# SUMMARY AND CONCLUSIONS

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This study was carried out at the Tissue Culture Lab. Horticulture Research Institute, Giza in co-operation with Tissue culture Unit, Hort. Dep. Faculty of Agriculture Moshtohor, Zagazig University during 1998 – 2002. This investigation aimed to evaluate different in vitro germplasm preservation and cryopreservation methods on physical, chemical and viability percentage.

Nodal cuttings of *in vitro Ceratonia siliqua, Acacia salicina* and *Rosa polyantha* were cultured on full strength Murashige and Skoog medium with or without some additive substances [sorbitol (5,10 and 15 g/l), coumarin at (0.5,1.0 and 1.5 mg/l), PEG (10,15 and 20g/l)], and soaking or overlaying in liquid paraffin wax, encapsulation with aglineic salts (sodium alginate 2% w/v) and conserved under low temperature (at 4°C in darkness) or under normal condition (room temperature (at 24°C) under light. Besides exposed the explants to pretreatment of cryopreservation before plunged in liquid nitrogen, the explants were desiccated under $CaCl_2$ 5g/Jar 200ml or Silica gel 5g/Jar, or soaking in cold cryoprotectants solutions PEG + DMSO (5 and 10%) for 10min then soaking in cold solution of ethylene glycol 10 and 20g/l for 10min. Or cultured on MS containing 1.5mg/l coumarin (pregrowth 1) and cultured on MS supplemented with DMSO 10% for two days then plunged in liquid nitrogen and finally using encapsulation dehydration with $CaCl_2$ or Silica gel. After soaking in L.N. for 24 hrs. The explants were thawed directly in water bath at 40 ± 2 ◦C for 5 mint. Then cultured on MS media. All conservation treatments preserved for 6,12 and 18 months, except with pretreatment of

cryopreservation preserved for 24hrs. After which, longevity rate and plantlets characters and chemical constituents were recorded.

Explants taken from the preserved plantlets (for the different periods) were recultured on the micropropagation MS media without any of the additive substances for 8 weeks under the lab. normal conditions. Both longevity rate and growth characters were recorded the data obtained were statistically analyzed and the results may be summarized as follow:

### 1. *Ceratonia siliqua L.*

- The highest survival percentages were achieved by culturing the explants on medium supplemented with 1.5 mg/l coumarin or 15 g/l PEG treatments.
- Adding of 0.5 ,1.0 and 1.5 mg/l coumarin or 15 g/l sorbitol to the *in vitro* storage medium induced the highest shoot numbers.
- Growth parameters i.e. callusing percentage , shoots characters (length, number and leaves number), moisture contents (both shoots and callus) were maximized by using dehydration, coumarin, L.P.W. overlaying treatments.
- Encapsulation and low temperature 4°C storage were the lowest effect on growth development parameters, which is preferable for germplasm preservation.
- Increasing of storage durations from 6 to 12 and 18 months led to decrease in most physical properties under study.
- Storing of the explants, on medium supplemented with 20 g/l PEG or 0.5 mg/l coumarin induced a positive effect on total carbohydrate, phenols and indole contents in the

explants. Similar effect was achieved by using 15 g/l PEG in the medium and dehydration treatments for increasing TSS and osmotic potentiality parameters.

- Chemical characters under study were responded negatively to increase in storage duration from 6 to 12 months. However, increasing storage duration to 18 months resulted in sharp decrease of chemical characters except polyphenyloxidase and peroxides enzyme activities which showed an increase in their activation when storage duration increased from 6 to 12 months while declined again by increasing the storage duration to 18 months.
- The highest viability percentages and growth parameters occurred when stored explants were recultured on medium supplemented with 1.0 mg/l coumarin.
- L.P.W. overlay treatment induced the highest increase of shoots number. However, the reveres were true when encapsulation treatments was used.
- The growth characters of regenerated plantlets i.e. shoot length , shoot number and leaves number parameters were improved when L.P.W. soaking and 1.0 mg/l coumarin treatments were used
- The lowest increase was occurred in preservation when L.P.W. soaking and 1.0 mg/l coumarin treatment were used.
- The lowest increase was occurred in preserved explants during different storage durations were noticed when encapsulation treatment was used.
- The growth parameters of regenerated plantlets was negatively affected by increasing of storage durations from 6 to 12 and 18 months.

## 2. *Acacia salicina*

- Adding the coumarin at 0.5 or 1.0 mg/l was more effective in increasing survival percentage and most parameters under study for all storage durations.
- Number of shoots was increased with most treatments used, while decreased when low temperature 4°C and drying were used.
- Liquid paraffin wax soaking or overlying and desiccation as well as adding various concentration of coumarin and supplementary sorbitol at 5 or 10g/l have improved the callusing potential of the explants.
- low temperature (4°C) and encapsulation gave low callus formation.
- All the preservation treatment had failed to induce root during all storage periods.
- Coumarin and sorbitol showed the highest values of shoot number, shoot length and leaves number. Meanwhile, using overlaying of liquid paraffin oil increases the moisture contents to the highest value for shoots and callus.
- Both of survival rate and callus ability were influenced with storage periods especially 6 months storage. Meanwhile, 12 months periods were the best for the other sprouting percentage and shoot number and their length, but the other character of growth (leaves number – moisture content for shoot and callus) the best result for all these characters were after 6 months storage.
- Overlying of liquid paraffin wax treatments recorded the highest contents of chlorophyll- A and – B. Meanwhile, PEG at 15 g/l raised total carotenoids and total phenols.

Also, total indols and total carbohydrates were affected drying conditions and coumarin.

- The total soluble solids and osmotic potentiality were greatly affected by using of sorbitol at either 5 or 10 g/l level.
- The enzyme (polyphenoloxidase PPO and peroxidase POD activation recorded the highest values for the explants preserved under PEG and desiccation.
- An indirect relationship was noticed between most chemical parameters (pigments, total chlorophyll, TSS and osmotic potentiality as well as activation of peroxidase (POD) enzyme and storage durations .Increasing of storage duration from 6 to 12 and 18 months resulted in the decline of most chemical parameters except total phenols, total indoles and activity of polyphenoloxidase enzyme parameters which maximized by increasing storage durations up to 18 months
- Viability percentages and the growth characters of explants treated with either coumarin or sorbitol were maximized after different storage durations of treated explants

### 3. *Rosa polyantha*

- Coumarin added to the preservation MS media at 0.5 mg/l resulted the highest value of survival percentage. Also, coumarin at 1.0 mg/l and low temperature (4°C) raised the survival rate of the preserved explants
- Low temperature (4°C) in darkness have the highest callus formation percentage.

- The growth characters (shoot number and length , leaves number) increased when growth retardant coumarin at 1.0 mg/l was used except with shoots length and number were increase when exposed to low temperature (4°C) in darkness.
- Regarding the chemical characters, the explants preserved under 0.5 mg/l and 1.0 mg/l coumarin gave increase in the pigments except with total carotenoids which resulted in high value under osmotic stress of sorbitol at 10 g/l. Meanwhile, total phenols, total soluble indols, osmotic potentiality, total soluble solids, contents had increased when coumarin at 1.0 mg/l was used.
- On the enzyme activity of Polyphenoloxidase (PPO) and Peroxidase (POD) both of them resulted in high when (coumarin at 1.0 mg/l) was used .
- Different storage periods gave an adores effect on chemical composition for the explant taken specially for 12 months.
- The explants taken from the preserved plantlets under low temperature (4°C) and darkness or coumarin adding to MS media at 1.0 and 0.5 mg/l increase regeneration viability on micropropagation stage after storage periods.
- Low temperature (4°C) in darkness raised the callusing ability to the highest value.
- On viability percentage the best periods of preservation was 6 months duration on all the growth characters for the explants taken from the conservation treatments.

## On pretreatment of cryopreservation:

- ***Ceratonia siliquia***

  The survival rate recorded the high value for the explants treating with coumarin at 1.5 mg/l for 1 week (Pregrowth I) and encapsulation dehydration under $CaCl_2$.

- ***Acacia salicina***

  Both of dehydration under $CaCl_2$ and soaking in cryoprotactant solution (PVS3) and encapsulation dehydration under the $CaCl_2$ gave the best value of survival rate for the explants plunged in liquid nitrogen

- ***Rosa polyantha***

  All the pretreatment of cryopreservation has a harmful effect on survival percentage whereas all treated explants and plunged on liquid nitrogen have not survived.

## On chemical constituents:

- On *Ceratonia siliquia* the same treatment (drying under $CaCl_2$) gave the highest value. Similarly on *Rosa polyantha* the total phenols, indols and total carbohydrates were resulted the highest value under drying conditions with $CaCl_2$. Meanwhile on *Acacia salicina* explants were soaked in cryoprotactant solution (PVS 1) and pregrowth 2, also, plunged in liquid nitrogen directly without any pretreatment (control) raised the chemical constituents of the treated explants.
- On the endogenous pigments (chlorophyll – A and – B or total carotenoids) both of *Rosa polyantha* and *Ceratonia siliqua* increasing under drying conditions of $CaCl_2$. Meanwhile, on *Acacia silicon* the contents of the pigments increase after soaking in cryoprotactant solution ( PVS3)

- On the enzyme activation of Peroxidase (POD) increasing up to the highest value of *R. polyantha* under soaking in cryoprotactant solution (PVS4) or growing on MS media containing coumarin at 1.5 mg/l for 1 week on *C. siliquia* or encapsulation dehydration under $CaCl_2$ on *A. salicina*. Furthermore, the activation of polyphenoloxidase was increased after soaking in PVS4 for *Ceratonia siliqua* and *Acacia silicon*. Meanwhile, on *Rosa polyantha* gave the highest value after plunged directly in liquid nitrogen.