SUMMARY and CONCLUSION

This investigation was carried out during three successive years of 1991, 1992 and 1993, at Tissue Culture Laboratory of Horticulture Department, Faculty of Agriculture, Moshtohor, Zagazig University. Various date palm explants of Zaghloul cv. were the plant material used in this work to study the following:-

1- Phenolic compounds in four explants as affected by antioxidant and charcoal inclusion:-

Phenolic compounds were determined in, shoot tip, sub-shoot tip, axillary bud and leaf primordium before and after soaking in antioxidant solutions. Moreover, phenols level in both cultured explants and employed medium itself in response to adding charcoal to nutrient media was periodically determined 2 and 4 weeks from culturing.

2- Some factors affecting producing ability and characteristics of callus from different explants :-

In this regard four main factors were included and some experiments were conducted to study their influences as follows:-

2.1. Nutrient media constituents :

2.1.a. Auxins (source and rates):

In this connection five factorial experiments were done to evaluate the interaction effect between three different kinds of Zaghloul date palm explants *i.e.*, shoot tip, sub-shoot tip and leaf primordium from one side and any of :-

a)- three auxins *i.e.*, IAA, NAA and 2,4-D each at 10 mg/liter, **b)-** two levels of , IAA (2.0/4.0 mg/liter) or **c)-** NAA level (5.0/10.0 mg/liter) from the other side in 1st, 2nd and 3rd experiments , respectively. While the fourth and fifth experiments were developed for studying the effect of different combinations between two auxins namely 2,4-D (0.1 or 5.0 mg/liter) and IAA (1.0, 2.0, 4.0 or 6.0 mg/liter) on callus formation from shoot tip or sub-shoot tip explants in former and latter experiments, respectively.

2.1.b. Effect of cytokinen " Benzylamino purine/BAP":

For this purpose an experiment was conducted to investigate two levels of BAP (0.0 & 0.1 mg/liter) as a component of nutrient culturing medium, since both levels of cytokinen were combined with the above mentioned three explants of Zaghloul cv. (shoot tip, sub-shoot tip and leaf primordium).

2.1.c. Effect of some complex additives :

Addition of coconut milk to nutrient medium [(0.0 and 5% (v:v)] in combination with three explants (shoot tip, sub-shoot tip and leaf primordium) was studied. Besides, three other undefined complex matters namely malt extract, casine hydrolesate and yeast extract at 150, 200 and 150 mg/liter, respectively were also studied regarding their effect on producing callus from four explants (shoot tip, sub-shoot tip, leaf primordium and axillary bud).

2.2. Effect of explant type :-

Three explant kinds of Zaghloul date palm cultivars namely shoot tip, sub-shoot tip and leaf primordium were evaluated regarding characteristics and

producing ability of callus. Beside segments of cotyledonary sheath as clonal explant was also used for the same purpose.

2.3. Effect of explanting date :

Six excision dates of shoot tip explant at two months interval all the year around *i.e.*, (January, March, May, July, September and November) in combination with 2 rates (0.1 or 5 mg/liter) of 2,4-D were compared through culturing on base medium containing four IAA levels *i.e.*, 1, 2, 4, 6 mg/liter in experiment 1, 2, 3 and 4, respectively.

2.4. Effect of some preculturing treatments:

Shoot tip segments were soaked for two hours just before culturing in one of the following solutions :

a- Kinetin at (2, 4 or 8 mg/liter).

b- IAA at (2, 4 or 8 mg/liter).

c- Vitamin mixture.

d- Coconut milk (10%, v:v).

e- Kinetin + IAA each at 2.0 mg/liter.

f- Vitamin mixture + IAA (2 mg) + kinetin 2 mg.

g- Distilled water (control).

3- Availability of establishing somatic embryogenesis :

Establishment of somatic embryogenesis (embryogenic callus) from the yellowish aggregated type callus through culturing and alternate sub-culturing on different nutrient media was studied. Such employed media were charcoal omission but including a relative higher adenine sulfate level. However, both

auxins and cytokinen were presented at lower rate or completely omitted. Since, five nutrient media were prepared for this purpose *i.e.*, **1-** kinetin and auxins omission base medium, **2-** base medium supplemented with (kinetin + malt extract + IAA at 0.5, 300 and 0.1 mg/liter, respectively), **3-** kinetin omission base medium supplemented with [BAP (0.0, 0.1 and 0.5 mg) + NAA + malt extract at 0.1 and 300 mg/liter, respectively], **4-** Base medium supplemented with IAA + IBA each at 1.0 mg/liter, and **5-** medium of M & S. salts + IAA + IBA each at 1.0 mg + vitamin mixture.

4- Histological examination :-

Developmental stages of callus initiated by shoot tip into somatic embryogenic callus was histologically studied.

5- Maturation and development of embryogenic callus into new plantlets:

In this connection, embryogenic callus (white creamy developed colonies) were segmented and cultured onto two charcoal omitted media containing relative higher level of both NaH_2PO_4 and adenine sulfate but an intermediate rate of kinetin and auxins.

6- Direct Proliferation :-

The possibility of both shoot tip and axillary bud as clonal explants, beside pollen grains (androgenesis) to proliferate directly into new plantlets through culturing each as intact organ onto 5 various nutrient media was studied. Variation in such employed media were mainly depended on either

presence or rates of kinetin, BAP and auxins, beside other additives such as NaH_2PO_4 and malt extract.

Data obtained could be summarized as follows :-

1- Phenolic compounds :-

Different explants were greatly varied in their phenols content, where both precultured shoot tip and leaf primordia showed the maximum level especially free from. On the other hand, phenols level in all antioxidant tested explants, particularly shoot tip were acutely decreased as compared to untreated ones, however the decrease was more pronounced in free form of phenols.

As for periodical changes of phenols in cultured shoot tip and axillary bud (after 2 and 4 weeks) it was clear, that axillary bud contained higher level than shoot tip, especially after 2 weeks, while the difference after 4 weeks was obviously minimized. Charcoal was generally ineffective, except with free form in shoot tip. Meanwhile, phenols content was increased in nutrient media by time and charcoal increased it particularly when it was cultured by axillary bud.

2- Factors affecting production, and characteristics of callus initiated from clonal date palm explants of Zaghloul cv.

2.1. Nutrient media constituents :

2.1.a. Auxins (source and rate):

From the life factorial experiments devoted for this purpose, data obtained could be concluded into the following topics:

Specific effect on callusing measurements :

Production and type of callus, both were in closed relationship to source and rate of auxin, where IAA and NAA were more effective than 2,4-D as each was added at 10 mg/liter. The higher rate of IAA (4 mg) and NAA (10 mg) was more desirable than their lower rates *i.e.*, 2 and 5 mg, respectively (experiment, 2 and 3). On the contrary the lower rate of 2,4-D (0.1 mg/liter) exhibited an abundance of callus with more aggregated type than higher rate (5 mg), however the differences were significant and more pronounced with shoot tip than sub-shoot tip explants (experiment, 4 and 5).

With regard to both necrosis and browning, the response to auxin treatments was of minor importance, since no considerable effect could be noticed in relation to auxin source *i.e.*, IAA, NAA and 2,4-D (experiment, 1). Beside, rates of investigated auxins, i.e. IAA (experiment, 2) NAA (experiment, 3) and 2,4-D with shoot tip (experiment, 4) or sub-shoot tip (experiment, 5) all were nearly the same in this concern.

Regarding specific effect of explant kind, results revealed the superiority of shoot tip as compared to either sub-shoot or leaf primordium (experiment, 1, 2, 3).

Interaction Effect :-

Data obtained revealed that the most preferable combinations for enhancing callus production and improving its type (nodosity) were as follows: **a-** IAA at 10 mg/liter × shoot tip (experiment, 1), **b-** Combinations between shoot tip and IAA at 2 or 4 mg/liter (experiment, 2), **c-** Combinations of (shoot tip × NAA at 10 mg/l) followed by (shoot tip × NAA at 5 mg/l), (experiment, 3), **d-** Combination of 2,4-D at 0.1 mg and IAA either at 1.0 or

6.0 mg/l for shoot tip explants (experiment, 4) and **e-** The combination of 2,4-D at 5 mg × IAA at 4 mg/l for sub-shoot tip explant (experiment, 5).

2.1.b. Effect of cytokinen (BAP) in combination with 3 date palm explants :

Specific effect of (BAP):

Obtained data revealed that inclusion of BAP at 0.1 mg/one liter of nutrient media was of minor importance for both callus production and necrosis occurrence, but it improved type of callus produced (more aggregated structure).

Interaction effect (BAP × explant):

It could be concluded that the most desirable combination was that of (BAP at 0.1 mg/l × shoot tip) where more abundant callus of the best type (nodular) was related to such treatment.

2.1.c. Effect of some organic additives combined with different explants :

This topic included two factorial experiments, the first dealing with (coconut milk × 3 explants" shoot tip, sub-shoot tip and leaf primordium") while the second [other additives " malt extract, casine hydrolsate and yeast extract " × four explants " shoot tip, sub-shoot tip, axillary bud and leaf primordium"].

Specific effect of organic additives :-

From both experiments, data obtained revealed that :-

1- Coconut milk not only resulted in inducing more abundant callus with higher granular texture, but also eliminated to some extent occurrence of both browning and necrosis.

2- Malt extract was statistically more effective than two other additives *i.e.*, casine hydrolysate and yeast extract for stimulating and improving callus production and quality, respectively.

Interaction effect :

Data obtained declared that both combinations of (coconut milk at 5% × shoot tip explant) and (malt extract at 150 mg × shoot tip explant) were the most preferable ones for all investigated callusing measurements.

2.2. Effect of explant kind :

Data obtained proved the superiority of shoot tip explants over two other ones (sub-shoot tip and leaf primordium) in stimulating callus production of best type (more granular texture), but leaf primordium was the inferior in this respect. Moreover, cotyledonary sheath explant produced smooth callus.

2.3. Effect of explanting Dates :

Data obtained from the conducted four experiments regarding effect of combinations between 6 explanting dates and 2 levels of 2,4-D could be concluded as follows :

Specific effect :

With regard to specific effect of explanting dates it is quite clear that excising dates either at March or July were the superior, where both induced significantly more abundant callus with the most aggregated callus as compared to four other explanting dates, regardless of IAA level of nutrient media employed for culturing shoot tip segments *i.e.*, 1, 2, 4 and 6 mg/liter. On the contrary explanting date in May was the inferior in this respect. Generally, it could be safely concluded that excising shoot tip explant at March and July " especially later" were the most suitable dates while the reverse was true in May as the three callus measurements *i.e.*, produced amount, type and necrosis were concerned.

As for the effect of 2,4-D rate, it could be noticed that the lower rate (0.1 mg) was more effective than higher rate (5.0 mg), especially in cases where culturing media contained 1 or 6 mg IAA per liter.

Interaction effect explanting date × 2,4-D rates :-

From data obtained it could be concluded that the interaction effect of explanting date × 2,4-D level on the different callus measurements was greatly influenced by the IAA content in employed culture media. Since, both combinations between explanting dates at March or July and the lower rate of 2,4-D *i.e.*, 0.1 mg/liter were the most desirable ones when culturing was carried out onto media contained 1 or 2 mg IAA/liter. While with media of 4 or 6 mg IAA/liter the combinations of explanting dates at March and July reflected their superiority with production and type of callus not only when combined with the lower rate of 2,4-D (0.1 mg) but also with its higher rate (5.0 mg). Nevertheless, spreading necrosis was more pronounced in

developed callus of excised shoot tip at May either combined with the lower (0.1 mg) or the higher (5.0 mg) 2,4-D per liter. However, differences between various combinations tended to be decreased with increasing rate of IAA included in the employed nutrient media.

2.4. Effect of some preculturing treatments :-

Data obtained pointed out that all preculturing treatments (soaking for 2 h. in solution of kinetin, IAA, coconut milk, vitamin mixture, either solely or some in combination) resulted in a considerable increase in callus production and its granulation, except kinetin solutions. However, coconut milk 10%, followed by (kinetin + vitamin mixture + IAA) and IAA at 8 mg/liter were the superior for both production and granulation (type) of callus, besides soaking in solution of vitamin mixture with produced mass of callus only. Meanwhile, other pretreatments were of less importance as compared to control (soaking in distilled water).

With respect to browning and necrosis both showed less response to various pretreatments. However, they were wide-spread with soaking in kinetin solution (4 mg/liter), but most treatments showed a relative lower (insignificant) decrease in their occurrence below control.

3. Establishment of embryogenic callus :-

Various media employed for culturing and repeated reculture of yellowish aggregated type callus initiated from either shoot tip or axillary bud of Zaghloul cv. that aimed to establishing embryogenesis revealed the following:

1- Charcoal, kinetin and auxins omitted base media was capable and enhanced callus development for establishing embryogenic callus.

2- Inclusion of kinetin, IAA and malt extract at (0.5, 0.1 and 300 mg/l) in charcoal omission base medium resulted also in inducing embryogenic callus, but extended the number of subcultures needed.

3- However, BAP at 0.1 mg/l encouraged embryogenesis process over no adding, but with 0.5 mg/l forced cultured callus to produce leaves only.

4- Base medium supplemented with IAA + IBA each at 1.0 mg/l failed to show embryogenesis, but stimulate cultured callus to grow and multiply only.

5- The most preferable medium for establishment embryogenic callus was that consisting of M. & S. salts supplemented with IAA + IBA each at 1.0 mg/l + vitamin mixture.

4. Histological examination :-

Histological examination throughout asexual embryogenic sequences in callus tissue initiated from shoot tip segments, revealed the following developmental stages and their own characteristics :-

1- White glabrous friable callus stage, (4 weeks from culturing origin explant) shows the homogenous tissue with no differentiated organs/structures.

2- Stage of yellowish aggregated type callus (8 weeks from culturing onto high auxin media). Two types of tissues are noticed :- 1st refers to multitude compact clumps of smaller cells (known also as embryogenic compact aggregates), these clumps are interdispersed among 2nd type *i.e.*, loose friable matrix tissue of larger sized cells.

3- Dark yellow, large lobed callus stage (after some repeated subcultures onto medium devoid of /containing low auxin). Where spherical proembryos of 2, 4, 8 ...etc. and multicelled globular proembryos could be observed.

4- Stage of dark brown callus with white creamy spots showing the ovate and bipolar embryoids after further subculture onto medium devoid of / contained low auxin, as well as cylindrical mature embryoids may be also occurred.

5- Germination of the somatic asexual embryoid takes place when embryonic cotyledon starts to elongate and its haustorial end folds away from medium surface.

5- Germination of new plantlets from embryogenic callus :-

Culturing and sub-culturing of white creamy colonies onto medium (a) which consisting of charcoal omitted base medium supplemented in mg/liter with 170 mg NaH_2PO_4, 80 mg adenine sulfate and 6.0 mg IAA enhanced germination of asexual embryoids into new planlets. Beside on other sub-culture onto medium (b) resulted in further growth of such developed plantlets.

6- Direct regeneration :-

6-1- Direct regeneration of clonal explants:-

Data obtained revealed that both shoot tip and axillary bud having the potentiality to be proliferated directly after culturing onto favourable media. Since, medium (c) reflected its superiority over the three other investigated ones in this respect. While, culturing onto medium (a) did

not succeed in inducing new plants through direct proliferation. On the other hand number of regenerated/proliferated new plants per an intact cultured shoot tip or axillary bud varied obviously from one explant to anther. Hence, only one vigorous plant was induced per an intact cultured axillary bud, while with shoot tip the initiated plants were usually 2 or more of less vigorous.

6-2- Androgenesis "Tissue cultured pollen grains":-

Culturing pollen grains on kinetin omitted base medium + 2 ip 3mg + NAA 100 mg + NaH_2PO_4 170 mg per liter. Scarcely induced new plant not capable to survive.