IV. RESULTS AND DISCUSSIONS

Data obtained from the differential experiments conducted during the whole stages of the *In Vitro* micropropagation of Zaghloul date palm cultivar through either the direct or indirect somatic embryogenesis methods in the present work will be discussed under the following two main topics "Parts":

IV.I. Part one " direct somatic embryogenesis " :

In this division of the present dissertation i.e, the *In Vitro* regeneration of Zaghloul date palm cv. through the direct somatic embryogenesis formation, data obtained during various stages of this part were as follows:

IV.I.1.Establishment stage:

In this stage leaf primordia explants were cultured on 3/4 MS basal medium supplemented with higher auxin level 100.0 mg/L 2,4-D; 3mg/1 2ip ; 200mg/1 glutamine; 3g/1 activated charcoal;0.4mg/1 theamine-HCI and 7.0g/1 agar. The original cultured explants (leaf primordia) were successively subcultured for 8 times (at 4 weeks interval) till their tissues became dark brown in colour associated with occurrence of swelled structures formed directly on the original explant itself.

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

In this stage an experiment was conducted to investigate the specific effect of four relative lower auxin levels (2.5; 5.0 ; 7.5 and 10.0 mg/L 2,4-D) added to 3/4 MS medium and presence/absence of cytokinin (2ip at 3 mg/L), as well

interaction effect of their combinations on number of somatic embryos formed directly per each explant (leaf primordium) through 3 successive subcultures. Data obtained are presented in **Table (2) and illustrated by photo (1).**

A. Specific effect:

Referring the specific effect of 2,4-D level added, **Table (2)** displays obviously the superiority of the lowest rate (2.5 mg/L) over three other investigated levels (5.0 ; 7.5 and 10.0 mg/L) for inducing somatic embryos directly. Herein, the 2.5 mg/L 2,4-D supplemented 3/4 MS medium was the unique culture on which cultured leaf primordia explants exhibited their capability for inducing somatic embryos directly. Such trend was true during three successive subcultures, however number of somatic embryos formed per each explant (leaf primordium) was gradually increased as the repeated subcultures were advanced, where average number of somatic embryos directly formed per each leaf primordium corresponding to the end of 1^{st} ; 2^{nd} and 3^{rd} - subcultures were 6.17 ; 8.83 and 12.0, respectively. On the contrary, three other 2,4-D levels (5.0; 7.5 and 10.0 mg/L) failed completely to stimulate direct somatic embryogenesis process.

As for the specific effect of providing 3/4 MS medium with cytokinin (3 mg/L 2ip) on number of somatic embryos formed directly per each explant, **Table** (2) reveals clearly that the 3 mg/L 2ip supplemented 3/4 MS media surpassed statistically the corresponding ones (cytokinin omitted media). The difference between presence and absence of cytokinin (3mg/L 2ip) was significant through three subcultures however rate of variance was increased with aging.

B. Interaction effect :

With regard to the interaction effect of different combinations between four investigated auxin levels (2.5; 5.0 ; 7.5 and 10.0 mg/L 2,4-D) form one hand and omission or presence of cytokinin (at 3 mg/L 2ip) from the other, **Table (2) and photo (1)** show obviously the great variance in response. Anyhow, 3/4 MS medium supplemented with the least 2,4-D level (2.5 mg/L) + 3 mg/L 2ip was statistically the superior, descendingly followed by the omitted 2ip 3/4 MS provided with 2.5 mg/L 2,4-D which ranked second. Such trend was true during 3 successive subcultures, however differences between the aforesaid two combinations was gradually increased as the number of subculture was advanced. On the contrary, 6 other combinations i.e, those of 5.0 ; 7.5 and 10.0 mg/L 2,4-D irrespective of 2ip was added or not failed completely to force direct formation of somatic embryos by Zaghloul date palm explants leaf (primordia).

The present result pertaining the stimulative effect of lower auxin level goes in line with the finding of **Abo EL-Soaud** *et al.,* **(2002-b),** who reported the possibility of direct embryogenesis from cells of parent tissues by transferring the oldest explants from high auxin media into low auxin ones. Moreover, finding of **Abo EL-Soaud** *et al.,* **(2002-a)** gave support to the present result regarding the beneficial effect of using 2,4-D and 2ip combination on stimulating the direct somatic embryos production in date palm.

Table (2): Specific and interaction effects of providing 3/4 MS medium with four auxin levels (2,4-D at 2.5; 5.0; 7.5 and 10.0 mg/l) added solely or combined with 3 mg/l 2ip on number of somatic embryos directly formed per each original cultured leaf primordium explant of Zaghloul date palm cv. during three successive subcultures.

[illegible]	No. of somatic embryos directly formed per leaf primordium through three successive subcultures								
	ibculture I			Subculture H			Subculture III		
[illegible]	Without 2ip	OT	uva	mpiouf cl!z	dfr 2111	*	Without 2ip	cl!z 2tu 0•i	Mean *
2.5 mg/1	ffS	e OWL	VL1 9	q ET L	10.33 a	VER'S	qa 6	L9'171	V 00 ZI
Vsui 0 S	0 00	0 00	0.00 I I	0 00	0 00	0.00 U	0.00	0 00	0.00 I I
UlmS L	0 00	0.00	0.00 U	0.00	0 00	0.00 EI	0 00	0.00	0.00 II
10.0 mg/1	0.00	0 00	0.00 U	0 00	0.00	0.00 U	0 00	0 00	
	1.34 B				Z'S9 V		I117£I		

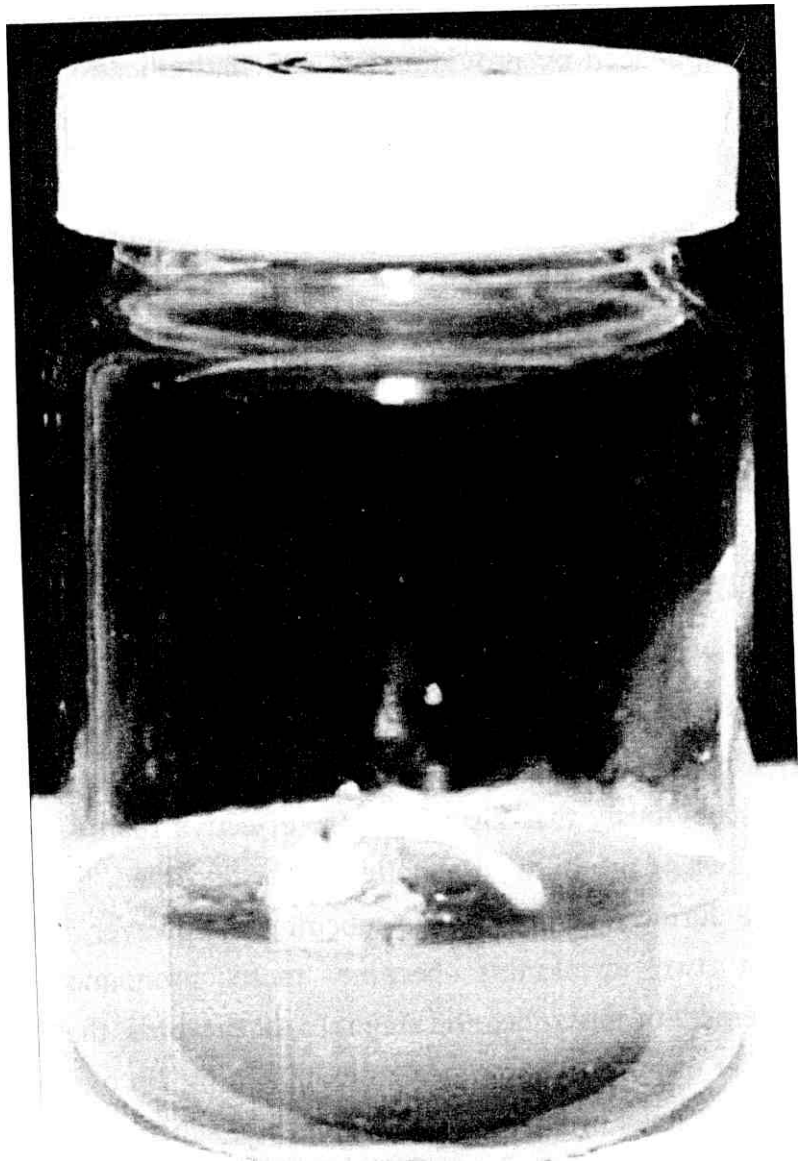


Photo.(1): Developed somatic embryos directly formed after culturing on MS medium supplemented with 2.5 mg/L 2,4-D + 3 mg/L Zip after transferring on auxin omitted MS medium for 4 subcultures.

IV.I.3. Multiplication stage:

For investigating the response of shoots *multiplication* rate as influenced by providing 3/4 MS multiplication medium with cytokinins (type & concentration) and silver thiosulphate (STS) two experiments were conducted.

IV.I.3.1. Experiment, I Effect of cytokinin type and concentration:

In this experiment two cytokinin types (kinietin & 2ip) each at either 0.1 or 1.0 mg/L added to 3/4 MS multiplication medium were investigated regarding their effect on number of proliferated shootlets per each somatic embryo and their average length. Data are presented in **Tables (3&4) and** illustrated by **photo (2).**

Number of proliferated shootlets:

A-Specific effect:

Referring the specific effect of cytokinins type, **Table (3)** displays obviously that 2ip was more effective than Kinetin as the number of proliferated shootlets was concerned. Such trend was true during three successive subcultures, however difference between two cytokinins became more pronounced with advancement of multiplication stage. In other words, the increase in number of proliferated shootlets exhibited by 2ip over Kinetin didn't reach level of significance during first subculture, while in two latter subcultures variance was more pronounced and significant .

Concerning the specific effect of cytokinins concentration, **Table (3)** reveals that cytokinin omitted 3/4 MS

multiplication medium exhibited statistically the least number of proliferated shootlets per each cultured somatic embryo. Meanwhile, providing 3/4 MS multiplication medium with cytokinin at either 0.1 or 1.0 mg/L increased significantly the proliferation process. However, higher rate of cytokinin was relatively more effective but differences didn't reach level of significance through three subcultures. Such trend was true during 3 subcultures.

B- Interaction effect :

Referring the interaction effect of cytokinins type and concentration on number of proliferated shootlets per each cultured somatic embryo (directly formed from leaf primordia explants) of Zaghloul date palm cv., **Table (3) and photo(2)** declare obviously that each investigated factor (type & concentration of cytokinin) directly reflected its own specific effect on their combinations. Herein, providing 3/4 MS multiplication medium with 2ip at either 0.1 or 1.0 mg/L exhibited statistically the greatest number of proliferated shootlets. Such trend was true during 3 subcultures, however the 2ip combination of its higher level tended to be more effective, especially during 0 and 2[1] subcultures, but the reverse was true during 3[1-1] subculture. Difference between two combinations of 2ip were significant during 2[11]-and P-[1] subcultures. In addition, during 0 subculture difference between two 2ip combinations i.e, 2ip at 0.1 and 1.0 mg/L were approximately the same regarding their influence on number of proliferated shootlets from statistical point of view, in spite of higher 2ip level tended relatively to be more effective. On the other hand both kinetin

combinations provided to 3/4 MS multiplication medium at either 0.1 or 1.0 mg/L were statistically less *effective* as compared to both analogous ones of 2ip. On the contrary the cytokinins omitted 3/4 MS multiplication medium was statistically the inferior during three successive subcultures.

Effect on average length of proliferated shootlets :

Regarding the specific and interaction effects of cytokinin type and concentrations added to 3/4 MS medium on average length of proliferated shootlets from culturing the directly induced somatic embryos from leaf primordial explants of Zaghloul date palm cv., data obtained during 3 successive subcultures are presented in **Table (4)** and illustrated by **photo(2).**

A- Specific effect :

It is quite clear that the average length of proliferated shootlets during 3 subcultures of multiplication stage followed to great extent the same trends previously mentioned with number of proliferated shootlets per each cultured somatic embryo directly derived from leaf primordial explants regarding their response to the specific effect of two investigated factors i.e, cytokinin type and provided level to 3/4 MS medium.

However, the response of shootlets length to cytokinin type was less pronounced as compared to the analogous one previously discussed with number of proliferated shootlets, especially during both and $2B^4$ subcultures, whereas difference between 2ip and kinetin didn't reach level of significance. On the other hand, from the statistical point of view both parameters of multiplication stage i.e, number and average length of

proliferated shootlets per each cultured somatic embryo followed typically the same trend regarding their response to specific effect of cytokinin concentration.

B- Interaction effect :

Table (4) and photo(2) display that the specific effect of each investigated factor i.e, cytokinin type and added rate to 3/4 MS medium was directly reflected on its own combinations. Anyhow, the tallest proliferated shootlets were in closed relationship to such somatic embryos cultured on % MS multiplication medium supplemented with the highest 2ip concentration (1.0 mg/L). Differences were significant as compared to those of the other five investigated cytokinins combinations added to MS multiplication medium during 3 successive subcultures except 2nd subculture, whereas both combinations of (1.0 mg/L kinetin) and (0.1 mg/L 2ip) did not significantly vary than 1.0 mg/L 2ip combination. On the contrary, the shortest proliferated shootlets were always in concomitant to the cultured somatic embryos on cytokinins omitted 3/4 MS medium. In addition, other combinations were in between the aforesaid two extremes.

The present result is in congeniality with the finding of **Shaker *et A*, (1998)** regarding the suitability of one cytokinin type than other , whereas the suitability of 2ip over kinetin is coincident with our result. The same trend was supported by **El-Sharabasy.,(2001)** who found that 2ip was more preferable than two other cytokinins types (BA & kinetin) regarding their effectiveness on shoots proliferation parameters for both Zaghloul and Sewi date palm cultivars. Meanwhile, the present

Concentrations(mg	Subculture I	Subculture II	Subculture III
No cyto. added (0.0)			

*, ** refer to specific effect of cytokinin type and concentration added to MS medium, respectively.
Capital and small letters were used for distinguishing between specific and interaction effect values, respectively. Mean followed by the same letter/s did not significantly differ at 5% level.

Cytokinins treatments	Average length of proliferated shootlets during different subcultures (cm)								
	Subculture I			Subculture II			Subculture III		
Type / Concentrations(mg/l)	Kinetin	2ip	Mean**	Kinetin	2ip	Mean **	Kinetin	2ip	Mean **
No cyto. added (0.0)	3.23 c	3.23 c	**3.23 B**	3.53 c	3.53 c	**3.53 B**	4.10 d	4.10 d	**4.10 B**
0.1 mg/l	3.50 c	3.96 b	**3.73 A**	4.06 b	4.50 a	**4.28 A**	4.80 c	5.30 b	**5.05 A**
1.0 mg/l	3.91 b	4.30 a	**4.10 A**	4.33 ab	4.60 a	**4.46 A**	4.85 c	6.00 a	**5.42 A**
Mean *	**3.55 A**	**3.83 A**		**3.97 A**	**4.21 A**		**4.58 B**	**5.13 A**	

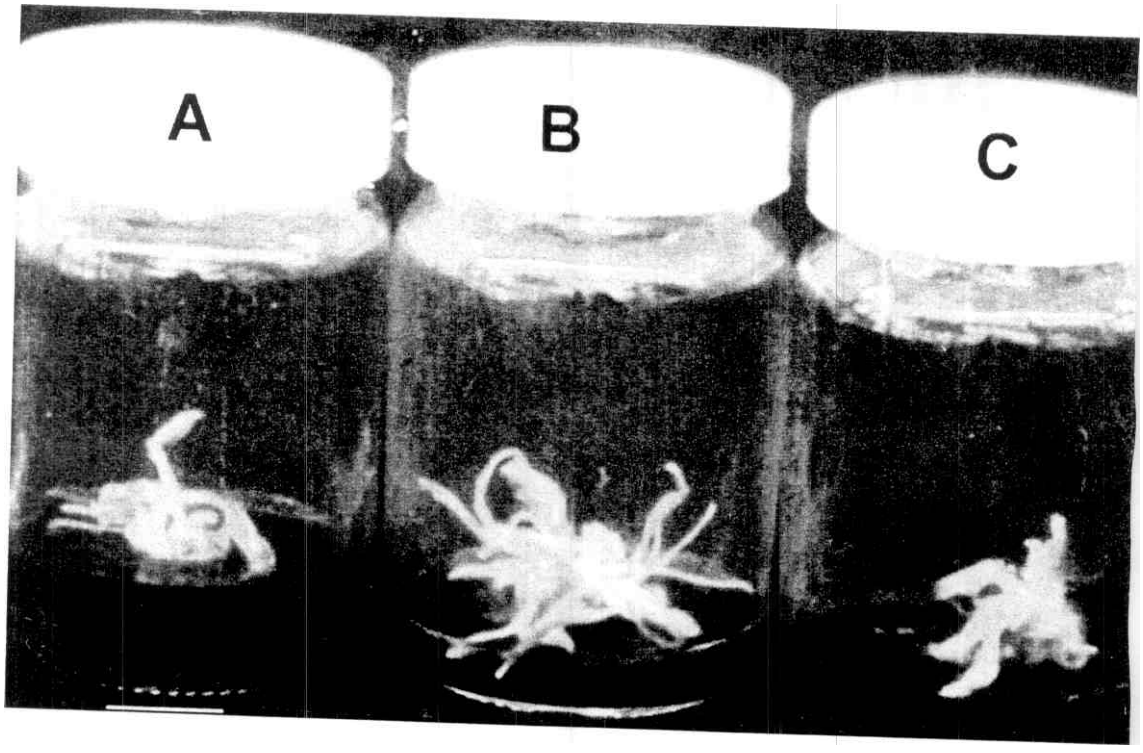


Photo.(2): Effect of providing MS multiplication medium with two cytokinin types (kinetin & 2ip) each at two concentrations (0.1 & 1.0 mg/L) on number of proliferated shootlets per each somatic embryo directly induced.

(A)= Cytokinens omission (control) Pi subculture.

(B)= 0.1 mg/L 2ip supplemented MS medium (3 111 subculture).

(C)= 1.0 mg/L kinetin supplemented MS medium (3rd subculture).

result regarding the effect of cytokinin concentrations goes generally in line with the early finding of several investigators i.e, **Abd-El-Baky.,(2001) and Abd- El-Hamid *et al.*, (2001).**

IV.I.3.2.Experiment, II " Effect of silver thiosulphate (STS) :

In this experiment providing 3/4 MS multiplication medium with silver thiosulphate (STS) at 3 rates (0.25; 0.50 and 1.0 ml/L 4 mM STS besides STS omitted 3/4 MS as control were investigated regarding the effect on some proliferation measurements (NO of proliferated shootlets per each cultured somatic embryo directly derived from Zaghloul leaf primordium explants and their average length). Data obtained during four successive subcultures included in multiplication stage are presented in **Table(5)** and illustrated **by photo (3).**

Number of proliferated shootlets:

Concerning the influence of silver thiosulphate (STS), data obtained through four successive subcultures of multiplication stage as shown in **Table (5)** and **photo (3)** revealed obviously that 3/4 MS multiplication medium supplemented with STS increased significantly number of proliferated shootlets (regardless of added level). However, the rate of increase exhibited by providing 3/4 MS medium with STS over control (STS omitted medium) varied from one subculture to another. Anyhow, the response to STS level during ^{1g} subculture proved that two higher concentrations (0.50 and 1.0 ml/L STS) not only were statistically more effective than lower one (0.25 ml/L) but also had the same effectiveness from the statistical point of view. Meanwhile, during 2 ^{114} subculture, three STS levels provided to 3/4 MS multiplication medium

increased significantly number of proliferated shootlets with comparison to control (STS omission) from one hand but differences were statistically absent as three 0.25; 0.50 and 1.00 ml/L concentrations compared each other in this concern.

In addition, the trend of response took the other way around during both two later subcultures (3rd & 4th ones), since the least concentration (0.25 ml/L of 4 mM STS) was statistically the superior, where the greatest number of proliferated shootlets was resulted. On the other hand beneficial effect of STS on shoots proliferation process was gradually decreased with increasing provided rate. Herein, 1.0 ml/L 4 mM STS showed statistically the least increase in number of proliferated shootlets per each cultured somatic embryo. In other words, 3 investigated STS levels (0.25; 0.50 and 1.00 ml/L of 4 mM STS) increased statistically number of proliferated shootlets over control during both 3rd and 4th subcultures of multiplication stage and they could be significantly arranged regarding their stimulative effect in a descending order from lowest level up to highest one.

Average length of proliferated shootlets:

Regarding the effect of providing silver thiosulphate to the 3/4 MS multiplication medium on average length of proliferated shootlets, **Table (5)** displays obviously the beneficial effect of different investigated STS concentrations during four successive subcultures. However, the least concentration (0.25 ml/L 4 mM STS) was statistically the superior during four subcultures of multiplication stage, whereas the tallest shootlets were detected . On the contrary, the highest added level i.e, 1.0 ml/L of 4 mM STS was the least effective,

while intermediate concentration was in between. Such trend was true during four successive subcultures regarding the superiority and the inferiority of the lowest and highest STS provided levels to 3/4 MS multiplication medium, respectively. However differences between the intermediate concentration of STS as compared to either the least or highest levels from other hand didn't follow firm trend , especially during 1[2] ; 2[nd] and 3[rd] subcultures.

Generally, it could be safely recommended the necessity of providing multiplication medium (3/4 strength basal nutrient MS) with the lowest STS concentration (0.25 ml of 4 mM STS solution per one liter medium) not only for its superiority and the beneficial effect on multiplication process (number and length of proliferated shootlets per each cultured somatic embryo directly derived from leaf primordial explants of Zaghloul date palm cv. from statistical point of view, but also as the economical standpoint was taken into consideration .

The beneficial effect of providing 3/4 MS multiplication medium with silver thiosulphate may be attributed to that silver (Ag^+) is known as an ethylene antagonist, **Edward *et al.*, (1983)** who demonstrated that ethylene production could be restricted (reduced) by applying inhibitors of ethylene biosynthesis . In this regard, findings of several investigators i.e, **Balletti *et* aL,(1994); Le, (1996) and El-Shobaky and Ibrahim, (1997)** all found that addition of silver thiosulphate to culture medium improved the explant growth (proliferated shoots, leaflets and rootlets) of different potato cultivars, however the response varied according to genotypes.

Treatments	Average shootlets length(cm)				
Subculture / STS concentration (ml) 4 mM STS/L	Subculture I	Subculture II	Subculture III	Subculture IV	Subculture IV
1.00 ml . / L					

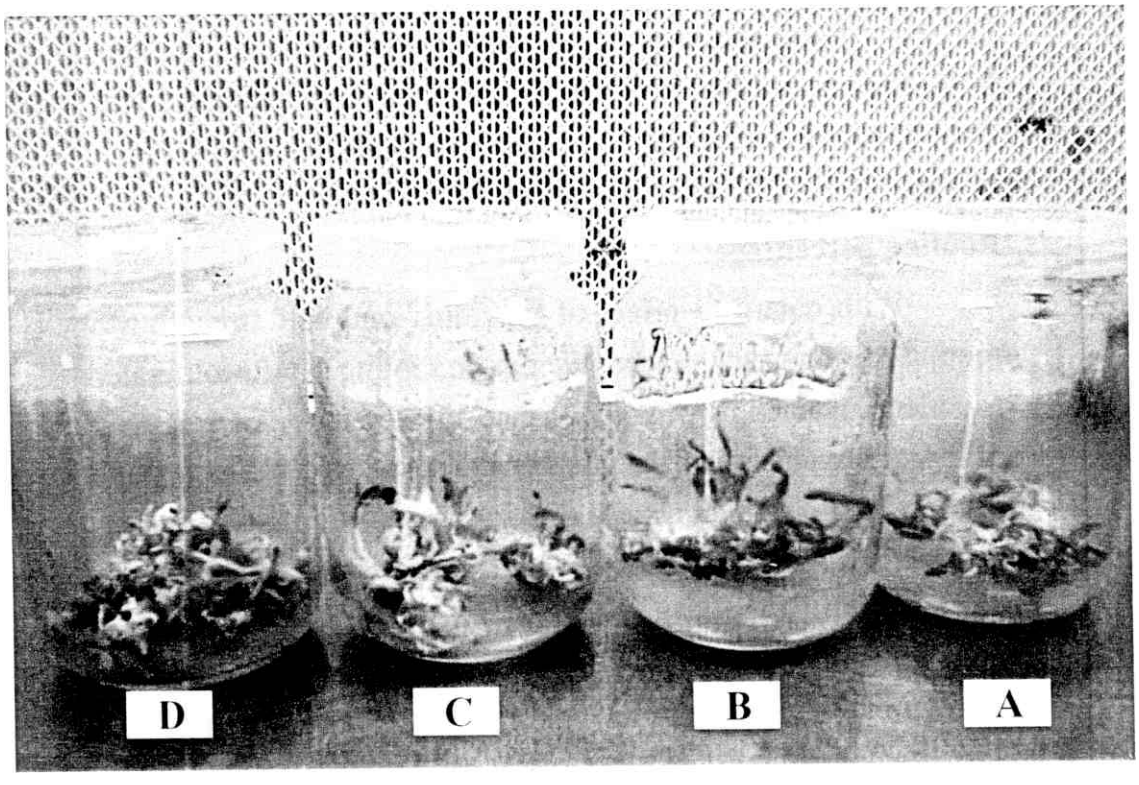


Photo.(3): Effect of silver thiosulphate level provided to 3/4 MS multiplication medium on number of proliferated shootlets and their length (cm).

(A)= Control (no STS added) (4th subculture).
(B)= 0.25 milL of 4 mM STS (4th subculture).
(C)= 0.50 ml/L of 4 mM STS (41 subculture).

IV.I.4. Rooting stage :

In this stage an experiment was conducted to investigate the effect of providing one half strength MS rooting medium with phloroglucinol (PG) at three concentrations (20 ; 40 and 80 mg/L PG, beside no PG added as control) on some rooting measurements (rooting % ; number of rootlets/each plantlet and average rootlets length) during three subcultures of rooting stage for proliferated shootlets of directly induced somatic embryos from leaf primordial explants of Zaghloul date palm cultivar. Data obtained during three successive subcultures are presented in Table (6) and illustrated by photo (4).

Rooting percentage:

With regard to effect of providing one half strength MS rooting medium with phloroglucinol on rooting % of proliferated shootlets (induced by directly formed somatic embryos from leaf primordia explants) of Zaghloul date palm cv., Table (6) and photo (4) show obviously that all PG concentrations stimulate statistically rooting process. The response to the stimulative effect of providing MS rooting medium with phloroglucinol through rooting stage varied not only from one concentration to other, but also the number of rooting subculture reflected its own characteristic on trend of response to different PG concentration.

Anyhow, at the last (3`d) subculture all 3 PG investigated levels (20 ; 40 and 80 mg/L) exhibited 100% rooted in shootlets compared to 66.66 `)/0 for the PG omitted MS rooting medium (control). However, during 2 [111] subculture, both 20 and 40 mg/L PG levels exhibited 100 % rooting for cultured shootlets

corresponding to the same rooting % of control resulted in the aforesaid subculture (66.66 %).

As for the response during 1[8] subculture, however 3 PG levels resulted in significant increase in rooting percentage over control (PG omitted MS rooting medium), but the intermediate level (40 mg/L PG) surpassed both 20 and 80 mg/L PG.

On the other hand, rooting % was increased with advancement of subculturing numbers for any of the investigated PG treatments. However, rooting % reached its peak (100%) during 2[24] subculture for cultured shootlets on either 20 or 40 mg/L PG supplemented MS rooting media. The 80 mg/L PG supplemented MS rooting medium resulted in 100 % rooting during 3rd subculture only.

Generally, it could be safely concluded that providing one half strength MS rooting medium with PG increased rooting percentage with approximately 33 % over control. However, both 20 and 40 mg/L PG concentration were the most effective especially during 2nd and 3rd subcultures, whereas both resulted in 100 % rooting . From the other side the lowest PG level (20 mg/L) was the most preferable as the economical standpoint was taken into consideration.

Number of rootlets / plantlets :

Regarding the effect of phloroglucinol (PG) on number of rootlets developed per each plantlet, data in **Table (6)** reveal that the response was not so pronounced during both earlier subcultures (1[8] and 2nd ones) as compared to control, except the moderate level (40 mg/L PG) , whereas it resulted in increasing rooting % during both two subcultures with approximately 2

folds as much as control. Herein, the number of initiated rootlets per each plantlet during subculture by those cultured on either phloroglucinol omitted media (control) or 80 mg/L PG supplanted MS media was only one rootlet as compared to the analogous ones on 40 mg/L PG medium which exhibited 1.99 rootlets/plantlet. The same was also observed in the second subculture, whereas number of rootlets/plantlet ranged from 1.00:1.66 for those cultured on MS rooting medium without PG (control) or supplemented with PG at 20 / 80 mg/L phloroglucinol in comparison with those cultured on MS supplemented with 40 mg/L PG which showed 3.99 rootlets per each plantlet.

Nevertheless, during subculture differences in number of rootlets per each developed plantlet due to various phloroglucinol treatments (omission or provided at 20 ; 40 and 80 mg/L PG) were too clear to be considered from the statistical standpoint. Herein, both control (PG omission) and PG supplemented MS rooting media at the highest level (80 mg/L) were statistically the inferior, whereas the least number of rootlets per each plantlets was found (1.6 & 1.99 rootlets/plantlet). However, the MS rooting medium supplemented with 40 mg/L PG was statistically the superior and resulted in the greatest number of rootlets per each plantlet (6.44). In addition, 20 mg/L PG supplemented MS rooting medium was statistically more effective as compared to those of either control or provided with 80 mg/L PG.

Average length of rootlets:

Table (6) displays that the response of rootlets length to the different phloroglucinol treatments added to MS rooting medium during three successive subcultures included in rooting stage was obviously less pronounced as compared to the aforesaid differences detected with two former rooting measurements (rooting % and number of developed rootlets per plantlet), especially during two earlier subcultures (1^{st} & 2^{nd} ones). Herein, average rootlets length of plantlets on 80.0 mg/L PG supplemented MS medium didn't significantly differ than those of control during both 1^{st} and 2^{nd} subcultures. Besides, difference was also so few to be considered during PI subculture as 20 mg/L PG supplemented rooting medium was compared to control. On the other hand, one half strength MS rooting medium provided with the moderate phloroglucinol (PG) level i.e, 40 mg/L was statistically the superior and exhibited the tallest rootlets i.e, 1.87; 2.72 and 3.00 cm. during 1^{st} ; 2^{nd} and 3^{rd} subcultures, respectively.

The stimulative effect of providing MS rooting medium with phloroglucinol on rooting measurements is supported by the earlier findings of several investigators. However, most of these researches were carried out on other fruit species i.e, **Chongshum *et al.,* (1998)** on apricot ; **Zanol *et al.,* (1998)** on apple ; **Madgi *et al.,* (1999)** on apple ; **Schmildt *et al.,* (2000)** on Citrus sinesis, **Erbenova *et al.,(2001)*** on sweet cherry and **Mona Hussain, (2005)** on date palm.

Besides, the beneficial effect of phloroglucinol (PG) on improving two rooting measurements (No and length of

Table (6):Effect of phloroglucinol (PG) provided to MS rooting medium at three concentrations on some rooting measurements of proliferated shootlets from somatic embryo directly derived from leaf primordium explant of Zaghloul date palm cv. through

Treatments	Rooting °A			No. of rootlets/plantlet			Average rootlets length (cm)		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Control (0.0mg/L)	55.55 C	D 99'99	11 99'99	D001	ɟIES'I	D 091	ɟIVO'I	H 06'I	D 00 Z
20 mg/ L	ɟILLLL	100.0 A	100.0 A	1117I"1	ɟI 991	ɟISO'ɟ	V ZL'I	H ZL'I	11 0S7
40.0 mg/L	88.89 A	100.0 A	100.0 A	V 661	V 66 E	V 147'9	V L8 I	V ZCZ	V OUT
80.0 mg/L	77.77 B	88.89 B	100.0 A	D 001	ɟI00'I	66'I	1.30 AB	ɟI 19'I	

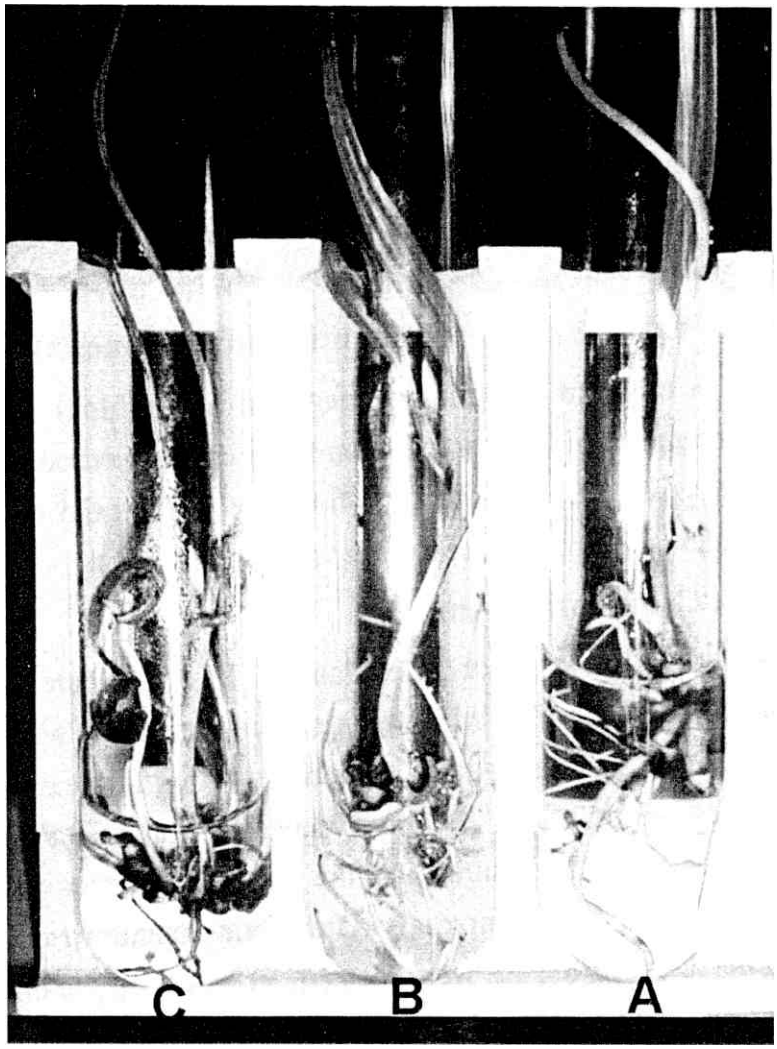


Photo.(4): Effect of phloroglucinol (PG) level added to 1/2 MS rooting medium on rooting of shootlets proliferated from directly derived somatic embryos during 3 I1 subculture.

(A)= 20 mg/L PG supplemented MS rooting medium.
(B)= 40 mg/L PG supplemented MS rooting medium.
(C)= 80 mg/L PG supplemented MS rooting medium.

developed rootlets) goes generally in line of Madgi *et al.,* (1999) on apple.

Conulusively, it was so clear that providing one half strength MS rooting medium with phloroglucinol at 40 mg/L proved its effectiveness on stimulation rooting of cultured date palm shootlets.

IV.H. Part two (indirect somatic embryogenesis):

In this part i.e, micropropagation of Zaghloul date palm cv. through the indirect somatic embryogenesis method using the shoot tip explant, data obtained from the differential conducted experiments during different stages were as follows:

IV.II.1. Callus formation:

In this stage shoot tip explants of Zaghloul date palm cv. were cultured on 3/4 MS medium supplemented with 100.0 mg/L 2,4-D;3g/1 activated charcoal; 0.4mg/1 theamine-HC1 ;7.0g/1 agar;3mg/1 2ip; 200 mg/1 glutamine and 30g/1 sucrose and repeatedly subcultured on the same fresh medium eight times at four weeks interval. After four subcultures a white compacted callus tissues were formed. However, at the end of 8 1 subculture callus tissues became creamy-yellowish in colour and having friable structure.

IV.II.2. Indirect formation of somatic embryos as influenced by auxin and 2ip treatments:

In this experiment formed callus through the former stage was aseptically transferred on 3/4 MS medium to investigate the influence of three 2,4-D levels (5.0; 7.5 and 10.0 mg/L) with absence or presence of 2ip at 3.0 mg/L regarding the number of

somatic embryos formed indirectly per/each cultured jar. Data obtained during three successive subcultures regarding the specific effect of 2,4-D provided levels and presence/absence of cytokinin at 3.0 mg/L 2ip, as well as their combinations are presented in **Table (7)** and **photo (6).**

A. Specific effect :

With regard to specific effect of providing 3/4 MS medium with 2ip (3.0 mg/L) as compared to the cytokinin omitted MS medium, it is quite evident that presence of 2ip at 3.0 mg/L stimulated relatively the somatic embryogenic callus formation during three successive subcultures. However, difference between 3.0 mg/L 2ip supplemented medium and 2ip omitted one was not so pronounced to reach level of significance, except in 2^{1} subculture, where the 2ip provided 3/4 MS medium resulted significantly in increasing number of indirectly formed somatic embryos.

Referring, the specific effect of three relative lower 2,4-D levels added to 3/4 MS medium, **Table (7)** reveals obviously that the number of indirectly formed somatic embryos per each cultured jar was significantly increased with raising provided 2,4-D rate from 5.0 up to 10.0 mg/L. Variances in number of indirectly formed somatic embryos due to specific effect of 2,4-D level added to 3/4 MS medium were more pronounced than those exhibited by presence/absence of 2ip, where differences between three 2,4-D concentrations were significant as they compared each other during 3 successive subcultures.

In other words, the 10.0 mg/L 2,4-D provided 3/4 MS medium exhibited statistically the highest number of indirectly

formed somatic embryos i.e, 2.30; 2.40 and 2.81 embryos during 2n and 3[11] subcultures, respectively. On the contrary, the least number of somatic embryos was significantly coupled with the lowest 2,4-D level (5.0 mg/L) i.e, 1.05; 1.16 and 1.41 embryos during l S ; 2'[l] and 3't subcultures, respectively. On the other hand, as both highest (10.0 mg/L) and least (5.0 mg/L) 2,4-D concentrations compared each other through a given subculture, it was quite clear that number of formed somatic embryos was reduced by the 5.0 mg/L 2,4-D to approximately one half of the corresponding one resulted by the 10.0 mg/L 2,4-D

B. Interaction effect :

Table (7) and **photo (6)** show that the specific effect of each investigated factor(2,4-D level and presence/absence of 2ip) was directly reflected on its own combinations as number of indirectly formed somatic embryos was concerned. Anyhow, the greatest number of somatic embryos was always in significant concomitant to the cultured callus on 10.0 mg/L 2,4-D provided 3/4 MS medium in presence of cytokinin at 3 mg/L 2ip. The superiority of 3/4 MS medium supplemented with (10.0 mg/L 2,4-D + 3 mg/L 2ip) was true during 3 subcultures with comparison to five other (2,4-D x 2ip) combinations. The numbers of indirectly formed somatic embryos exhibited by the aforesaid superior combination (10.0 mg/L 2,4-D x 2ip at 3 mg/L) were 2.52; 2.70 and 3.43 embryos through la ; 2`-L[l] and 3KL[l] subcultures, respectively. Moreover, both (10.0 mg/L 2,4-D without 2ip) and (7.5 mg/L 2,4-D + 3 mg/L 2ip) ranked statistically second as compared to the superior combination

(10.0 mg/L 2,4-D + 3 mg/L 2ip) regarding their stimulative effect on indirectly formed somatic embryos. On the contrary, providing 3/4 MS medium with the least 2,4-D level (5.0 mg/L) was statistically the inferior, regardless of 2ip at 3 mg/L was added or not during 3 successive subcultures. In addition, other combinations were in between the aforesaid two extremes.

Conclusively, it could be generally said that providing 3/4 MS medium with the relative lower auxin rate (10.0 mg/L 2,4-D) plus cytokinin at 3mg/L 2ip was the most effective for embryogenic callus production (yellowish aggregated callus), where such nodular callus type due to its nature considered the precursors of asexual embryos originating from small single meristematic cells in the callus tissues.

The present results regarding the influence of 2,4-D level (auxin) and presence of 2ip (cytokinin) on production of embryogenic callus are supported by the early findings of **Tisserat *et al.*, (1979); Gabr and Tisserat, (1985) and Mater,(1986-a)** all demonstrated the markedly relationship between auxin concentration and good callus production and development. Besides, the response to auxin x cytokinin combinations goes generally in the line with finding of **Abo-El-Soud *et al.*, (2002).**

IV.II.3. Callus differentiation:

Yellowish friable embryogenic callus formed on the relative lower auxin level supplemented MS media during former stage was transferred to growth regulators free MS medium at 3/4 strength. After four successive subcultures (at 4 weeks interval) well mature developed embryoids were noticed

Table (7): Effect of providing 3/4 MS medium with 3 relative lower auxin levels (5.0; 7.5 and 10.0 mg/2,4-D) solely or combined with cytokinin (at 3 mg/L 2ip) on number of indirectly formed somatic embryos through repeatedly culturing callus tissues derived from shoot tip explant of Zaghloul date palm cv.

Treatments	Number of indirectly formed somatic embryos through 3 subcultures											
	Subculture I				Subculture H						lture III	
2,4-D mg/I Presence o cyotIcinin												
No cytokinin added												
Zip at 3 mg/1												
Mean**												

* and **refer to specific effect of and small letters were used for distingueshing between spec the same letter/s did not significantly differ at 5% level.

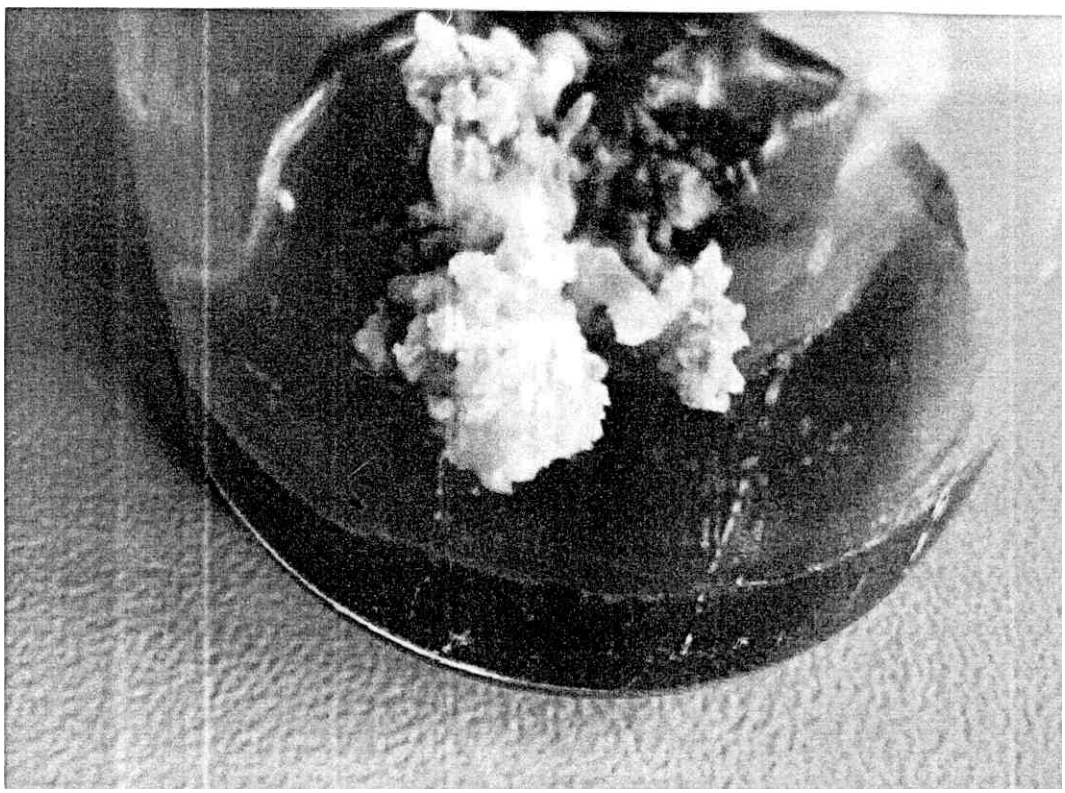


Photo (6): Embryogenic callus formation (indirectly formed somatic embryos) as influenced by 2,4-D and 2iP combinations provided to 3/4 MS medium during 3 subcultures. (MS provided with 10 mf.'//L 2,4-D +3mg/l_e 2iP at the end of 3 ^{12} subculture).

Photo (7) :Callus differentiation after transferring of friable callus on growth regulators free 3/4 medium(4th subculture) .

either in clusters aggregation or occurred individually as shown from **photo (7).**

This resulted is early supported by **Tisserat** *et* **a/.,(1980)** who found that transferring callus to medium devoid of auxin allowed further embryoids development.

IV.II.4. Multiplication stage:

During this stage an experiment was performed to evaluate the response of shoots proliferation to cytokinin type (kinetin & 2ip each at 2 levels) added to 3/4 MS medium in combination with NAA (provided at either 0.1 or 0.5 mg/L) through three successive subcultures (at 4 weeks interval). In this regard specific effect of three investigated factors i.e, cytokinin type (kinetin and 2ip); cytokinin concentration (0.1 & 0.5 mg/L) and NAA level (0.1 & 0.5 mg/L), as well as interaction effect of their combinations were evaluated concerning their influence on shoots proliferation process expressed as number and average length of proliferated shootlets per each individual jar cultured with indirectly formed somatic embryos during three successive subcultures included in this stage. Data obtained are presented in **Tables (8&9)** and **photo(8).**

IV.II.4.1. Number of proliferated shootlets:

A- Specific effect :

As for the specific effect of cytokinin type, data obtained as shown in **Table (8)** and **photo (8)** revealed obviously the superiority of 2ip over kinetin. Herein, cultured embryogenic clusters on 2ip supplemented 3/4 MS medium proliferated statistically numerious shootlets per each jar as compared to the analogous ones of other cytokinin type (kinetin supplemented

3/4 MS medium). Such trend was true during 3 successive subcultures, whereas the increase exhibited by 2ip over kinetin reached approximately 50 % . On the other hand, number of proliferated shootlets was gradually increased by advancement of subculture number. Hence, kinetin supplemented MS medium resulted in 3.79; 5.17 and 6.04 shootlets per each jar, while numbers of shootlets exhibited by 2ip supplied medium were 4.54 ; 8.46 and 9.73 per each jar during 1 ; 2, 11- and 3 24 subcultures, respectively.

Referring the specific effect of NAA concentration, **Table(8)** reveals clearly that variance between both investigated auxin levels i.e, 0.1 and 0.5 mg/L NAA was not so pronounced pertaining the response of number of proliferated shootlets. However, number of shootlets tended relatively to be increased by providing 3/4 MS medium with higher NAA level (0.5 mg/L) as compared to those of 0.1 mg/L NAA, especially during both 1.i and 2nd subcultures, where difference didn't reach level of significance. In addition, higher NAA level (0.5 mg/L) surpassed statistically its lower rate (0.1 mg/L) during 3'll subculture as its effect on number of proliferated shootlets was concerned.

Regarding the specific effect of provided cytokinin rate (0.1 & 0.5 mg/L), it was quite clear that variation in number of proliferated shootlets per each jar due to cytokinin rate (0.1 & 0.5 mg/L) added to 3/4 MS medium was not significant and it could be safely neglected.

B. Interaction effect :

With regard to the interaction effect of three investigated factors i.e, cytokinin (type & concentration) and NAA

concentration on number of proliferated shootlets per each cultured jar, **Table (8)** and **photo (8)** clearly prove that specific effect of each factor was reflected directly on its own combinations. Herein, the greatest number of proliferated shootlets was always in concomitant to such indirectly formed somatic embryos of Zaghloul date palm cv. cultured on 3/4 MS medium supplemented with both 2ip and NAA each at 0.5 mg/L . The superiority of such combination over other investigated ones was significant during three successive subcultures. Moreover, 3/4 MS medium supplemented with 0.1 mg/L 2ip + 0.5 mg/L NAA, especially during both 2nd and PL subcultures ranked statistically second as the number of proliferated shootlets per each jar was concerned.

On the contrary, four kinetin combinations exhibited generally the least number of proliferated shootlets, however the 3/4 MS medium supplemented with 0.5 mg/L kinetin x 0.1 mg/L NAA was statistically the most depressive one, especially as an average of three subcultures was concerned.

In addition, other cytokinins X NAA combinations were in between the aforesaid two extremes regarding their influence on number of proliferated shootlets.

IV.II.4.1. Average length of proliferated shootlets :

A. Specific effect:

Concerning the specific effect of cytokinin type on average length of proliferated shootlets of Zaghloul date palm cv., **Table (9)** reveals that the response followed generally the same trend previously detected with the number of proliferated shootlets. However, the rate of variance was less pronounced,

where the increase exhibited by 2ip over kinetin in average length of proliferated shootlets was significant during 3 ^{III} subculture only and didn't reach level of significance during two earlier subcultures (1$^{'1}$ & 2 ^{1} ones).

Referring the specific effect of NAA concentration added to 3/4 MS medium on average length of proliferated shootlets from the indirectly formed somatic embryos, **Table (9)** displays obviously that difference due to two investigated auxin levels (0.1 & 0.5 mg/L) was completely absent during 3 subcultures from statistical point of view.

As for the specific effect of cytokinin concentration, tabulated data in **Table (9)** indicated also that variations in length of proliferated shootlets during 3 successive subcultures were too little to be considered in this regard except in 3 ^{III} one, where higher cytokinin level (0.5 mg/L) slightly increased it.

B- Interaction effect:

Regarding the response of average shootlets length proliferated from culturing the indirectly formed somatic embryos of Zaghloul date palm on 3/4 medium supplemented with different combinations between (2 cytokinin types each at two levels x 2 NAA concentrations) during multiplication stage, **Table (9)** and **photo (8)** reveal obviously that the aforesaid discussed trends for specific effect of each investigated factor was directly reflected on its own combinations. Herein, due to the absence of specific effect of added concentration for either cytokinin or NAA from one hand which associated with the less pronounced response to cytokinin type from the other, so four 2iP combinations i.e, (2 levels of 2iP x 2 NAA levels), especially

during 1st and 2nd subcultures resulted in the tallest shootlets and were equally the same as compared each other. However , in the 3rd subculture, the trend took the other way around, where the tallest shootlets were significantly in closed relationship to such indirectly derived somatic embryos cultured on 3/4 MS medium supplemented with 0.5 mg/ L 2iP + 0.5 mg/L NAA. The shift in trend of response to investigated cytokinin x auxin combinations from one subculture to another is a real reflection to the more pronounced response to cytokinin type in 3rd subculture rather than two other ones. In other words the superiority of 2iP over kinetin from statistical standpoint during 3rd subculture only influence of concentration for both cytokinins is the real reasons for explaining the variance in response of proliferated shootlets length to the different cytokinin (type & concentration) combined with NAA levels from one subculture to another.

The present results regarding the influence of cytokinin x NAA combinations go generally in line with the findings of several investigators i.e, **Nasir** *et aL,* **(1994); AL-Khayri and AL-Maarri, (1997); Bekheet and Saker., (1998); Saker** *et aL,* **(1998) and Ahmed, (1999)** all demonstrated the necessity of the proper rate of cytokinin x auxin combinations for the best shoots proliferation in date palm. Moreover, the suitability of 2iP than kinetin as cytokinin resource, data obtained in this regard is in accordance with the findings of **Abd- EL-Baky (2001); Abd-**EL-Hamid *et al.,* **(2001) and Tahg** *et al.,* **(2001),** all indicated the superiority of 2iP. However, **Zaid, (2003)** found a conflicted trend to that detected in the present study, suggested that kinetin was more effective than 2iP for stimulating number and length of

Cytokinin treatments		Number of proliferated shootlets per jar cultured with indirectly formed somatic embryos through 3 subcultures of multiplication stage									
		Subculture I			Subculture II			Subculture III			
Type	concentration. mg/l	NAA mg/l			NAA mg/l			NAA mg/l			
		0.1 mg/l	0.5 mg/l	Mean*	0.1 mg/l	0.5 mg/l	Mean*	0.1 mg/l	0.5 mg/l	Mean*
Kinetin	0.1 mg/l	4.00 cde	3.83 de	**3.79 B**	6.33 d	4.17 g	**5.17 B**	7.17 d	6.00 e	**6.04 B**
	0.5 mg/l	3.66 e	3.66 e		4.67 f	5.50 e		5.17 f	5.83 e	
2ip	0.1 mg/	4.66 ab	4.16 cd	**4.5 4 A**	8.00 c	9.08 b	**8.46 A**	9.50 c	10.17 b	**9.73 A**
	0.5 mg/l	4.33 bc	5.00 a		6.75 d	10.00 a		7.08 d	12.17 a	
Mean**		**4.16 A**	**4.16 A**		**6.44 A**	**7.19 A**		**7.23 B**	**8.54 A**	
Mean* for 0.1 & 0.5 mg/l**		**4.16 A & 4.12 A**			**6.90 A & 6.73 A**			**8.21 A & 7.56 A**		

in various
oped from
tip during

Cytokinin treatments		Average length of proliferated shootlets from cultured somatic emb subcultures of multiplication stage								
	Concentration. mg/1	SubcultureI			SubcultureII			SubcultureIII		Mean* I
[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
[illegible]	[illegible]	2.20 ab	[illegible]	[illegible]	2.34 abc	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	1.83 bc	[illegible]		2.12 bc	2.33 abc		[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	2.04 abc	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	0.5 mg/1	2.05 abc	2.02 abc		[illegible]	[illegible]		[illegible]	[illegible]	
[illegible]		[illegible]	[illegible]		[illegible]	[illegible]		[illegible]	[illegible]	
	** for 0.1 & .5mg/I		[illegible]		[illegible]					

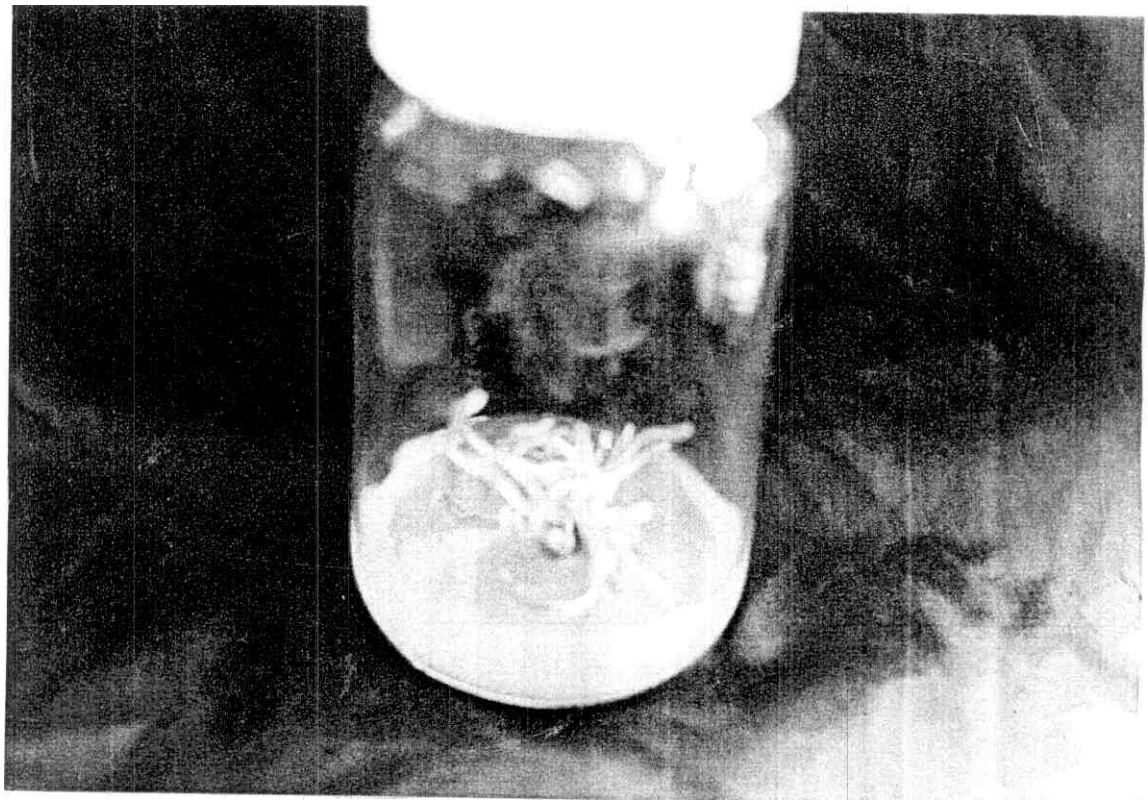


Photo (8):Effect of cytokinin (type& concentration) in combination with 2 NAA levels on shootlets proliferation of Zaghloul date palm cv. during multiplication stage (2ip+NAA each at 0.5 mg/L).

proliferated shootlets through micropropagation of date palm (actually multiplication stage).

IV.II.5. Rooting stage:

During this stage three experiments were conducted to investigate the influence of providing one half strength MS rooting medium with different auxins combinations; silver thiosulphate and silver nitrate treatments on some rooting measurements.

IV.II.5.1. Experiment one "Effect of three auxin types and their combinations":

In this experiment one half strength MS rooting medium was provided with 3 auxin types (NAA; IAA and IBA) each at 0.5 mg/L added either solely or in different combinations to investigate their effect on rooting of proliferated shootlets after they had been subjected to pre-rooting application (cultured on 3/4 MS basal nutrient medium devoid of growth regulators). Data obtained during three subcultures included in this stage regarding the response of 3 investigated rooting measurements (rooting % ; number of induced rootlets per each developed plantlets and their average length) are presented in **Table (10)** and **photo (9).**

Rooting percentage :

Table (10) and photo(9) display clearly that providing one half MS rooting medium with any of the seven investigated auxins (NAA; IAA and IBA) treatments increased significantly rooting % of cultured shootlets of Zaghloul date palm cv. as compared to the control (auxin free MS rooting medium) during three subcultures. Herein, auxin free MS rooting (control)

exhibited 55.6 rooting % during 3 successive subcultures. However, seven investigated NAA; IBA and IAA treatments resulted in 100 °A) rooted shootlets during both 2 nd and 3 rd subcultures. Meanwhile, in l S[t] subculture rooting % ranged from 77.8 to 88.9% for the investigated auxin treatments provided to one half strength MS rooting medium.

Number of initiated rootlets per each plantlet

Data obtained during three successive subcultures conducted during rooting stage as shown in **Table (10)** and illustrated by **photo (9)** declared that the number of rootlets formed per each plantlet of Zaghloul date palm cv. significantly varied by the different auxin treatments added to one half strength MS rooting medium. Anyhow, the least number of rootlets resulted per each Zaghloul date palm plantlet was in closed relationship to the cultured shootlets on the auxin free MS rooting medium (control). However, the inferiority of the auxin omitted MS rooting medium was significant as compared to auxin provided MS media during 3 successive subcultures, except the 0.5 mg/L IAA supplemented MS rooting medium which showed significantly the same effectiveness of control as the number of rootlets resulted per each plantlet was concerned.

Nevertheless, the greatest number of rootlets formed per each individual Zaghloul date palm plantlet was markedly coupled with the *In Vitro* regenerated ones (rooted shootlets) on the NAA+IBA+IAA supplemented half strength MS rooting medium during three successive subcultures. Moreover, both MS rooting media supplemented with IBA either solely or combined with NAA (each auxin at 0.5 mg/L) exhibited also the same

effectiveness of the aforesaid superior rooting medium during the la subculture only as the number of resulted rootlets per each plantlet was concerned.

In addition, other combinations, especially those of IAA i.e, both (NAA+IAA) and (IBA+IAA) were in between the aforesaid two extremes during 3 successive subcultures.

Average length of rootlets:

Regarding the influence of the differential auxin treatments provided to one half strength MS rooting medium on average length of rootlets, **Table (10)** reveals that the response was less pronounced as compared to the analogous ones detected with the aforesaid two rooting measurements (rooting % & number of rootlets), especially during the la—subculture. Anyhow, the shortest rootlets were in closed relationship to Zaghloul date palm plantlets developed on the auxin omitted MS medium followed by those on the MS rooting medium supplemented with one only of IBA; NAA or IAA, especially later one. On the contrary, the tallest rootlets were to great extent coupled with Zaghloul date palm plantlets developed on (NAA+IBA) or (NAA+IBA+IAA) supplemented MS rooting medium during two earlier subcultures and later one, respectively.

From the above mentioned results discussed regarding the influence of different NAA; IBA and IAA treatments (provided to half strength MS rooting medium on 3 rooting measurements (rooting % ; number of initiated rootlets and their average length), it could be safely concluded that both (NAA+IBA) and (NAA+IBA+IAA) auxin treatments were statistically the

superior either the response during each subculture or an average of 3 subcultures were concerned.

The present results is in general agreement with the findings of **Tisserat, (1982; 1983 and 1984-a),** who postulated that axillary shoots could be detached from the parent plant and rooted separately in a medium containing 0.1 mg/L NAA to produce a viable normal plantlet. **Zaid and Tisserat, (1983-a)** observed that the best rooting results was detected on charcoal omitted medium but provided with 0.1 mg/L NAA.

Brackpool, (1988) postulated in date palm micropropagation that once a good healthy shoot has developed, adventitious roots formation could be normally induced with presence of a considerable NAA level. In addition **Shaheen., (1990) ; Nasir** *et al.,* **(1994); Bekheet and Saker., (1998); Sharon and Shanker (1998); Abd EL-Baky, (2001); EL-Sharabsy** *et al.,* **(2001) and Gadalla., (2003)** all demonstrated that root initiation is a critical stage in palm micropropagation which governs the subsequent success of free living date palm production. All pointed out the necessity of auxin level and type for adventitious root formation in the medium and subsequent the root quality (number and length).

IV.II.5.2- Experiment two "Effect of silver thiosulphate / STS":

In this experiment three silver thiosulphate rates (STS) i.e, 0.25 ; 0.50 and 1.0 ml of 4 mM STS solution added to one liter of one half strength MS rooting medium were investigated regarding the response of some rooting measurements (rooting %; number of rootlets per plantlet and average rootlets length)

Table (10): Effect of auxin treatments (NAA; IBA and IAA added solely or in different combinations) to 1/2 MS rooting medium on some rooting measurements of Zaghloul date palm shootlets proliferated from indirectly formed somatic embryos during rooting stage.

Auxin treatments added each at 0.5 mg/1 to 1/2 MS rooting medium	Rooting % Subcultures (I)	(II)	(III)	No of rootlets per plantlet Subcultures (I)	(II)	(III)	Average rootlets length Subcultures (I)	(II)	(III)
1- auxin free (control)	88.6	SS.6H	88.6 [EI	I.00 IC	3 CC I	IC8	0 83 D	I.000	I.00
Z NAA	88.9	100.0 A	100.0 A D	ZZZ H D	2.66 BC	Oc3	L81)		ZrZ
3- HEA	77.8	100.0 A	100 0 A	887 A	3 ZZ H	3 16	0 9Z D	700 D	766 D
IAA	77.8	100.0 A	100.0 A	100 I)	I 33 3	I 3LL7	(19L1E	3 00.Z	I.10
5- NAA + IBA	88 9	100.0 A	100.0 A	766 A	2.77 BC	Oct	V 178 Z	3 00 A	3 00 8
6 I.IAA + IAA	88.9	100 0 A	100.0 A	Z.rZ	2.22 CD	ZCZ	EI8.I Z	Z SO II	Z 50 3
7 IHA + IAA	77.8	100.0 A	100.0 A	W	507	3 51Z	700	Z 50	Z 50
8- NAA + IBA + IAA	88.9	100.0 A	100.0 A	887 A	77 E A	16.66 A	H50•	Z 7L1I3	3 60 A

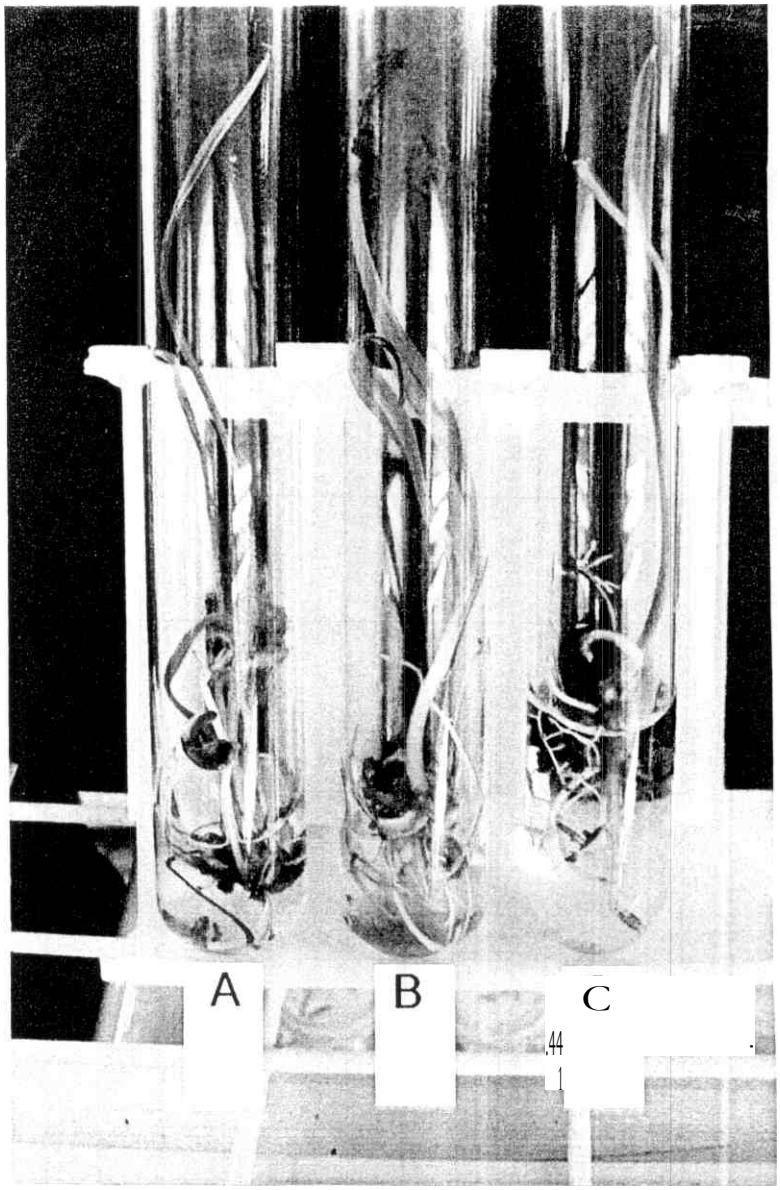


Photo (9): Effect of auxin treatments (NAA;IBA and IAA) each added to MS rooting medium at 0.5 mg/L either solely or in **combination some rooting measurements .**

(A)=Control (auxin omitted 1/2 MS rooting medium).

(B)=One half strength MS rooting medium supplemented with(NAA+ IBAeach at 0.5mg/l .

(C)= On half strength MS rooting medium supplemented with (NAA+IBA+IAA each at 0.5mg/L .

during three successive subcultures included during rooting stage of cultured shootlets. Data obtained are presented in **Table (11)** and **photo(10).**

Rooting percentage:

It is quite clear as shown from tabulated data in **Table (11)** that rooting percentage of cultured Zaghloul shootlets responded positively to three investigated silver thiosulphate (STS) rates as compared to the STS omitted MS rooting medium(control). However, the beneficial effect of providing STS to MS rooting medium on rooting % of cultured shootlets was more pronounced with both lower and intermediate levels (0.25 & 0.5 ml of 4 mM STS solution per each liter of MS rooting medium), especially during 2r[1] and subcultures, whereas rooted shootlets on both 0.25 and 0.50 ml/L STS reached 100 % . On the other hand, providing MS rooting medium with the highest silver thiosulphate rate (1.0 ml/L 4 mM STS solution) resulted also in increasing rooting % over control but difference was significant during P-[I] subculture only.

Number of initiated rootlets per plantlet :

It is quite evident as shown from tabulated data in **Table (11)** that the rooted shootlets of Zaghloul date palm cv. varied in their rootlets number by providing rooting medium with various silver thiosulphate levels. Herein, the poorest Zaghloul date palm plantlets in their rootlets were those rooted in the STS omitted MS rooting medium. However, regenerated plantlets on the 0.25 ml 4 mM STS solution /L supplemented MS rooting medium were statistically the richest in this concern. On the other hand, Zaghloul date palm plantlets could be descendingly arranged

regarding the number of rootlets per each after the aforesaid richest category by those rooted on MS rooting medium supplemented with 0.50 ml then those on MS rooting medium provided by the highest STS rate (1.0 ml/L of 4 mM STS solution). Differences between the above mentioned four categories i.e, control and MS provided with 0.25 ; 0.50 and 1.0 ml/L 4 mM STS solution were significant as compared each other during three subcultures, except when control and 1.0 ml STS/L were compared during 1^{8} subculture.

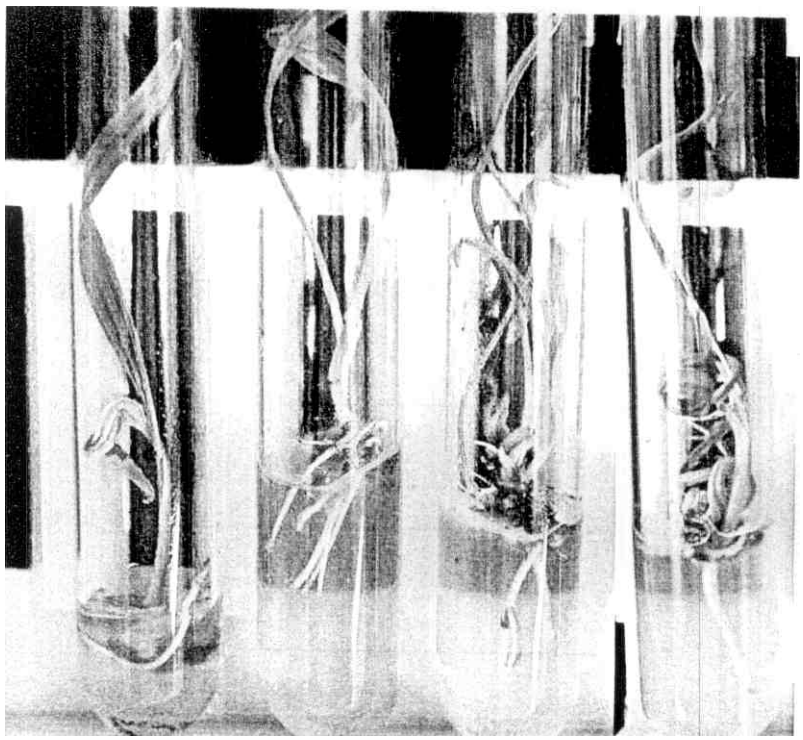
Average length of rootlets :

As for the influence of providing one half strength MS rooting medium with silver thiosulphate at 3 levels on average length of developed rootlets, it was so clear to be noticed that the response followed to great extent the same trend previously detected with the former rooting measurement (number of rootlets per plantlet). Hence, both lower and intermediate STS rates added to MS rooting medium increased significantly rootlets length over control (STS omitted MS medium) from one hand but the lower level was statistically the superior from the other.

From the aforesaid discussed results dealing with the influence of providing MS rooting medium with silver thiosulphate (STS) at 3 rates on three investigated rooting measurements of cultured Zaghloul date palm shootlets, it could be safely concluded that all STS levels increased obviously these measurements, however the least level (0.25 ml/L) followed by the intermediate one (0.50 ml/L) were the most effective.

Table (11): Effect of adding silver thiosulphate (STS) to 1/2 strength MS rooting medium on some rooting measurements of Zaghloul date palm shootlets proliferated from indirectly formed somatic embryos through rooting stage.

silver thiosulphate treatments ml of (4 mM STS solution added per one liter medium)	[illegible]			NQ. of rootlets perplantlet			Average rootlets length		
		[illegible]		[illegible]		[illegible]		[illegible]	[illegible]
[illegible]		[illegible]				[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]		[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	
[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]



Photo(10):Effect of providing one half strength MS rooting medium with 3 silver thiosulphate levels (0.25; 0.50 and 1.0 ml of 4 mM STS solution) on some rooting measurements at 3ak subculture.

(A)= control (STS omitted MS rooting medium).
(B)= 0.25 mg/L STS supplemented MS rooting medium.
(C)= 0.50 mg/L STS supplemented MS rooting medium.
(D)= 1.0 mg/L STS supplemented MS rooting medium.

The present results regarding the beneficial effect of silver thiosulphate (STS) is in congeniality with the findings of several investigators on some plant species. Depending upon the function of silver which appears unique among the heavy metals acting as an ethylene antagonist (playing as an inhibitor of ethylene biosynthesis) **Beyer, (1976); Veen and Van De Geun, (1978) ; Edwards *et al.,* (1983); Mollers *et al.,* (1992) and Balletti *et al.,* (1994)** all suggested that beneficial effect may be attributed to such reason: However, the STS complex was more preferable than the uncomplexed silver ion may be attributed mainly to the much greater mobility of the former form (STS complex) in plant tissues than the mineral ion,Veen **and Van De Geun,(1978).**

IV.II.5.3. **Experiment three" Effect of silver nitrate/$AgNO_3$":**

In this experiment three silver nitrate levels (0.25; 0.50 and 1.0 mg/L $AgNO_3$) added to one half strength MS rooting medium supplemented with 0.1 mg/L NAA were investigated regarding the response of the above mentioned 3 rooting measurements in two former experiments during three successive subcultures. Data obtained are presented in **Table(12)** and illustrated by **photo (11).**

Rooting percentage:

It is quite evident as shown from tabulated data in **Table (12)** and **photo (11)** that providing MS rooting medium with any of the three investigated silver nitrate levels (0.25; 0.50 and 1.0 mg/L $AgNO_3$) increased obviously rooting % of Zaghloul date palm shootlets as compared to the AgNO3 omitted medium (control) during three successive subcultures. However, the

intermediate $AgNO_3$ level (0.50 mg/L). exhibited the highest rooting percentage during three subcultures. Rooted shootlets of Zaghloul date palm cultivar derived from its indirectly formed somatic embryos resulted by the one half strength MS rooting medium supplemented with 0.5 mg/L $AgNO_3$ reached 66.66; 88.89 and 100 % during 1st, 2nd and 3rd subcultures, respectively. On the other hand, two other silver nitrate levels i.e, the lowest and highest (0.25 & 1.0 mg/L $AgNO_3$) rates ranked second and exhibited the same value of rooting % during 1st subculture (55.55 %) , as well as 77.78 % during both 2nd and 3rd subcultures as an average of two subcultures was concerned.

Number of rootlets per plantlet:

Data obtained during three subcultures of rooting stage as shown from **Table(12)** and **photo (11)** displayed obviously that number of rootlets formed per rooted Zaghloul date palm shootlets cultured on 1/2 MS rooting medium was influenced significantly by adding $AgNO_3$. The highest increase in number of rootlets per plantlet was significantly gained by the moderate silver nitrate level (0.5 mg/L $AgNO_3$) added to one half strength MS rooting medium, where 2.33 ; 3.00 and 3.33 rootlets per each plantlet were resulted during 1st ; 2nd and 3rd subcultures, respectively.

Nevertheless, number of rootlets formed per each plantlet was also increased by two other investigated silver nitrate levels (0.25 & 1.0 mg/L $AgNO_3$). However, the highest and lowest SN levels added to rooting medium were equally the same from statistical standpoint regarding their influence on number of obtained rootlets and surpassed statistically the control ($AgNO_3$

omitted medium) during 0 and 2nd subcultures from one hand, but difference was insignificant during 3rd subculture from the other.

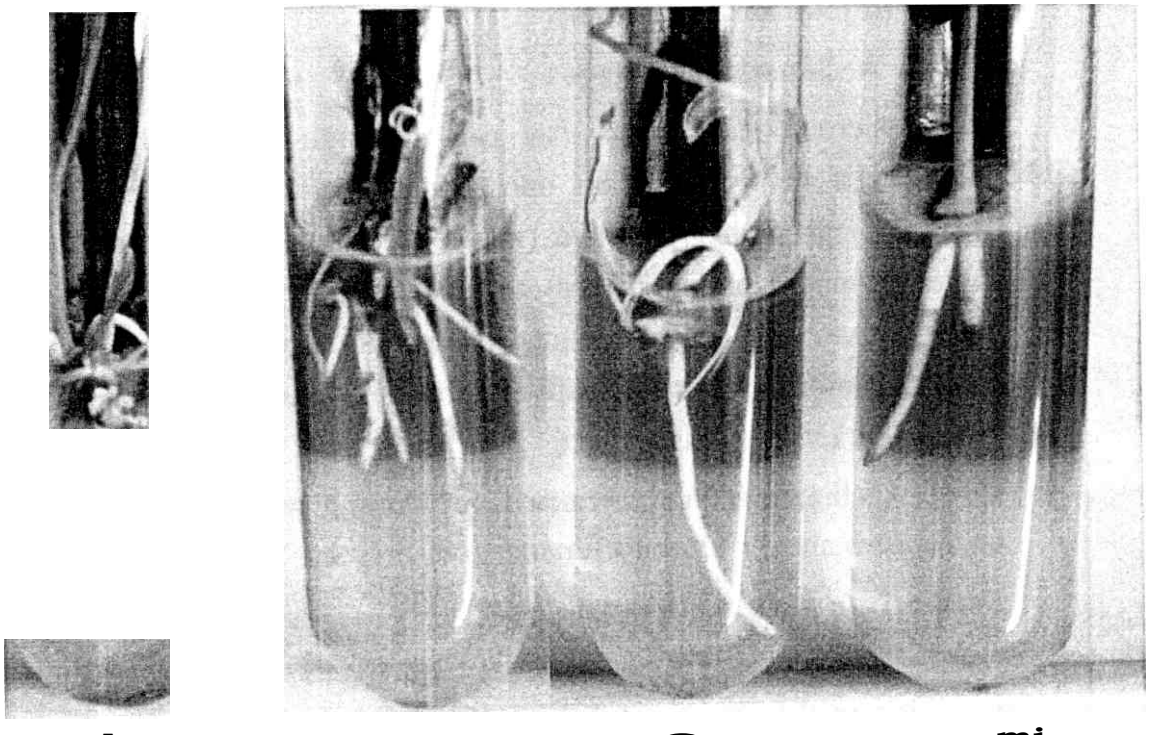
Average length of rootlets :

Regarding the influence of providing 1/2 MS rooting medium with silver nitrate on average length of rootlets of Zaghloul date palm plantlets, **Table (12)** displays that the response was relatively less pronounced than those exhibited with both formers rooting measurements (rooting % & number of rootlets per plantlet). Herein, the lowest level (0.25 mg/L $AgNO_3$) didn't significantly differ than control ($AgNO_3$ omitted MS rooting medium) during three subcultures. However, the intermediate silver nitrate (0.50 mg/L $AgNO_3$) added to one half strength MS rooting medium resulted significantly in the tallest rootlets as compared to either control or two other SN rates during three successive subcultures. Moreover, the highest SN rates added to MS rooting medium (1.0 mg/L $AgNO_3$) increased significantly average length of developed rootlets as compared to either control or 0.25 mg/L $AgNO_3$ level during 0 and 2nd subcultures. While in 3rd subculture rootlets length of Zaghloul date palm plantlets on three MS rooting media i.e, $AgNO_3$ omitted MS(control) and MS medium supplemented with $AgNO_3$ at either 0.25 or 1.0 mg/L didn't significantly differ.

Generally, it could be safely concluded that providing one half strength MS rooting medium with silver nitrate improved 3 rooting measurements (rooting % ; number of rootlets and their average length) of Zaghloul date palm plantlets regenerated by culturing shootlets proliferated from the indirectly formed

Table (12): Effect of adding silver nitrate (SN) to $^1/_2$ strength MS rooting medium on some rooting measurements of Zaghloul date palm shootlets proliferated from indirectly formed somatic embryos through rooting stage.

Silver nitrate treatments mg/l$AgNO_3$	Rooting %			No. of rootlets per plantlet			Average rootlets length		
	Sub. (I)	Sub. (II)	Sub (III).	Sub. (I)	Sub. (II)	Sub.(III)	Sub.(I)	Sub. (II)	Sub.(III)
1-$NOAgNO_3$ added (control)	44.44 B	44.44 C	55.55 C	1.16 C	1.33 C	1.67 B	0.30 C	0.50 C	1.00B
2- 0.25 mg/l $AgNO_3$	55.55 AB	66.66 B	88.89 AB	1.66 B	2.11B	2.05 B	0.33 C	0.50 C	1.64 B
3- 0.50 mg/l Ag NO_3	66.66 A	88.89 A	100.00 A	2.33 A	3.00 A	3.33 A	0.49 B	1.86 A	2.7 8 A
4- 1.0 mg/l Ag NO_3	55.56 AB	77.78 AB	77.78 B	1.66 B	2.11 B	2.11 B	0.94 A	1.22 B	1.2 9 B



A ob,w4st. C minumm

Photo(11):Effect of providing 1/2 MS rooting medium with 3 AgNo3 levels on some rooting measurements during 3 III subculture.

(A)= control (0.0) (AgNo3 omitted MS rooting medium).
(B)= 0.25 mg/L AgNo3 supplemented MS rooting medium.
(C)= 0.50p mg/L AgNo3 supplemented MS rooting medium.
(D)= 1.0 mg/L AgNo3 supplemented MS rooting medium.

somatic embryos. However, the intermediate level (0.5 mg/L $AgNO_3$) was statistically the superior.

The present results regarding the stimulative effect of providing MS rooting medium with silver nitrate on three investigated rooting measurements may be attributed to the physiological role of $Ag^{\pm}$ ion could be played as an antiethylene agence (**Fjeld and Moe, 1985).**

IV.11.6. Acclimatization stage :

Micropropagation of date palm through tissue culture technique is mainly depending upon transferring the succeptable *In Vitro* regenerated plantlets (induced by direct or indirect somatic embryogenesis means) to remain alive and continue their growth and development successfully *Ex Vitro* . So acclimatization for such sensitive plantlets is one of the most essential practice in this respect.

Acclimatization was carried out through two main steps i.e, *In Vitro* and *Ex Vitro* acclimatization.

IV.1I.6.1. *In Vitro acclimatization:*

This step was extended for three weeks under aseptic condition in the growth chamber (27° C ± 1 + the same light exposure of rooting stage). Since, recently *In Vitro* regenerated Zaghloul date palm plantlets were transferred to 2.8 x 25.0 cm. tubes contained one half strength liquid MS basal medium as shown from **photo (12).** Tubes were capped securely with aluminium foil through the 1— week of this step. One week later i.e, during $2^{1\text{-}1}$ week ventillation was allowed gradually by punching holes in aluminium foil caps periodically (one hole

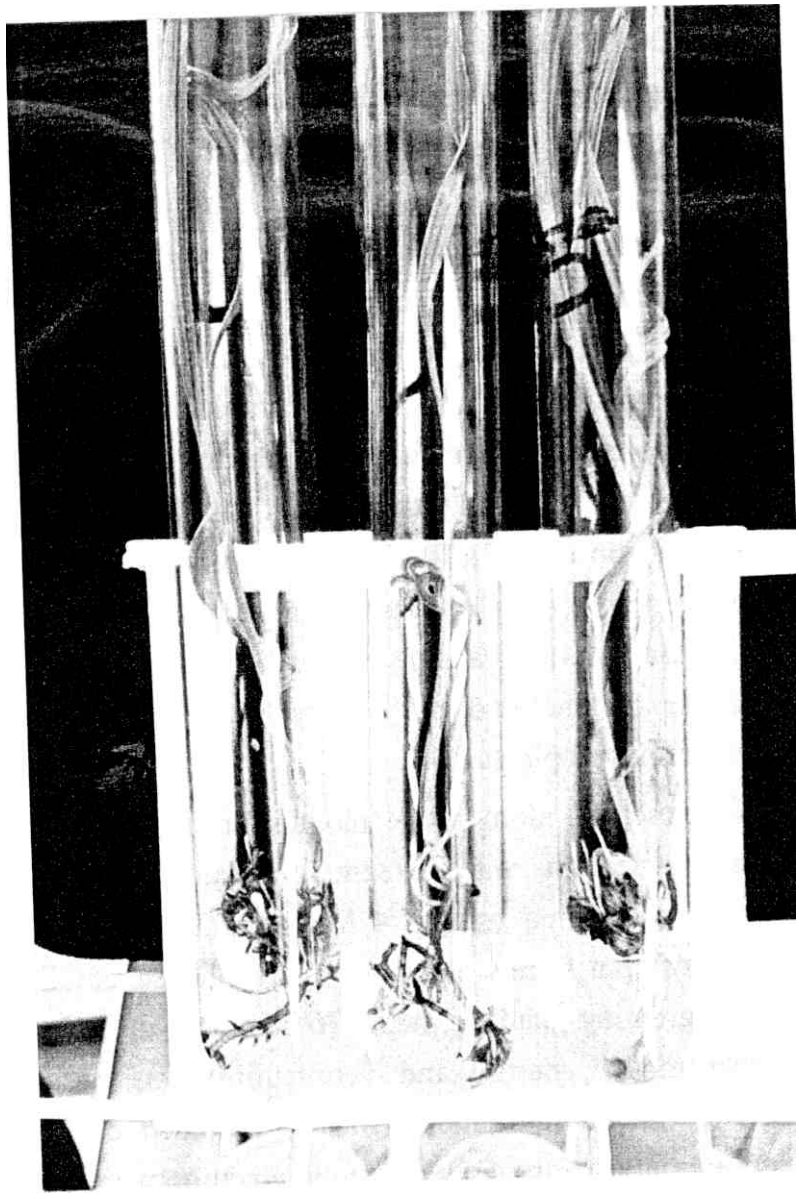


Photo (12): Microregenerated Zaghloul date palm plantlets grown in one half Strength liquid MS basal medium at the last week (P^{I}-one) of the *In vitro Acclimatization.*

daily through the earlier five days of week), then caps were completely removed next day.

IV.II.6.2. *Ex Vitro acclimatization* "second step" :

The *Ex Vitro* acclimatization extended for three months, where Zaghloul date palm plantlets subjected to the *In Vitro* acclimatization were transferred to a semi-free living condition of the green house (polyethylene tunnel). Plantlets were transplanted individually each in plastic pot (5 X 18 cm, dimensions) filled with one of the six investigated growing media (sand ; peat moss ; perlite and vermiculite mixtures at different ratios). Each plantlet was completely covered with white plastic bag just before transferring to greenhouse (polyethylene tunnel) and during first month of *Ex Vitro* acclimatization **photo (13).** Taking into consideration that after one week of transferring to plastic tunnel relative humidity of ambient condition was gradually reduced by punching holes in plastic cover till the end of 1^{2} month, where covers were completely removed **photo (14).**

Nevertheless, along three months during which the *Ex Vitro* acclimatization was extended , Zaghloul date palm plantlets were irrigated using 1/4 MS inorganic salts. Survival percentage of transferred plantlets to greenhouse condition in relation to growing/planting media (6 mixtures investigated of sand, peat moss ; perlite and vermiculite) was periodically recorded (at one month interval). Data obtained during three successive months of the *Ex vitro* acclimatization were presented in **Table (13)** and illustrated by **photo (15).**

Regarding, the influence of different mixtures of sand ; peat moss ; perlite and vermiculite substrates as growing media on survival % , it was quite evident that a significant variances were clearly detected during 3 months of Ex Vitro acclimatization. Herein, at the end of 1 ^{8} month three mixtures (planting media) of peat moss + sand at 3:1 ratios ; peat moss + perlite + vermiculite at equal proportions (1:1:1) and peat moss + sand +perlite + vermiculite at (1:1:1:1) exhibited statistically the highest survival percentage (100 %). On the contrary both mixtures of peat moss + sand at (1: 3 ratio) and peat moss + perlite at (1 : 1 ratio) resulted significantly in the least survival percentage (50%). In addition, peat moss + sand at equal proportion (1 : 1 ratio by volume) was statistically in between, where it showed 75 % survived plantlets during 1 ^{8} month.

Meanwhile, in month both planting media comprised of (peat moss + sand at 3V : 1V ratio) and (peat moss + perlite + vermiculite at equal proportions by volume) were still the superior and each resulted in 100 % survived plantlets. Moreover, both (peat moss + sand at 1 : 1 ratio) and (peat moss + sand + perlite + vermiculite at equal proportions by volume) ranked statistically second, where each induced significantly the same survival percentage (75 %) . In addition, two mixtures of (peat moss + sand at 1: 3) and (peat moss + perlite at 1 : 1) were statistically the inferior, where each resulted in 25 `)/0 survived plantlets at the end of 2^{31} month of the *Ex Vitro* acclimatization.

At the end of the third month since *Ex Vitro* acclimatization was terminated, it was quite clear that response of survival percentage of Zaghloul data palm plantlets through

Ex Vitro acclimatization to the differential investigated mixtures used as planting media followed typically the same trend previously detected during 1 2 month. Hence, three mixtures of (peat moss + sand at 3:1); (peat moss + perlite + vermiculite at 1:1:1) and (peat moss + sand + perlite+ vermiculite at equal proportions) were statistically the superior from one hand and showed equally the same survival percentage (75 %) from the other. The reverse was true with both (peat moss + sand at 1 : 3) and (peat moss + perlite at 1:1) mixtures, where each resulted in the least survival percentage (25%). In addition the peat moss + sand mixture at equal proportion (1: 1 by volume) was in between the aforesaid two extremes (50%).

On the other hand, survival percentage of Zaghloul date palm plantlets was decreased with the advancement of *Ex Vitro* acclimatization in most case. Such decrease was true, especially with comparing the survival percentage exhibited by a given planting medium mixture at the end of 1 2 month to the analogous one of the 3 ml month. However, as the comparison was dealing with the survival percentage resulted by any of the investigated mixtures in two successive months i.e, between (1 2 and 2 IIII) or (2 lr K and 3 III) months two trends were detected in this concern. In other words, with comparing survival percentage exhibited by a given mixture at the end of 1 2 and 2 24 months, three mixtures of (peat moss + sand at 3 : 1) , (peat moss + sand at 1:1) and (peat moss + perlite + vermiculite at 1: 1: 1) each showed the same value during two months i.e, 100 ; 75 and 100 % for 1 2 , 2 nd and 3 11 mixtures, respectively. However, with three other investigated mixtures i.e, (peat moss + sand at 1: 3); (peat

moss + perlite at 1 : 1) and (peat moss + sand + perlite + vermiculite at 1:1:1:1) the survival percentage was decreased from 50 to 25 % for both 1[8] and 2[n] mixtures and from 100 to 75 % for P- mixture as comparison was dealing with 1[8] and 2[11] months of *Ex Vitro* acclimatization.

Meanwhile, as survival percentage resulted in 2' and 3'[l] months of the *Ex Vitro* acclimatization by each investigated mixture was concerned, **Table (13)** reveals that no difference was observed during both months for the plantlets grown in (peat moss + sand at 1: 3) ; (peat moss + perlite at 1: 1) and (peat moss + sand + perlite + vermiculite at 1:1:1:1) , where each of these three growing media showed the same survival percentage i.e, 25 ; 25 and 75 % during 2n and 3'[l] months for 1[8] ; 2'[l] and 3'[l] mixtures, respectively. However, with three other investigated ones survival percentage was decreased by aging from 2"[l] to 3"[l] month where with both (peat moss + sand at 3 : 1) and (peat moss + perlite + vermiculite at 1:1:1) the percentage was decreased from 100 in 2"[l] month to 75 % in 3"[l] month. Beside, with the (peat moss + sand mixture at 1: 1), survival percentage was decreased from 75 % in n[l] month to 50 % in 3"[l] month.

Generally, it could be safely concluded that survival percentage of Zaghloul date palm plantlets through the *Ex Vitro* acclimatization stage was obviously influenced by the different substrate mixtures used as growing media. Herein, three mixtures of (peat moss + sand at 3:1) ; (peat moss + perlite + vermiculite at 1:1:1) and (peat moss + sand + perlite + vermiculite at 1:1:1:1) were statistically the superior since they exhibited 100 and 75 % survived plantlets during 1[8] and 3'[l]

months of *Ex Vitro* acclimatization stage, respectively. However, two mixtures of (peat moss + sand at 1: 3 ratio) and (peat moss + perlite at equal ratio) were statistically the inferior and showed equally the same depressive effect on survival percentage, where each resulted in the least survival percentage i.e, 50 , 25 and 25 °A) during 1 ^{8}- , 2 ^{2}= and 3L`- months, respectively. In addition, (peat moss + sand at equal proportion) was intermediate as compared to the aforesaid two extremes, where it resulted in 75, 75 and 50 % survived plantlets after 1 ^{2} , 2nj and 3`` months of *Ex Vitro* acclimatization, respectively.

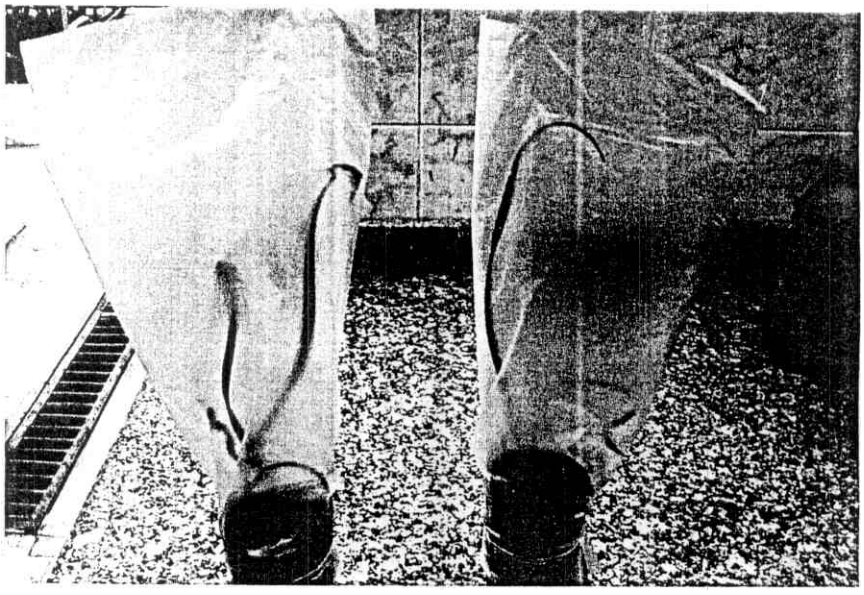
Neverthless, survival percentage was decreased with all investigated growing medium, especially as data of 1 ^{2} and P months of *Ex Vitro* acclimatization for a given planting medium was concerned.

Obtained results regarding the response of *Ex Vitro* acclimatized plantlets to the suitability of different investigated mixtures used as planting media are in general agreement with the earlier findings of several investigators. In this respect **Tisserat (1989,82,84b,87)** who mentioned that plantlets can be successfully transferred to a 1 : I peat moss : vermiculite mixture in shaded greenhouse. **Ibrahim** *et al.,* **(1999)** reported that the best survival percentage (67 after 3 months under uncontrolled greenhouse condition, especially when transplanted in sand and peat moss mixture at 1 : 2/3 ratios. **EL-Bohre** *et al.,* **(2003)** on Egyptian date palm cv. Zaghloul found that the highest survival percentage of plantlets i.e, 23 % was observed with transplanting in mixture of washed sand + peat moss + vermiculite + perlite at equal ratio (1:1:1:1).

2- 1-

Table (13): Survival percentage of Zaghloul date palm plantlets (*In Vitro* micropropgated by direct and indirect somatic embryogenesis methods) as influenced by planting media used during the *ex vitro* acclimatization stage (3months).

Planting media (mixtures ratios by volume)			
Peat moss + sand (3 :1)	O		
Peat moss + sand (1 :3)	O		
3- Peat moss + perlite (1 :1)		N	
4- Peat moss + sand (1 :1)			
5- Peat moss + perlite+vermiculite (1 :1 :1)	O		
6- Peat moss + sand + perlite+vermiculite (1 :1 :1:1)	O O	N	



Photo(13): Covering plantlets with white polyethylene bags through 1[8] **month of *Ex Vitro acclimatization***

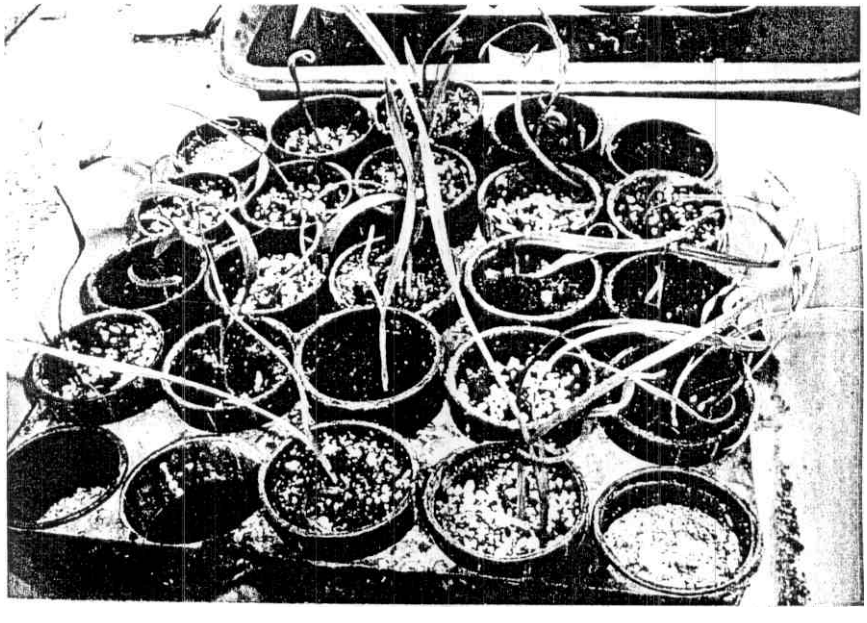


Photo (14) : **Plantlets after removing polyethylene covers during ex vitro acclimatization**

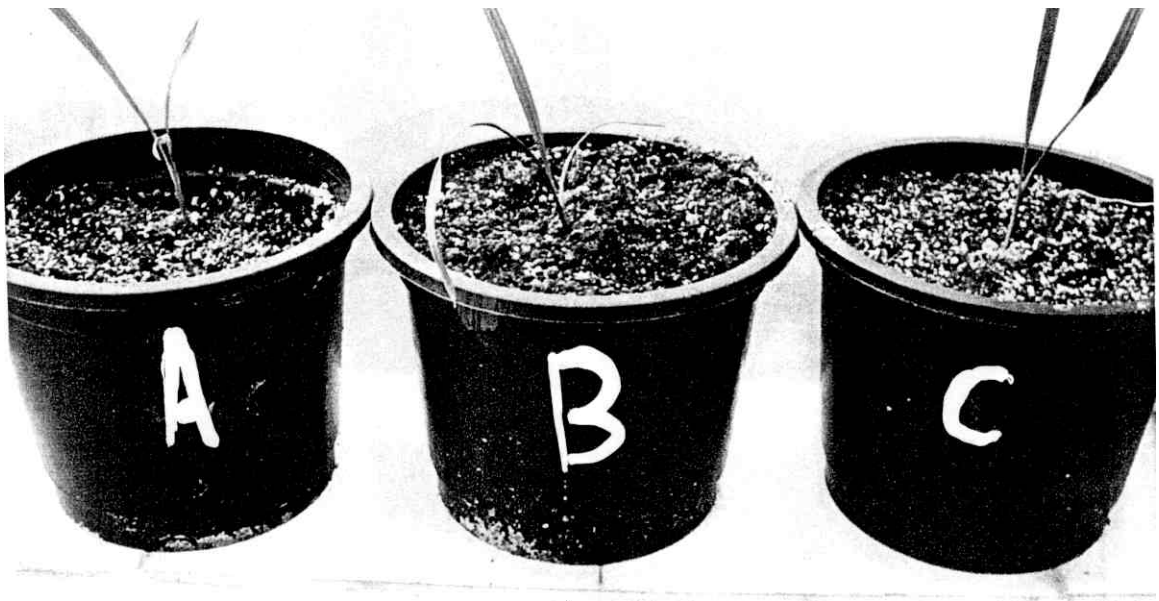
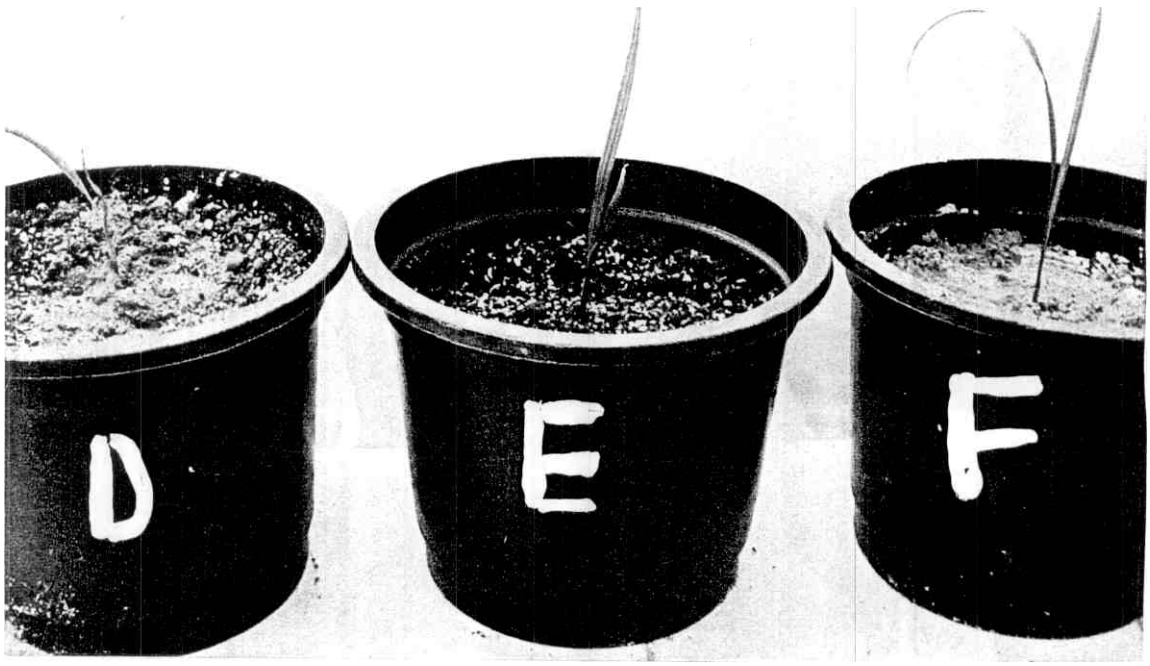


Photo (15): Adapted microregenerated Zaghloul date palm plantlets grown on different planting media mixtures after three months of *Ex vitro* acclimatization.

(A) = (peatmoss+sand 3:1) ;B = (peatmoss+perlite+vermiculite at 1:1:1);C = (peatmoss+sand+perlite+vermiculite at 1:1:1:1).



Photo(15) : **Adapted microregenerated Zaghloul date palm plantlets grown on different planting media mixtures after three months of** *Ex vitro* **acclimatization.**

(D) = **(Peatmoss+sand at 1:3);E = (Peatmoss+Perlite at 1:1)** and (Peatmoss+sand at 1:1) .

IV. III. Some chemical analysis :

Three chemical constituents i.e, total soluble sugars ; total phenolic compounds and total free amino acids contents in either the directly formed somatic embryos(on leaf primordial explants) or the indirectly derived somatic embryos (embryogenic callus developed by shoot tips explants) of Zaghloul date palm cv. in response to different auxin (2,4-D) and cytokinin (2ip) treatments were investigated. Data obtained are presented in **Tables (14) and (15).**

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Total soluble sugars:

Regarding the response of total soluble sugars content in somatic embryos directly formed by leaf primordial explant of Zaghloul date palm cultivar as affected by the 2,4 — D added at 2.5 mg/L level and 2iP at 3 mg/L rate to 3/4 MS medium, Table (14) displays that formed somatic embryos on 3/4 MS medium supplemented with 2.5 mg/L 2,4—D + 3 mg/L 2iP was significantly richer in their total soluble sugars as compared to either those derived on 2iP omitted MS medium supplemented with 2.5 mg/L 2,4—D. However, (leaf primordium) was

statistically the richest. In other words presence of either 2,4—D of 2iP at 2.5 / 3 mg/L, respectively influenced total soluble sugars content in directly formed somatic embryos derived from leaf primordial explant of Zaghloul date palm cultivar.

As for the indirectly derived somatic embryos from shoot tip explant of Zaghloul date palm explant as influenced by different 2,4-D levels (10.0; 7.5 and 5.0 mg/L) combined with 2ip at 3mg/L , it is quite evident as shown from tabulated data in **Table (15)** the pronounced response. Herein, the undifferentiated callus (due to the omission of growth regulators) was statistically the richest in its total soluble sugars contents. On the other hand total soluble sugars content was in significant positive relationship to the 2,4-D level added to 3/4 MS medium. In addition presence of 2ip at 3 mg/L in 3/4 MS medium also increased total soluble sugars in indirectly formed somatic embryos. However, such increase in most cases was less pronounced than that detected with 2,4-D level.

Generally, it could be safely concluded that total soluble sugars content was significantly higher in either the original explant (leaf primordia) or the undifferentiated callus formed by shoot tip explant as compared to the directly or indirectly formed somatic embryos, respectively. Meanwhile, the presence of 2ip relatively increased total soluble sugars, while an obvious positive relationship was also detected with 2,4-D added level to 3/4 MS medium.

The present result is in harmony with that found by **Zein EL-Din, (2005)** regarding the developmental stage. However, the trend of response detected in this study regarding the

influence of auxin level added to MS medium is conflicted with that observed by **Abd EL- Sattar, (2005).** So, further study are needed to throw some lights on the real reasons factors may be the responsible for such confliction.

Total phenolic compounds:

Table (14) reveals that the original leaf primordial explant contained significantly the highest phenolic compounds. However, the directly derived somatic embryos by leaf primordium on 3/4 MS medium supplemented with either 2.5 mg/L 2,4-D solely or combined with 2ip at 3 mg/L showed not only lower total phenols but also contained the same level from statistical stand point. Whereas, difference in phenolic content of the directly formed somatic embryo due to presence or absence of 2ip at 3 mg/L in 3/4 MS medium was not so worthy to be taking into consideration.

The present result regarding the response of phenolic compounds level in tissues at different developmental stages is in congeniality with that reported by **Zein EL-Din, (2005)** on some data palm cultivars. However, the response to 2ip is in partial agreement with **Abd EL- Sattar, (2005).**

As for the effect of different 2,4-D level and 2ip added to 3/4 MS medium on phenols content in the indirectly formed somatic embryos by Zaghloul date palm shoot tip explant, it is quite clear as shown from tabulated data in **Table (15)** that a negative relationship between 2,4-D level and total phenolic compounds was observed. Herein, the reduction in total phenols of the indirectly formed somatic embryos due to raising 2,4-D level added to 3/4 MS medium was significant as three 2,4- D

levels were compared each other, regardless of Zip was present or absent. Meanwhile, the reduction in total phenolic *compounds* content of the indirectly formed somatic embryos by using shoot tip explant exhibited by providing 3 mg/L Zip to MS medium, especially as both absence and presence of 2ip (at 3mg/L) were compared at providing two MS media at the same 2,4-D level. On the other side the undifferentiated callus (produced on the growth regulators free MS medium) showed significantly the highest phenolic compounds as compared to the indirectly formed somatic embryos on 3/4 MS medium provided with different 2,4-D levels either 2ip at 3 mg/L was added or not.

Such trend of response is in general agreement with the finding of **Abd EL-Sattar, (2005)** regarding the influence of 2,4-D level and 2ip.

Total free amino acids content :

Table (14) shows that the original leaf primordial explant contained significantly the highest values of total free amino acids content. However, the somatic embryos directly derived from the leaf primordial explant had lower free amino acids. On the other hand, presence of 2ip 3 mg/L in 3/4 MS medium provided with 5 mg/L 2,4-D reduced the total free amino acids as compared significantly to the analogous ones induced on 3/4 MS medium supplemented with 2,4-D only at 2.5 mg/L.

As for the total free amino acids content in somatic embryos indirectly derived from shoot tip explants of Zaghloul date palm cultivar, **Table (15)** displays obviously that the undifferentiated callus was significantly the richest as compared to the embryogenic callus (somatic embryos indirectly formed),

	Total free amino acids
T_1-No G.R. added (undifferentiated callus control).	
T_3 - 2.5 mg 2,4-D	

Growth regulators treatments added per liter	Content as mg/g F.Wt.embryogenic callus		
	Total sugars	Total phenols	Total free amino acids
T_1-No G.R. added (undifferentiated callus control).	8.26 A	0.70 A	3.36 A
T_2 - 10.0 mg/2,4-D+3mg 2ip	5.09 B	0.16 E	1.75 C
T_3 - 10.0 mg 2,4-D	4.00 C	0.20 E	1.83 B
T_4 -7.5 mg 2,4-D+3 mg 2ip	3.60 D	0.30 D	1.18 E
T_5 - 7.5 mg 2,4-D	3.40 D	0.35 CD	1.30 D
T_6 - 5.0 mg 2,4-D+3mg 2ip	3.00 E	0.39 BC	0.92 F
T_7 - 5.0 mg 2,4-D	2.02 F	0.45 B	0.92 F

Means within each column followed by the same letter are not significantly different at 5% level.

regardless of the 2,4-D level added to 3/4 MS medium either 2ip was present or not. On the other hand, total free amino acids contents of the indirectly formed somatic embryos on 3/4 MS medium was in positive relationship to the 2,4-D rate added. In addition, provided 3/4 MS medium with 2ip (3 mg/L) decreased significantly total free amino acids content especially with providing 1/4 MS medium with two higher 2,4-D levels (10.0 & 7.5 mg/L), however with the 5.0 mg/L 2,4-D MS medium presence of 2ip was not effective.

The present result regarding the influence of 2,4-D levels added on total free amino acids content is in general agreement with the finding of **Abd EL-Sattar, (1999).**