# V. SUMMARY AND CONCLUSION

This investigation was conducted in the Tissue Culture Unit, Department of Horticulture, Faculty of Agriculture, Moshtohor, Benha University during the period from 2002 to 2005 to point out a complete protocol for gradual adaptation and acclimatization of monocotyledonous *in vitro* plants specifically pineapple and banana plants. Also, histological changes occurred during acclimatization under different treatments. Healthy and well rooted *in vitro* acclimatized pineapple plantlets *Smoth Cauun* cv. and banana *Grand Naine* cv. were selected and taken away from the Jars. The chosen rooted plantlets were washed thoroughly with running tap water to get rid of medium residues then dipped in anti-fungal solution (Vitavax) at the rate of 0.2% for 15 minutes. In addition, the roots were washed with sterilized distilled water and planted in black polyethylene pots of (8 cm) in size and covered with transparent white plastic bags. The plantlets of pineapple and Grand Naine used in this study were subjected to Laboratory acclimatization (*in vitro* and *in vivo*) as recommended by **Abd El-Gawad (2001).** Different agricultural media, microclimate spaces, pot size, growth retardants and $GA_3$ were applied during acclimatization. Also, nutrients medium salts, medium vitamins and organic additives were studied in nutritional studies on both pineapple and banana plants. However, flowering, fruiting and histological studies were only carried out on pineapple plants.

**The obtained results could be summarized as follow:**

## 5.1. Pineapple plants:

### 5.1.A. Acclimatization:

1- Application of combination agricultural media consisted of 50% vermiculite + 50% peatmoss induced the highest percentage of survival percentage, shoot length, number of leaves, leaf length, leaf width, root length and number of roots. While, the combination of (33.3% vermiculite + 33.3% perlite + 33.3% peatmoss) was superior for shoot thickness.

2- Either plastic bags or plastic cage increased both survival percentage and shoot length as compared with those of greenhouse.

3- Large pot size (11 cm) increased survival percentage, shoot thickness, leaf length, leaf width and root length followed 9 cm pot size then (6 cm) as compared with (10 cm). while, other pots sizes (9 & 11 cm) increased shoot length and number of leaves.

4- Cycocel surpassed coumarin and paclobutrazol in all parameters under study i.e. shoot length root length and number of roots. While, coumarin induced an increase in leaf length.

5- Application of higher concentration of CCC (0.5 mg/L) encouraged an increase in survival percentage, shoot length, leaf width, root length and roots number. However, higher concentration of $PP_{333}$ (0.5 mg/L) enhanced a significant increase in shoot thickness.

6- The higher $GA_3$ concentrations (300 and 400 ppm) an increase in shoot length, shoot thickness, leaf length, leaf width, root length and number

of roots as compared with those of lower concentration 100 and 200 ppm).

7- Pineapple plantlet roots treated under greenhouse conditions with coumarin showed elongation in root tissues which increased with increasing of concentrationand showed large cortex and lacked fibers as well as small xylem and phloem with small pith. Roots of plantlets treated with cycocyl (CCC) showed reduction in elongation which increased with increasing concentration beside cortex consists of two large layers separated by large ring of sclerenchymatous tissues (fibers), extensive xylem and phloem. Meanwhile, roots of plantlets treated with paclobutrazol ($PP_{333}$) showed an increase in diameter with increasing concentration with two layer cortex of collenchyma, lacked to fibers, had a suitable viscular bundle surrounded with clear healthy pericycle. On the other hand, roots of plantlets previously treated with 0.50 mg/L $PP_{333}$ treated again with 100, 200, 300 and 400 ppm of gibberellins ($GA_3$) increased root-hairs, cortex size, root diameter, viscular bundle significantly higher concentration, fibers of sclerenchymatous tissues were clear and intensive at 100 and 200 ppm concentrations, while less or no fibers at 300 or 400 ppm concentrations.

8- Pineapple plantlet stems treated under greenhouse conditions with coumarin encouraged the growth as well as increased the elongation of cells when the concentration increased. Moreover, epidermis covered with thickness cuticle, two layer parenchyma cortex, suitable viscular bundles. On the other hand, stems of plantlets treated with cycocyl (CCC) showed a decrease in cell elongation as the concentration increased. Anatomical studies showed large

cortex layer, epidermis with thin cuticle, clear small viscular bundles. Meanwhile, stem of plantlets treated with paclobutrazol ($PP_{333}$) showed decrease in cell elongation and xylem size as concentration increased, with medium cortex. On the other hand, stems of plantlets previously treated with 0.50 mg/L $PP_{333}$ treated again with 100, 200, 300 and 400 ppm of gibberellins ($GA_3$) enhanced the cuticle thickness, increased cortex size, viscular bundle, plastid size, as the concentration increased.

9- Pineapple plantlet leaves treated under greenhouse conditions with coumarin encouraged the growth and increased the elongation of cells when the concentration increased. Anatomical studies showed that epidermis covered with thickness cuticle. Moreover, spongy mesophyll depth was greater than palisade mesophyll, which had little plastid and increase in viscular bundles. Leaves of plantlets treated with cycocyl (CCC) showed increase in cell elongation as the concentration increased, epidermis covered with thin cuticle. Meanwhile, leaves of plantlets treated with paclobutrazol ($PP_{333}$) showed a decrease in the cell elongation and xylem size with the in increasing concentration, epidermis covered with clear cuticle layer, Spongy mesophyll depth was greater than palisade mesophyll which had intensive plastid. On the other hand, leaves of plantlets previously treated with 0.50 mg/L $PP_{333}$ and treated again with 100, 200, 300 and 400 ppm of gibberellins ($GA_3$) enhanced the cuticle thickness, viscular bundle, plastid size, as the concentration increased, Spongy mesophyll depth seems the approximately equal with palisade mesophyll, all concentrations induced hypertrophy in the abaxial leaf side only which showed crinkle.

## 5.1.B. Change occurred during different acclimatization phase:

1- The leaves of acclimatized pineapple plants increased in thickness and length by advancing acclimatization phases.

2- The anatomy of unacclimatized plantlets roots showed smaller roots, cortex consists of two layers of parenchyma without fibers, the cortex and collateral vascular bundles forming a ring around the pith.

3- The anatomy of acclimatized roots reflected larger roots, exodermis had less intense root hairs, cortex consists of three layers, scleenchymatous tissue inbetween two layers of parenchymatous tissues, pericycle cells were larger, primary xylem were larger and surrounded by phloem and separated by zone of parenchyma cells, and pith appears like star sclerenchymatous tissues.

4- The anatomy of unacclimatized plantlet stems were smaller epidermis with less cuticle thickness, smaller vascular bundles and without pith. However, the acclimatized stems were larger in diameter, epidermis had very thickened cuticle, large vascular bundles with no pith.

5- The anatomy of unacclimatized pineapple plantlet leaves were relatively thin, poorly formed palisade cells and large air spaces, while, the acclimatized ones had distinct palisade, well developed cuticle, high cell density, spongy tissues mesophyll and vascular bundles.

## 5.1.C. Nutritional study:

1- Combination of Murashige & Skoog and Gamborg salts were superior in improving of all parameters of growth and rooting under

study (*i.e.* shoot length, shoot thickness, number of leaves, leaf length, leaf width, root length and number of roots).

2- Murashige and Skoog vitamins were more preferable than either Nitsch and Nitsch (N & N) or Gamborg (B5) vitamins in maximizing all parameters under study, *i.e.* shoot length, shoot thickness, number of leaves, leaf length, leaf width, root length and number of roots.

3- 2.00 mg/L of yeast profoundly increased all parameters under investigation followed by sulphate adenine and coconut milk.

4- Combination of 2 g/L yeast + 0.1 g/L adenine + 8% coconut milk are important for inducing an increase in all parameters under study

### 5.1.D. Flowering and fruiting:

1- Combination of amino and yeast extract maximized most growth parameters, which in turn maximized shoot length, shoot thickness and leaf length while, ethephone increased flowering length and flowering width.

2- Higher concentration of ethephone (1 ml/L) induced an increase in crown width, number of crown leaves, and neck thickness while lower concentration (0.25 ml/L) increased number of fruitlets and neck length parameters.

## 5.2. Banana plants:

### 5.2.A. Acclimatization:

1- The combination treatment (25% vermiculite + 25% perlite + 25% sand + 25% loam) induced the highest percentage of survival and

improved shoot length, shoot thickness, No. of leaves, leaf length, root length and roots number parameters.

2- Plastic bags increased survival percentage, shoot length, leaf length, leaf width and number of roots followed by plastic cage as compared with greenhouse climate space.

3- Pots size (10 cm) increased survival percentage, shoot length, number of leaf, leaf length, leaf width and root length followed by (9 cm) then (6 cm) as compared with (11 cm).

4- Coumarin growth retardants surpassed $PP_{333}$ and CCC in most parameters used *i.e.* survival percentage, number of leaves, leaf length, leaf width and root length.

5- The higher concentration of paclobutrazol (0.5 ml / L) surpassed coumarin and cycocel in shoot thickness, leaf width and number of roots while lower concentration (0.125 ml/L) encouraged leaf length followed with coumarin and cycocel. While, coumarine concentrations, specially higher ones, had the best effect an increasing root length and number of leaves.

6- Higher concentration of $GA_3$ (500 ppm) had an adverse effect on the most growth parameters under study. However, lower concentrations had less harmful effect.

## 5.2.B. Nutritional study:

1- Combination of Murashige & Skoog + Gamborg + White salts treatment induced superior effect on growth and rooting parameters.

2- Murashige & Skoog vitamin was superior in all parameters of growth (*i.e.* shoot length, shoot thickness, number of leaves, leaf length, leaf width, root length and number of roots).

3- 2.00 mg/L level of yeast profoundly increased all parameters under investigation *i.e.* shoot length, shoot thickness, number of leaves, leaf length, leaf width, root length and number of roots.

4- Combination of 2 g/L yeast + 0.25 g/L adenine sulphate + 2% coconut milk treatment is important for all parameters under study *i.e.* shoot length, shoot thickness, number of leaves and roots, leaf length and width and number of roots.

# CONCLUSIONS

Planting of *in vitro* acclimatized pineapple and banana plantlets in pots (11 cm for pineapple and 10 cm for banana) filled with mixture of agricultural media (50% vermiculite + 50% peat moss for pineapple or 25% vermiculite + 25% perlite + 25% sand + 25% loam for banana) and covered with transparent plastic bags. Also, 0.5 mg/L cycocell as growth retardant enhanced acclimatization, maximizing survival percentage and reducing plant losses during acclimatization. In the same time, using of medium salts (MS + Gamborg for pineapple or MS + Gamborg + White for banana) supplemented with MS vitamins and organic additives (yeast extract, adenine sulphate, and coconut milk) induced the best nutritional status and improved plant growth. Moreover, using of high levels of ethephone (1.0 mg/L) maximized flowering and fruiting parameters of pineapple plants. Furthermore, histological studies showed a significant anatomical changes occurred during acclimatization of pineapple plants.