# V. SUMMARY AND CONCLUSION

The present study was carried out during two consecutive experimental seasons through the four successive 2002 ; 2003; 2004 and 2005 years in the laboratory for Date palm Researches and Development , Agric. Rese. Center, Minis. of Agric., Giza Governorate, Egypt.

The main purpose was aimed to find out an efficient way by which adequate numbers of Zaghloul date palm offshoots could be obtained vegetatively through micropropagation technique in order to meet the increasing demand for establishing new orchards of such desirable soft cultivar. So, the present wark was conducted to induce somatic embryos through either direct or indirect plantlets differentiation *In Vitro* by which sufficient numbers of homogenious female Zaghloul date palm offshoots ( true to the mother trees) could be rapidly produced.

In early March 2002 and 2004 years during $1^{8}$ and $2^{114}$ experimental seasons, respectively offshoots (3-5 kg weight) detached from fruitful Zaghloul date palms grown in Rashid region(El-Behara Governmante) were the clonal plant material used for preparing two explant types i.e, leaf primordia and shoot tip needed for the direct and indirect somatic embryogenesis formation, respectively.

In the laboratory two explant types leaf primordia and shoot tip were prepared and subjected to the recommended procedures mentioned previously (explants preparation, surface sterilization and different culturing media for various stages).

**Experimental layout :**

Two main parts included in the present dissertation were devoted to propagate Zaghloul date palm cultivar *In Vitro* through somatic embryogenesis induced by two ways (direct & indirect embryogenesis), whereas each part involved its own experiments as follows :

**V.I. Part one " direct embryogenesis":-**

In this part experiments devoted through different stages for the clonal *In Vitro* propagation of Zaghloul date cv. by producing somatic embryos directly on the initial (original) explant used (leaf primordia) itself without intervening callusing phase were as follows:

V.I.1. Establishment stage:

Aseptic explants (leaf primordia with a thin layer of soft shoot tissue) were cultured individually on 3/4 strength MS (1962) basal nutrient medium supplemented with other additional constituents 200 mg/L glutamine +170 mg $kH_2PO_4$ 100 mg/L myo-inositol + 3 mg/L Zip + 0.4 mg/L thiamine—HC1 + 100 mg/L 2,4-D + 3 g/L activated charcoal + 7 g/L agar. Cultured jars with leaf primordia explants were incubated at 27° C± 1 under complete darkness through the whole duration of this stage ( 8 successive subcultures at 4 weeks interval) until they became dark brown in colour.

V.I.2.Direct formation and germination of somatic embryos:

At the end of the last subculture ($8^{th}$ one) during establishment stage the remained alive original explants (leaf primordia) were transferred to 3/4 strength MS medium

supplemented with the same additional components except auxin level was reduced to 2.5; 5.0 ; 7.5 and 10.0 mg/L 2,4-D and 2ip was present at 3 mg/L or completely omitted, beside activated charcoal at 3 g/L was also added. Transference of the original explants was repeated 3 times at 4 weeks interval on the same fresh 3/4 MS media and under the same incubation condition of the establishment stage, whereas number of somatic embryos formed per each explant(jar) was counted at the end of each of the 3 subcultures.

At the end of the 3`s subculture of this stage, formed somatic embryos(swellings exhibited on subcultured leaf primordia) were bigger enough to be separated, they were individually subcultured four times at 4 weeks interval on the same 3/4 MS medium(except both auxin and charcoal were omitted while Kinetin at 3 mg/L was added), then incubated under 27° C± 1 with daily 16/8 hrs light/dark periods.

**V.1.3. Multiplication stage:**

To investigate the influence of cytokinins (type and concentration) and silver thiosulphate (STS) to multiplication media two experiments were conducted.

In the experiment two cytokinins types (2ip & kinetin) each at two concentrations (0.1 & 1.0 mg/L) were investigated regarding their stimulating effect on multiplication rate through 3 successive subcultures on auxin omitted 3/4 strength MS medium supplemented with the same other additional constituents used for establishment culture plus 3 g/L activated charcoal + the corresponding cytokinin treatment (type & concentration) through 3 subcultures. However, in the 2'

experiment it was extended for four subcultures on 1/4 MS medium supplemented with the same constituents of the 1 2 experiment besides, ($GA_3$ & NAA each at 0.1 mg/L) and silver thiosulphate (STS) at (0.25; 0.50; 1.0 mg/L or omitted), then incubated under the same condition of 1 St experiment.

**V.I.4.Rooting stage :**

In this stage an experiment was conducted with main purpose to promote rooting process through investigating adding phloroglucinol (PG) to MS rooting medium. Rooting medium was prepared at one half strength of MS basal nutrient medium supplemented with (60 g sucrose + 170 mg $KH_2PO_4$ + 200 mg glutamine+ 100 mg myo-inositol + 0.4 mg thiamine — HC1 + 0.1 mg NAA + 7 g agar) per each liter. Three levels of phloroglucinol (PG) i.e, 20.0 ; 40.0 and 80.0 mg/L, besides no adding as control were investigated. Proliferated shoots derived from previous stage ( multiplication) were cultured individually in test tubes and incubated for 3 subcultures at 27' C and dailyl6 hrs light of 3000 LUX. At the end of each subculture the response to phloroglucinol (PG) level added to MS rooting medium was evaluated through the following rooting measurements :

1- rooting % ; 2- number of developed rootlets per each plantlets and 3- average length of root.

**V.H. Part two (indirect somatic embryogenesis) :**

Shoot tip explant of Zaghloul date palm cv. was the plant material used, where stages and experiments conducted for the somatic embryogenesis were as follows:

VIM. Callus formation:

The prepared shoot tip explants were aseptically cultured on ¾ strength MS basal nutrient medium supplemented with the same additional constituents previously provided to the establishment culture medium used for the direct somatic embryogenesis in the la- part. Cultured jars were incubated (at 27° C± 1 and complete darkness), then repeatedly subcultured 8 times (at 4 weeks interval) on the same fresh medium. At the end of 4th subculture formed callus was compacted and white, while at the end of the 8th subculture it showed friable structure and creamy yellowish colour.

**V.II.2. Maintaining, increasing callus and indirect formation of somatic embryos in response to auxin and cytokinin treatments :**

To maintain and increase formed callus from the former stage, it was aseptically transferred to culture jars contained the same 1/4 MS medium used for former stage (except auxin level) was added here at 3 lower rates (5; 7.5 and 10.0 mg/L either cytokinin was absent or added at 3 mg/L 2ip). Whereas, the response to 6 combinations ( 3 auxin levels X presence or absence of 2ip) was investigated through 3 subcultures (4 weeks interval) under the same incubation condition of the previous stage. At the end of this stage callus became of higher friability state (unattached embryogenic globular callus ).

**V.II.3. Callus differentiation :**

Small masses of the friable embryogenic callus tissues formed through the previous stage were transferred to growth regulators free MS medium at 1/4 strength and supplemented with

(30 g/L sucrose + 170 mg/L $KH_2PO_4$ + 7 g /L agar + 0.4 mg/L thiamin-HC1 + 200 mg/L glutamin+ 3 g/L activated charcoal), then incubated through four successive subcultures at 27" C± 1 and complete darkness. At the end of this stage mature developed embryoids could be obviously noticed either in clusters aggregation or individually distributed.

**V.II.4. Multiplication stage :**

Clusters composed of 3-4 shootlets were separated and inoculated in larger jars (1 cluster/each jar) contained 1/4 MS medium supplemented with (30 g/L sucrose + 3 g/L activated charcoal + 170 mg/L $KH_2PO_4$ + 7 g/L agar + 0.4 mg/L thiamin-HCl + 200 mg/L glutamin ) in order to investigate the influence of 2 cytokinins types (kinetin & 2ip) each at either 0.1 or 0.5 mg/L in combination with 2 NAA levels (0.1 or 0.5 mg/L). Cultured jars were incubated at 27" C and exposed daily to 16 hrs. light of 3000 LUX intensity followed by 8 hrs dark through the whole duration (3 subcultures) of this stage. Number of proliferated shootlets and their average length were recorded at the end of each subculture.

**V.II.5. Rooting stage :**

In this stage three experiments were conducted to investigate the influence of providing rooting medium (1/2 strength MS) with : a- auxins (NAA; IAA and IBA each at 0.5 mg/L added either solely or in different combinations) ; b- silver thiosulphate (STS) at 3 concentrations i.e, 0.25 ; 0.50 or 1.0 ml/L 4 mM STS solution and c- Silver nitrate (SN) at 0.25; 0.50 or 1.0 mg/L $AgNO_3$ in 1-[illegible] ; 2[illegible] and experiments, respectively. Taking into consideration, that pre-rooting application just

preceding rooting stage was done by transferring cultures (proliferated shootlets) obtained at the end of multiplication stage to 1/4 MS medium devoid of growth regulators but supplemented with 3 g/L activated charcoal and incubated under the same condition for two subcultures.

After the proliferated shoots had been developed enough to be easily separated at the end of pre-rooting stage, they were transferred individually to the different rooting media investigated in the following three experiments:

**V.II.5.1. Effect of 3 auxin types and their combinations :**

In this experiment the following eight treatments (auxin types in different combinations) added to half strength MS rooting medium were investigated [a) 1/2 MS auxin free (control); b) 1/2 MS + 0.5 mg/L NAA; c) 1/2 MS +0.5 mg/L IBA; d) 1/2 MS + 0.5 mg/L IAA; e) 1/2 MS + (NAA +IBA each at 0.5 mg/L ); f) 1/2 MS + (NAA+IAA each at 0.5 mg/L); g) 1/2 MS + (IBA +IAA each at 0.5 mg/L) and h) 1/2 MS +(NAA+IBA+IAA each at 0.5 mg/L)].The initially developed plantlets (proliferated shoots) through the pre-rooting stage were individually cultured in test tubes(250 x 25 mm) contained the corresponding rooting media of different eight auxin treatments. Then, incubated in growth room at $27^0$ C± 1 of 16 hrs light at 3000 LUX illumination for three subcultures.

**V.I1.5.2 Effect of silver thiosulphate :**

In this experiment adding silver thiosulphate (STS) to one half strength MS rooting medium supplemented with 0.1 mg/L NAA besides other additional constituents supplied to the

rooting media used in previous experiment (effect of auxin) was investigated regarding the response of rooting measurements.

In this regard developed shoots (plantlets) were individually transferred in test tube filled with one of the following 'A strength MS rooting media :

1- One half strength MS with no STS added as control.

2- One half strength MS + 0.25 ml/L 4mM STS solution.

3- One half strength MS + 0.5 ml/L 4 mM STS solution .

4- One half strength MS + 1.0 ml/L 4 mM STS solution .

Cultured test tubes were incubated for 3 subcultures under the same condition of former experiment.

V.II.5.3. Effect of silver nitrate ($AgNO_3$) :

Adding silver nitrate (SN) to $^1/2$ strength MS rooting medium used in the former two rooting experiments at 3 levels (0.25; 0.50 and 1.0 mg/L) besides no adding as control were investigated. Developed plantlets were transferred to test tubes filled with one of the following rooting media and subcultured for 3 subcultures:

1- One half strength MS without silver nitrate (control).

2- One half strength MS + 0.25 mg/L $AgNO_3$.

3- One half strength MS + 0.50 mg/L $AgNO_3$.

4- One half strength MS + 1.0 mg/L $AgNO_3$.

The complete randomized block design with three replications was used for arranging the investigated treatments in three rooting experiments and every replicate was represented by 3 cultured test tubes. Whereas rooting 'Yo, number of

rootlets/plant and average roots length were recorded at the end of each subculture.

**V.I1.6. Acclimatization stage :**

Acclimatization process was carried out through the following two main steps :

**1- First step** ***(In Vitro*** acclimatization) :

It was carried out through its whole duration (3 weeks) under aseptic condition of the same temperature (27 °C± 1) and light (intensity & period) prevailing through the previous stage (rooting). Herein, Zaghloul date palm plantlets were transferred in 2.8 x 25 cm. Tubes contained one half strength liquid MS basal nutrient medium, then securely capped with aluminum foil and kept for one week. From the beginning of 2 1/4 week ventilation was allowed gradually by punching holes (one hole every day during first five days), then caps were completely removed at the end of 2 1/2 week and along the rest period of *In Vitro* acclimatization.

**2- Second step** ***(Ex Vitro acclimatization) :***

In this step all attempts were devoted for offering all possible efforts to the susceptible succulent *In Vitro* regenerated date palm transplants to survive and continued their growth and development succefully after transferring from the aseptic culture environment *(In Vitro)* to the semi- free living condition of green house *(Ex Vitro).* So plantlets were transplanted individually in plastic pots contained different planting media (peat moss, sand, perlite and vermiculite mixtures), then each

plantlet was covered with white polyethylene bag and kept for three months in greenhouse (plastic tunnel construction). The investigated growing media mixtures were :

a- peat moss + sand (3:1) ; b- peat moss + sand (1:3) ; c- peat moss+ perlite (1:1) ; d- peat moss + sand (1:1) ; e- peat moss + perlite + vermculite (1:1:1) and f- peat moss + perlite + sand + vermculite **(1:1:1:1).** Suitability of the aforesaid 6 mixtures as growing media for the *In Vitro* regenerated Zaghloul date palm plantlets derived from either direct or indirect somatic embryogenesis techniques were evaluated through the *Ex vitro* acclimatization stage pertaining their influence on survival % ( recorded periodically 3 times at one month interval).

The complete randomized block design with three replications was used, each replicate was represented by (3) date palm plantlets.

**V.III. Some chemical analysis:**

In this respect total (soluble sugars; phenols and free amino acids) were determined in directly formed somatic embryos ( swellings formed on leaf primordia) and the embryogenic callus indirectly developed by shoot tip to investigate the effect of different Zip and 2,4-D combinations.

The obtained data could be summarized as follows :

**V.I. Part one (direct embryogenesis):**

**V.I.1. Establishment stage :**

Leaf primordia explants were repeatedly cultured on 1/4 strength MS supplemented with 100.0 mg/L 2,4-D for 8

subcultures (at 4 weeks interval) until tissues of the original explants became dark brown in colour.

**V.I.2. Direct formation and germination of somatic embryos:**

An experiment was conducted to investigate the specific and interaction effects of providing ¾ MS medium with four relative lower auxin levels (2.5; 5.0 ; 7.5 and 10.0 mg/L 2,4-D ) in combination with absence or presence of cytokinin (2ip at 3 mg/L) through 3 subcultures.

**A- Specific effect :**

Data obtained revealed obviously the specific effect of providing MS medium with cytokinin (3 mg/L 2ip) on number of somatic embryos formed directly per each original explant as compared to those cultured on cytokinin omitted media. Meanwhile, data regarding the specific effect of 2,4-D concentration displayed obviously the superiority of the least level (2.5 mg/L) over 3 other ones which failed completely to induce / form somatic embryos.

**B- Interaction effect :**

Providing MS medium used in this stage with 2.5 mg/L 2,4-D + 3 mg/L 2ip exhibited significantly the greatest number of somatic embryos during 3 subcultures.

**V.I.3. Multiplication stage :**

**V.I.3.1. Experiment I " Effect of cytokinins type and concentration" :**

In this experiment specific effect of cytokinin type (Kin. & 2ip) and concentration, as well as interaction effect of their combinations added to MS multiplication media regarding the

response of multiplication rate number and average length of proliferated shootlets per each cultured embryo were investigated.

**A- Specific effect :**

Data obtained, revealed that both parameters responded specifically to cytokinin type, whereas 2ip was more effective than kinetin during 3 subcultures. However, the response was more pronounced with number of proliferated shootlets than their length. As for the specific effect of cytokinin concentration, data obtained that two investigated levels (0.1 & 1.0 mg/L) did not significantly differ as compared each other regarding the response of both number and length of proliferated shootlets. However, two concentrations surpassed statistically control (cytokinin omission).

**B- Interaction effect :**

Data obtained displayed clearly that the specific effect of each investigated factor (cytoki. Type & concentration) reflected directly on their own combinations. Herein, the 2ip supplemented 3/4 MS multiplication medium (regardless of added level) surpassed statistically those of either kinetin or cytokinin omission. Whereas, the greatest number and tallest proliferated shootlets were resulted by culturing somatic embryos on both combinations representative of 2ip supplemented MS medium at either 0.1 or 1.0 mg/L with a relative tendency of variance declared the superiority of 1.0 mg/L 2ip combination in most cases.

**V.I.3.2. Experiment, II " Effect of silver thiosulphate-STS" :**

In this experiment the effect of providing 3/4 MS multiplication medium with silver thiosulphate (STS) at 0.25 ; 0.5 and 1.0 ml/L 4 mM STS solution on multiplication process (number and length of proliferated shootlets) per each cultured somatic embryo (directly derived from Zaghloul date palm leaf primordium).

Data obtained displayed obviously that all investigated silver thiosulphate levels (0.25; 0.50 and 1.0 ml/L 4 mM STS solution) added to 3/4 MS multiplication medium increased significantly both multiplication parameters (number & length of proliferated shootlets)over control (STS omitted medium). However, the least STS rate (0.25 ml/L-4 mM STS) was statistically the superior, since the greatest number and tallest proliferated shootlets were exhibited during four subcultures of multiplication stage with few exception during 1-g and 2 $^{2-1}$ subcultures, whereas both least and intermediate concentrations (0.25 & 0.50 ml/L) were statistically the same.

**V.I.4. Rooting stage :**

In this stage some rooting measurements (rooting % ; number of rootlets and their length) of the proliferated shootlets obtained from the directly formed somatic embryos of Zaghloul date palm cv. were investigated regarding their response to providing one half strength MS rooting medium with phloroglucinol (PG) at 20.0; 40.0 and 80.0 mg/L. Data obtained declared clearly that all 3 investigated PG rates added to MS rooting medium improved significantly three rooting measurements (rooting % ; number of rootlets and their length)

with comparing to the PG omitted medium (control). However, the rate of response to three PG investigated levels was slightly modified from one rooting measurement to another from one hand, but as the three measurements together were taking into consideration the superiority of the intermediate PG level (40 mg/L) was too firm to be noticed from the other side.

**V.II. Part two (indirect somatic embryogenesis):**

**V.II.1. Callus formation :**

Shoot tip explants were aseptically cultured on 3/4 MS medium supplemented with 100.0 mg/L 2,4-D and repeatedly subcultured on the same fresh medium for eight times at four weeks interval . After four subcultures a compacted white callus was formed , which it became of creamy yellowish colour and friable structure at the end of 8`[1] subculture.

**V.II.2. Indirect formation of somatic embryos as influenced by auxin and Zip treatments :**

**A-Specific effect :**

Data obtained displayed that the response of somatic embryos formed per each jar to the specific effect of two investigated factors i.e, 2,4-D levels (5.0; 7.5 and 10.0 mg/L) and presence of 2ip at 3.0 mg/L added to 3/4 MS medium was more pronounced with former factor (auxin level) than later one (presence of cytokinin). Anyhow, a positive relationship was obviously detected between 2,4-D level and number of somatic embryos formed. However, with presence of 2ip at 3 mg/L the increase was too slight to reach level of significance as compared to the cytokinin omitted MS medium.

**B- Interaction effect :**

It was so clear to be concluded that providing 3/4 MS medium with 10.0 mg/L 2,4-D + 3.0 mg/L Zip was the most effective, where the greatest number of somatic embryos per each jar was exhibited during 3 subcultures.

**V.II.3. Callus differentiation :**

In this stage yellowish friable embryogenic callus was transferred to growth regulators free MS medium and repeatedly subcultured on the same medium for four subcultures. Well mature developed embryoids were induced (in clusters aggregation or individually).

**V.II.4. Multiplication stage :**

In this stage proliferation process (expressed as number and average length of proliferated shootlets per each cultured jar with the indirectly formed somatic embryos) as influenced by specific effect of (cytokinin type, concentration and NAA level) added to 3/4 MS multiplication, besides their interaction effect during 3 successive subcultures were investigated.

**A- Specific effect :**

Data obtained displayed that both multiplication parameters i.e, number of proliferated shootlets and their average length responded to specific effect of cytokinin type. Herein, 2ip was more effective than kinetin, however the rate of response was more pronounced with number of proliferated shootlets as difference between two cytokinin types was significant during three subcultures. Meanwhile, variation in shootlets length was

relatively slight, especially during 1 2 and n- subcultures, where both types did,nt significantly differ.

As for the specific effect of either cytokinin or NAA concentration, variances were in most cases relatively slight and did,nt reach level of significance during 3 subcultures, except in 3 14 subculture as the influence of NAA level on number of proliferated shootlets was concerned. Herein, higher NAA rate increased significantly the number of proliferated shootlets.

**B- Interaction effect :**

Regarding the response of shootlets proliferation (number and length of shootlets) to the interaction effect of different cytokinin types x cytokinin concentration x NAA concentration added to 3/4 MS multiplication medium, data obtained displayed that 3/4 MS multiplication medium supplemented with (0.5 mg/L 2ip + 0.5 mg/L NAA) was statistically the superior (greatest number and tallest shootlets). On the contrary, four combinations of kinetin were the inferior, especially 0.5 mg/L kinetin + 0.5 mg/L NAA as an average of three subcultures were concerned.

**V.II.5. Rooting stage :**

Three experiments were conducted during this stage.

**V.II.5.1. Experiment one " Effect of three auxin types" :**

Investigated treatments in this experiment were representative of providing one half strength MS rooting medium with NAA; IBA and IAA auxins each at 0.5 mg/L (either solely or in different combinations). The influence was evaluated through the response of three rooting measurements (rooting % ;

number of rootlets/plantlet and average rootlets length). Data obtained revealed that all NAA ; IBA and IAA treatments improved statistically three rooting measurements i.e, rooting % number of rootlets and their average length as compared to control (auxin omitted MS medium). The response was more pronounced with rooting %, where all auxin treatments resulted in 100 % rooting during 2[111] and 3fi[d] subcultures. However, with both number and average length of rootlets two combinations of (NAA +IBA) and (NAA + IBA+IAA) were statistically the most effective.

**V.II.5.2. Experiment two" Effect of silver thiosulphate/STS":**

**Data obtained declared that three silver** thiosulphate (STS) levels added to one half strength MS rooting medium improved significantly three rooting measurements (rooting percentage ; number and length of developed rootlets). However, the lightest STS level (0.25 ml/L of 4 mM STS solution was the superior, while highest one (1.0 ml/L) was the inferior from statistical point of view.

**V.II.5.3. Experiment three " Effect of silver nitrate " :**

**Data obtained displayed that providing** MS rooting medium with silver nitrate improved 3 rooting measurements( rooting %; number of rootlets and their length) for Zaghloul date palm shootlets proliferated from indirectly formed somatic embryos. However, the 0.50 mg/L AgNO3 provided MS medium was the most preferable in this concern.

**V.II.6. Acclimatization stage :**

In this regard two main steps were employed namely *In Vitro* and *Ex Vitro* acclimatization for Zaghloul date palm

plantlets derived from either direct or indirect somatic embryogenesis

**V.I1.6.1. *In Vitro* acclimatization :**

During this step the *In Vitro* regenerated plantlets were transferred to capped tubes contained 1/4 liquid MS medium through 3 weeks in the growth chamber (under aseptic condition). Ventilation was allowed gradually by punching holes in aluminium foil caps during first five days of 2nd week.

**V.II.6.2. *Ex Vitro acclimatization :***

In this stage i.e, *Ex Vitro* acclimatization and after the *In Vitro* regenerated Zaghloul date palm plantlets had been subjected to the *In Vitro* acclimatization step they were transferred to the semi. Free living condition (polyethylene tunnel) for three months to investigate the suitability of six substrates mixtures regarding the influence on survival percentage.

Data obtained displayed that survival percentage of Zaghloul date palm plantlets was decreased with aging through 3 month of the *Ex Vitro* acclimatization stage. However, in some cases such reduction was absent as survival percentage exhibited by a given mixture during two successive months was concerned, but as the percentage during 1st and 3rd months of the same growing medium was compared the reduction was obviously detected.

Nevertheless, survival percentage was significantly influenced by the differential investigated planting media (sand, peat moss, perlite and vermiculite mixtures) along the whole duration (3 months) of the *Ex Vitro* acclimatization stage.

Herein, three mixtures of (peat moss + sand at 3:1) ; (peat moss + perlite + vermiculite at 1:1:1 ratio) and (peat moss + sand + perlite + vermiculite at equal proportions 1:1:1:1) were statistically the superior regarding their suitability for being used as planting media, where three mixtures exhibited 100 % and 75 °A) survived plantlets during 1 [8] and 3 [14] months of *Ex vitro* acclimatization stage, respectively. The reverse was true with two mixtures of (peat moss + sand at 1: 3) and (peat moss + perlite at 1 : 1), where both were statistically the inferior and showed equally the same depressive effect, where each resulted in the lowest survival percentage i.e, 50 , 25 and 25 % during 1 [8] ; 2 [1-111] and 3 [.11] months, respectively. On the other side peat moss + sand mixture at equal proportion (1 : 1 by volume) was in between the aforesaid two extremes, where it exhibited 75 ; 75 and 50 % survived plantlets at the end of 1 [8] ; 1 [i] and 3 [31] months of the *Ex Vitro* acclimatization, respectively.

**V.111. Some chemical analysis :**

In this respect three components i.e, total ( soluble sugars; phenolic components and free amino acids) in either the directly formed somatic embryos from leaf primordial explants or the indirectly derived somatic embryos (embryogrnic callus induced from shoot tip explants) in response to 2,4-D and 2ip treatments were investigated.

**Total soluble sugars:**

Regarding the total soluble sugars content, data obtained that both original leaf primordial explant and undifferentiated callus were statistically the richest as compared to either the directly or indirectly formed somatic embryos, respectively. On

the other hand, total soluble sugars content was in positive relationship to 2,4-D level and the presence of 2ip.

**Total phenolic compounds:**

**Both** original leaf primordial explant and the undifferentiated callus contained significantly higher level as compared to either directly or indirectly formed somatic embryos , respectively. In addition, a negative relationship was noticed between the 2,4-D added level and presence of 2ip in 3/4 MS medium especially auxin level with the indirectly somatic embryogenesis where differences were significant.

**Total free amino acids content :**

Total free amino acids content was significantly higher in both original leaf primordial explant and undifferentiated callus as compared to the directly and indirectly formed somatic embryos, respectively. However, the trend of response to 2,4-D and 2ip treatments took the other way around as compared to both detected ones with total soluble sugars and phenolic compounds.