemented with sucrose as osmotic pressure factor.

4- Incubating *in vitro* explant source for 20 hours in enzyme mixture solution succeeded in maximizing protoplast yield.

5- Shaking the incubated mixtures of enzyme solution and *in vitro* explant for 15 min. with speed rate 75 rpm encouraged the highest protoplast yield.

5.1.b. Filtration :

1- 25 µm pore size mesh sieve was superior in increasing the protoplast yield of *in vitro* explant.

2- By increasing pore size mesh sieve had reduced protoplast yield for both explants source used hence they increased cell residues.

3- Centrifugation at the rate of 1000 rpm maximized the protoplast yield of *in vitro* explant source.

5.1.c. Protoplast culture:

1- Kao and Michayluk medium was more preferable in increasing protoplast development. While, Gambourge (B5) medium failed to be effective in protoplast development.

2- Culturing protoplast at density rate 2.5×10^4 was preferable in maximizing protoplast development of *in vitro* explant source.

3- Addition of both 3.0 mg/L auxin (NAA) and 0.2 mg/L cytokinin (BAP) to culture medium succeeded in inducing the highest protoplast of *in vitro* explant source.

4- The best development of protoplsat as a result of reducing contamination was noticed when adding the combination of 0.4 g/L Ampicilin + 0.1 g/L Gentamycin + 0.1 g/L tetracycline was supplemented to the culture medium.

5.2. Banana:

5.2.a. Protoplast Isolation:

1- *In vitro* explant source was more superior than *in vivo* explant in increasing protoplast isolation.

2- Enzyme mixture consist of 1% cellulase + 1% Macerozyme + 1% pectinase was more preferable an increasing protoplast isolation.

3- The highest protoplast was obtained when *in vitro* explant source was immersed in solution supplemented with mannitol as osmotic pressure factor.

4- Incubating *in vitro* explant source for 24 hours in enzyme mixture solution induced an increase in protoplast yield.

5- Shaking the incubated mixtures of enzyme solution and *in vitro* explant source for 15 min. with speed rate 75 rpm caused the highest protoplast yield.

5.2.b. Filtration:

1- 25 µm pore size mesh sieve gave a good response concerning protoplast yield of *in vitro* explant source.

2- Increasing pore size mesh sieve gave negative result in reducing protoplast yield for both explant sources used since they increased cell residues.

3- Centrifugation at the rate of 1000 rpm maximized protoplast yield of *in vitro* explant source.

5.2.c. Protoplast culture:

1- Murashige and Skooge medium encouraged best protoplast development. While KM medium was inferior in this respect.

2- Culturing protoplast at density rate 2.5 x 10^4 increased protoplast development of *in vitro* explant source.

3- Adding the combination of 3.0 mg/L Auxin (NAA) and 0.3 mg/L cytokinin (BAP) to the culture medium induced the highest protoplast development of *in vitro* explant source.

4- Best development of protoplast as a result of reducing contamination was obtained when adding the 0.4 g/L Ampicilin + 0.1 g/L Gentamycin + 0.1 g/L tetracycline to the culture medium.