MICROPROPAGATION STUDIES ON *ZAGHLOUL* AND *SEWI CULTIVARS* OF DATE PALM (PHOENIX DACTYLIFERA L.) 1 –CALLUS INITIATION AND FORMATION

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ABSTRACT

The highest percentage of **Callus initiation** of ***Zaghloul*** and ***Sewi cultivars*** was obtained from MS-medium supplemented with 100 mg/l 2,4 -D +3 mg/l 2ip and 100 mg/l NAA + 3 mg/l 2ip respectively and both media formed the highest callus quantity.

The highest total percentage of callus formation of ***Zaghloul*** and ***Sewi cultivars*** was obtained from MS-medium supplemented with 100 mg/l 2,4 D +3 mg/l 2ip and 100 mg/l NAA + 3 mg/l 2ip, respectively and both media formed the highest callus quantity.

The highest total percentage of embryonic of ***Zaghloul*** and ***Sewi cultivars*** were obtained from using MS-medium supplemented with 10 mg/l 2,4 D +3 mg/l 2ip and 10 mg/l NAA + 3 mg/l 2ip, respectively.

INTRODUCTION

Palm crop improvement has been slow due to their long-lived nature, growth habit and lack of adequate methods of vegetative propagation. Propagation of most species of the palm family (*Arecaceae*) is dependent on seed germination and development. Seed-propagated palms do not bear true to type due to heterozygsity.

Offshoots, which grow from lateral buds to reproduce the parent clonally, are the most common types of vegetative propagation among palms. Some established clones of the date palm (*Phoenix dactylifera* L.) have been clonally propagated for centuries through the cutting and rooting of suckers (offshoots). Unfortunately, relatively few offshoots are produced during a date palm's lifetime and of these most occur during the Juvenile stage (Barrett, 1973). Thereafter, in mature date palms, lateral buds are devoted to inflorescence production with a few exceptions.

Attempts to induce suckering of branching in palms on demand through manipulation of the physical or chemical environment has been unsuccessful.

Tissue culture micropropagtion has been employed to aid in the clonal propagation of numerous plant species. The inherent advantage of tissue culture over field propagation is the greater plant production potential from a single plant. Tissue culture techniques may offer a possible method to produce large numbers of genetically uniform palms. Several reports dealing with tissue culture in palms have appeared in the literature in the 1970's. Production of a sexual embryos and their subsequent development into free-living plants in oil palms was the first published report in the literature has obtained free-living plants from clonal date palm explant tissues derived from shoot tips, lateral buds, inflorescence ***(Tisserat, 1983).***

Date palm can be propagated both by somatic embryogenesis and via axially buds (***Tisserat and De Mason 1980, Poulain et al. 1979). Gabr and Tisserat (1985***) concluded, on the basis of some preliminary results, that mass cloning of palms is only possible through somatic embryogenesis. There is little doubt that micropropagtion by somatic embryogenesis is more efficient in terms of rates of multiplication and production costs than micropropagtion by axillary branches.

The present investigation was planned to find out the most suitable treatments for the vegetative propagation (micropropagtion) and production of date palm (*Phoenix dactylifera* L.) Zaghloul and Sewi. CV. Seedlings via somatic embryogenesis by using tissue culture technique. And it using the explants of micropropagtion stages in chemical analysis

MATERIALS AND METHODS

*** Plant material:**

Shoot tips of 2-4 years old offshoots of Zaghloul and Sewi date palm CVs were used as explant sources in this study. Zaghloul date palm CV. offshoots were obtained from the trees grown in the Faculty of Agriculture Cairo University, while, Sewi CV. offshoots were obtained from Padrasheen, Giza.

*** Explant preparation:**

Explants were prepared, washed several times with sterile distilled water followed by soaking in sterile anti-oxidant solution of ascorbic acid (100 mg/L) and citric acid (150 mg/l). They were isolated under complete aseptic conditions to obtain shoot tip containing the apical meristem with 2-4leaf primordial. Explants were surface sterilized using ethyl alcohol (70%) for 1-2 minutes then rinsed once with sterile distilled water and transferred to 50% Clorox (2.5% Sodium hypochlorite) and two drops of tween 20 for 20 minutes. All traces of the used disinfectant were removed by soaking and rinsing three times using autoclaved distilled water.

*** The basic nutrient medium:**

All the following experiments were conducted with Murashige and Skoog (MS) basal medium (1962). The pH value of the nutrient media was adjusted at 5.7 to 5.8 with adding few drops of either 0.1 KOH or 0.1 HCL. The agar was added to the nutrient medium at concentration 6.5 g/L for callus experiments and rooting stage. The medium was distributed into the culture jars (200-ml) contained 35 ml of the medium/jar. The jars were immediately capped with polypropylene closure and autoclaved at 121ºC at 15 Ibs/ins^2 capped for 15 min

Experiment I: Callus initiation (starting) stage:

In this experiment the effect of plant growth regulators on callus initiation was studied. Therefore, shoot tip explants of Zaghloul and Sewi date palm CV were cultured on MS basal medium supplemented with different growth regulators as follows:

1.10 mg/L 2,4-D (2,4-Diclorophenoxy acetic acid
2.100 mg/L 2,4-D
3.10 mg/L 2,4-D + 3.0 mg/L 2ip. (2ip=ISO pentenyladenin)
4.50 mg/L 2,4-D + 3.0 mg/L 2ip.
5.100 mg/L 2,4-D + 3.0 mg/L 2 BA (BA = Benzyladenine)
6.100 mg/L 2,4-D + 5.0 mg/L BA
7.100 mg/L 2,4-D + 2.0 mg/L kin. (Kin = Kinetin)
8.10 mg/L NAA (NAA = Naphthalene acetic acid)
9.100 mg/L NAA.
10.10 mg/L NAA + 3.0 mg/L 2ip.
11.50 mg/L NAA + 3.0 mg/L 2ip.
12.100 mg/L NAA + 3.0 mg/L 2ip.
13.100 mg/L NAA + 5.0 mg/L BA.
14.100 mg/L NAA + 2.0 mg/L Kin.

Each treatment consisted of 6 jars.

Culture environment:

After planting of different shoot tip of Zaghloul and Sewi cultivars, which had been done on the various media that corresponding the investigated treatments of callus initiation, the explants of different treatments were incubated at 25-27ºC and complete darkness.
The following parameters were recorded after 8 weeks.

- Survival number. - Survival percentage.
- Swelling number. - Swelling percentage.
- Callus initiation number - No of explants forms/callus.
-Callus initiation total number of explants formed callus.
- Callus initiation (total percentage). - Callus initiation percentage.
- Average of callus value.

Where:

1< = + = Small value of callus.
2 < = ++ = Moderate value.
3 < = +++= High value.

Experiment II: Callus formation (Production) stage:

This experiment aimed to study the effect of plant growth regulators on callus formation. The developed callus from shoot tip of both Zaghloul and Sewi cultivars were transferred to MS basal medium supplemented with different growth regulators used in the 1st experiment. Each treatment consisted of 12 jars. Cultures were incubated under the same conditions in callus initiation. The following parameters were recorded after 6 weeks.

- Number of explants formed callus.
- Percentage of explants formed callus.
- Total number of explants formed callus.
- Average of callus value.

Where:

1< = + = Small value of callus.
2 < = ++ = Moderate value.
3 < = +++= High value.

Experiment III: Embryonic callus stage:

In this experiment, the aggregated soft callus (Yellowish callus) developed from callus formation stage was investigated through culturing

on MS basal medium supplemented with different growth regulators as follows:

1. Control. (Without growth regulators)
2. 1.0 mg/L 2,4 D
3. 10 mg/L 2,4 D
4. 10 mg/L 2,4 D + 3.0 mg/L 2ip.
5. 1.0 mg/L NAA
6. 10 mg/L NAA
7. 10 mg/L NAA + 3.0 mg/L 2ip.

Each treatment consisted of 12 jars.

Cultures were incubated under the same conditions in callus initiation.

The following parameters were recorded after 6 weeks.

-Number of explants (callus) formed embryonic callus.
- Percentage of explant formed embryonic callus.
- Total number of explants formed embryonic callus.
- Embryonic callus (total percentage).
- Average of callus value.

Where:

1< = + = Small value of callus.
2 < = ++ = Moderate value.
3 < = +++= High value.

RESULTS AND DISCUSSION

Survival percentage:

The presented in Tables (1and2) results clearly show that, survival percentage for Zaghloul and Sewi CVs. ranged 83.33 - 100%, whereas, the different treatments had 100% survival except 10-mg/l 2,4-D for both CVs and 10 mg/l NAA for Zaghloul CVs, which resulted in 83.33%.

Swelling percentage:

Swelling percentage was responded differently to the used treatments according to CV. In this concern, swelling percentage for Zaghloul CV. ranged from 33.33 to 100%. The highest swelling percentage 100% was recorded for 100 mg/l 2,4-D or/and 100 mg/l NAA + 3 mg/l 2ip and or/and 5 mg/l BA. While, the lowest swelling percentage 0.0% was recorded for 10 mg/l NAA. Kintine had depressing effect on swelling percentage when used with 100 mg / l 2,4-D. 2ip had positive effect on swelling % (Table 1).

With regard to swelling % for Sewi CV. (Table2) the obtained results show that 2,4-D had positive effect on this parameter with no differences between 10 and 100 mg/l 2,4-D which resulted in 83.33%. 2ip had depressing effect on swelling % comparing with 10 mg/l 2, 4-D. Combining treatments of 10 mg/l 2,4-D + 3 mg/l 2ip resulted in 50% swelling but increasing 2,4-D level increased swelling to 66.66% when 50 mg/l 2,4-D + 3 mg/l 2ip and 100 % when 100 mg/l 2,4-D was combined with 3 mg/l 2ip. The same result 100% swelling was recorded for 100 mg/l 2,4-D + 5 mg/l BA or 100 mg/l 2,4 D+ 2 mg/l kin. Swelling % was of positive correlation with increasing NAA level from 10 mg/l NAA, which resulted in 16.66 swelling to 100 mg/l NAA, which had 83.33%. Combining 3 mg/l 2ip with 10 mg/l NAA increased swelling % to 66.66% using 50 or 100 mg/l NAA with the different cytokinins tested in this study resulted in 100% swelling.

Callus initiation:

Data of Tables (1 and 2) and figs (1 and 2) show clearly that using auxins (2,4-D or NAA) had no effect on callus initiation (0.0%) when each used without cytokinins except 100 mg/l NAA with Sewi CV. However, the combined treatments of different 2,4-D (10, 50 and 100 mg/l) with 3 mg/l 2ip stimulate callus initiation on Zaghloul explants. The same result was found when 100-mg/l 2,4-D was combined with 2 mg/l kin. Combined NAA levels with 2ip or kin stimulate 16.66% of Zaghloul explants to initiate callus, while, combined 100 mg/l NAA with 5 mg/lBA stimulate 33.33% of Zaghloul explants to initiate small of callus. Moreover, combined 50 mg/l NAA with 3 mg/l 2ip stimulate 16.66% of each category (Small, moderate and high values) to initiate callus.

Treating Sewi CV. explants with the different 2,4-D levels had no effect on callus initiation. The same result was recorded for 10 mg/l NAA, while, 100 mg/l NAA stimulate 16.66% of explants to initiate callus of small value. The same result was obtained when 10 mg/l 2,4-D or NAA was combined with 3 mg/l 2ip or 50 mg/l NAA + 3 mg/l 2ip or/and 100 mg/l NAA + 2 mg/l kin. Were combined. In addition to using

Table (1): Effect of different concentrations of Auxins (2,4-D and NAA) and cytokinins (2ip or BA or Kin) on survival, swelling and callus formation of date palm (*Phoenix dactylifera* L.) Zaghloul derived from culture shoot tip after 8 weeks.

Treatments (mg/l)	No. of Explant	Survival		Swilling										Value
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
1. 10 2,4 D	6	5	83.33	2	33.33	0	0.0	0	0.0	0	0.0	0	0.0	1.0
2. 100 2, 4 D	6	6	100	5	83.33	0	0.0	0	0.0	0	0.0	0	0.0	1.0
3. 10 2,4 D + 3 2ip	6	6	100	4	66.66	1	16.66	0	0.0	0	0.0	1	16.66	1.17
4. 5100 2,4 D + 3 2ip	6	6	100	5	83.33	1	16.66	1	16.66	0	0.0	2	33.33	1.50
5. 100 2,4 D + 3 2ip	6	6	100	6	100	1	16.66	2	33.33	1	16.66	4	66.66	2.33
6. 100 2,4 D + 5 BA	6	6	100	6	100	2	33.33	1	16.66	0	0.0	3	50	1.67
7. 100 2,4 D + 2 Kin	6	6	100	3	50	1	16.66	0	0.0	0	0.0	1	16.66	1.17
8. 10 NAA	6	5	83.33	0	00	0	0.0	0	0.0	0	0.0	0	0.0	1.00
9. 100 NAA	6	6	100	4	66.66	0	0.0	0	0.0	0	0.0	0	0.0	1.00
10. 10 NAA + 3 2ip	6	6	100	3	50	1	16.66	0	0.0	0	0.0	1	16.66	1.17
11. 50 NAA + 3 2ip	6	6	100	5	83.33	1	16.66	1	16.66	1	16.66	3	50	2.00
12. 100 NAA + 3 2ip	6	6	100	6	100	1	16.66	1	16.66	0	0.0	2	33.33	1.50
13. 100 NAA + 5 BA	6	6	100	6	100	2	33.33	0	0.0	0	0.0	2	33.33	1.33
14. 100 NAA + 2 Kin	6	6	100	5	83.33	1	16.66	0	0.0	0	0.0	1	16.66	1.17
L. S. D					7.530									0.756

Table (2): Effect of different concentrations of Auxins (2,4-D and NAA) and cytokinins (2ip or BA or Kin) on survival, swelling and callus formation of date palm (*Phoenix dactylifera* L.) Sewi cultivar derived from culture shoot tip after 8 weeks.

Treatments (mg/l)	No. of Explant	Survival		Swilling										Value
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
1. 10 2,4 D	6	5	83.33	2	83.33	0	0.0	0	0.0	0	0.0	0	0.0	1.00
2. 100 2, 4 D	6	6	100	5	83.33	0	0.0	0	0.0	0	0.0	0	0.0	1.00
3. 10 2,4 D + 3 2ip	6	6	100	3	50	1	16.66	0	0.0	0	0.0	1	16.66	1.17
4. 5100 2,4 D + 3 2ip	6	6	100	4	66.66	2	33.33	0	0.0	0	0.0	2	33.33	1.33
5. 100 2,4 D + 3 2ip	6	6	100	6	100	2	33.33	1	16.66	0	0.0	3	50	1.67
6. 100 2,4 D + 5 BA	6	6	100	6	100	2	33.33	0	0.0	0	0.0	2	33.33	1.33
7. 100 2,4 D + 2 Kin	6	6	100	6	100	0	0.0	0	0.0	0	0.0	0	0.0	1.00
8. 10 NAA	6	6	100	1	16.66	0	0.0	0	0.0	0	0.0	0	0.0	1.00
9. 100 NAA	6	6	100	5	83.33	1	16.66	0	0.0	0	0.0	1	16.66	1.17
10. 10 NAA + 3 2ip	6	6	100	4	66.66	1	16.66	0	0.0	0	0.0	1	16.66	1.17
11. 50 NAA + 3 2ip	6	6	100	6	100	1	16.66	2	33.33	0	0.0	3	50	1.83
12. 100 NAA + 3 2ip	6	6	100	6	100	2	33.33	1	16.66	1	16.66	4	66.66	2.17
13. 100 NAA + 5 BA	6	6	100	6	100	2	33.33	1	16.66	0	0.0	3	50	1.67
14. 100 NAA + 2 Kin	6	6	100	6	100	1	16.66	0	0.0	0	0.0	1	16.66	1.17
L. S. D					6.471									0.677

50 mg/l NAA + 3 mg/l 2ip stimulate 33.33% of explants to initiate callus of moderate category.

The best results were recorded for Sewi explants, which were treated with 10 mg/l NAA + 3 mg/l 21ip, stimulating 33.33% explants to

initiate callus of small value, 16.66% of explants to initiate moderate callus and 16.66% of explants to initiate callus of high value. The obtained results show that combined 2,4-D with kin had no effect on callus initiation. During initiation stage, there are some problems faced explant (Shoot tip) in the media, some of them contamination, browning, response for the tissue to the medium used. Our observation clearly indicated that contamination and browning were solved during this stage by using the techniques which were mentioned before and incubated the cultures in complete darkness.

*** Callus formation stage:**

From t he presentation in Tables (3 and 4) data it is clear that callus formation responded to different to auxins (2, 4-D and NAA) and cytokinins (2ip or BA or kin) concentrations used in this study. Using 10 mg/l 2,4-D stimulated 33.33% of Zaghloul explants to format callus of the small value and 33.33% of them to format callus of the moderate value, while, no explant had stimulated to format callus of the high value. Increasing 2,4-D level to 100 mg/l stimulated 41.66% of Zaghloul explants to format callus of the small value and 25% of them to format callus of moderate value.

Using 3 mg/l 2ip with 10 mg/l 2,4-D stimulated 8.33% of Zaghloul explants to format callus of small value, 25% of them to format callus of moderate value and 16.66% to format callus of high value. Combined 50 mg/l 2,4-D treatment with 3 mg/l 2ip stimulate 16.66% of Zaghloul explants to format callus of the small value and 25% of them to format callus of the moderate value and 25% of them to format callus of high value. Combined treatment of 100 mg/l 2,4-D with 3 mg/l 2ip stimulated the different Zaghloul explants to format callus of the different categories reaching to 100%. This result was obtained when Zaghloul explants were subjected to 100 mg/l NAA + 3 mg/l 2ip treatment. BA (5 mg/l) was more active in stimulation of Zaghloul explants to format callus when it combined with 100 mg/l NAA (83.33%) than 100 mg/l 2,4-D (75%). However, 2 mg/l kin had the same effect on callus formation when combined with 2,4-D or NAA.

With regard to Sewi CV., the results show that callus formation on Sewi CV. explants was of positive correlation response with increasing auxin levels (2,4-D or NAA) from 10 mg/l to 100 mg/l. Table (4). Using 3 mg/l 2ip combined with auxin treatments (2,4-D or NAA) had highly significant effect and stimulate explants to format callus.

Table (3): Effect of different concentrations of Auxins (2,4-D and NAA) and cytokinins (2ip or BA or Kin) on callus formation of date palm (*Phoenix dactylifera* L.) Zaghloul cultivar after 6 weeks.

No	Treatments (mg/l)	No. of Explant	Callus formation								
			+		+ +		+ + +		total		Value
			No.	%	No.	%	No.	%	No.	%	
1	10 2,4 D	12	4	33.33	1	8.33	0	0.0	5	41.66	1.50
2	100 2, 4 D	12	5	41.66	3	25	0	0.0	8	66.66	1.92
3	10 2,4 D + 3 2ip	12	1	8.33	3	25	2	16.66	6	50	2.08
4	5100 2,4 D + 3 2ip	12	2	16.66	3	25	3	25	8	66.66	2.42
5	100 2,4 D + 3 2ip	12	2	16.66	4	33.33	6	50	12	100	3.33
6	100 2,4 D + 5 BA	12	3	25	4	33.33	2	16.66	9	75.0	2.42
7	100 2,4 D + 2 Kin	12	5	41.66	2	16.66	2	16.66	9	75	2.25
8	10 NAA	12	3	25	1	8.33	0	0.0	4	33.33	1.42
9	100 NAA	12	6	50	2	16.66	0	0.0	8	66.66	1.83
10	10 NAA + 3 2ip	12	2	16.66	2	16.66	2	16.66	6	50	2.00
11	50 NAA + 3 2ip	12	3	25	3	25	3	25	9	75	2.50
12	100 NAA + 3 2ip	12	3	25	5	41.66	4	33.33	12	100	3.08
13	100 NAA + 5 BA	12	3	25	4	33.33	3	25	10	83.33	2.67
14	100 NAA + 2 Kin	12	5	41.66	2	16.66	2	16.66	9	75	2.25
	L. S. D									2.984	0.800

Table (4): Effect of different concentrations of Auxins (2,4-D and NAA) and cytokinins (2ip or BA or Kin) on callus formation of date palm (*Phoenix dactylifera* L.) Sewi cultivar after 6 weeks.

No	Treatments (mg/l)	No. of Explant	Callus formation								
			+		+ +		+ + +		total		Value
			No.	%	No.	%	No.	%	No.	%	
1	10 2,4 D	12	3	25	1	8.33	0.0	0.0	4	33.33	1.42
2	100 2, 4 D	12	5	41.66	2	16.66	1	8.33	8	66.66	2.00
3	10 2,4 D + 3 2ip	12	1	8.33	2	16.66	3	25	6	50	2.17
4	5100 2,4 D + 3 2ip	12	1	8.33	3	25	3	25	7	58.33	2.33
5	100 2,4 D + 3 2ip	12	3	25	4	33.33	5	41.66	12	100	3.17
6	100 2,4 D + 5 BA	12	3	25	3	25	4	33.33	10	83.33	2.82
7	100 2,4 D + 2 Kin	12	3	25	3	25	3	25	9	75	2.50
8	10 NAA	12	3	25	1	8.33	0	0.0	4	33.33	1.42
9	100 NAA	12	5	41.66	2	16.66	0	0.0	7	58.33	1.75
10	10 NAA + 3 2ip	12	2	16.66	1	8.33	2	16.66	5	41.66	1.83
11	50 NAA + 3 2ip	12	3	25	2	16.66	3	25	8	66.66	2.33
12	100 NAA + 3 2ip	12	3	25	5	41.66	4	33.33	12	100	3.08
13	100 NAA + 5 BA	12	4	33.33	2	16.66	3	25	9	75	2.42
14	100 NAA + 2 Kin	12	4	33.33	3	25	2	16.66	2	75	2.33
	L. S. D									3.013	0.843

Using 5 mg/l BA + 100 mg/l 2,4-D was more active in order to format more callus formation (83.33%) than that of using 100 mg/l NAA (75%). The same result was recorded for 100 mg/l NAA or 2,4-D + 2 mg/l kin (75%).

3. **Embryonic callus stage:**

Regarding Zaghloul cultivar, data(5) indicated that MS basal medium supplemented with 10 mg/l 2,4 D + 3 mg/l 2ip gave the best results (83.33%) for callus differentiation to somatic embryo (embryogenesis). This ratio partition to low (8.33%) amount of embryonic callus at shoot tip explant, (33.3%) moderate amount of embryonic callus at shoot tip explant and high amount (41.66%) of embryonic callus at explants. The same trend was found when with 10 mg/l NAA + 3 mg/l 2ip, where the embryonic callus percentage was 75% (8.33 low, 33.3 moderate and 33.33 high). On the other hand, the lowest embryonic callus percentage was obtained with control (16.66%).

These results show the importance of auxins/cytokinins balance to callus formation and differentiation. The ratio was 2.5 and 4.0 times more than control when MS basal medium supplemented with 1.0 and 10 mg/l 2,4 D. The same trend was found for NAA but at a low ratio (1.5 and 3.5 times more than control). It can concluded that 2,4-D is very important for date palm callus formation and differentiation followed by NAA and the results improved by adding 2ip to auxins.

For Sewi cultivars NAA gave the best results (91.66%) comparing to 2,4-D (75%) and same trend was found when adding 2ip to MS basal medium containing NAA or 2,4-D as presented in table (6).

Data presented in tables (5 and 6) also showed that the highest average of callus value for both CV was recorded for 10 mg/l 2,4-D + 3 mg/l 2ip followed by 10 mg/l NAA + 3 mg/l 2ip, respectively. While, the control had the lowest average of callus value.

Results under discussion are in line with Tisserat (1979, 1981 and 1984), Khan *et al.*(1982), Sharma *et al.*, (1984), Gabr and Tisserat (1985), Mater (1986), Brackpool (1988), Dass *et al.*, (1989), Hervan *et al.*, (1993), Shakib *et al.*, (1994), Veramendi and Navarro (1996), Al-Kharyi and Al Maarri (1997) and Ibrahim (1999).

Table (5) Effect of different concentrations of Auxin (2,4-D or NAA) and 3,0 mg/L 2ip on Embryogenic callus of date palm (*Phoenix dactylifera* L.) Zaghloul cultivar after 6 weeks.

Treatments		No. of Explant	Embryogenic callus								
			+		++		+++		Total		Means of callus value
No.	Concentrations (mg/l)		No.	%	No.	%	No.	%	No.	%	
1.	Control	12	1	8.33	1	8.33	0	0.00	2	16.66	1.25
2	1.0 2,4-D	12	2	16.66	3	25	0	0.00	5	41.66	1.67
3	10 2,4-D	12	1	8.33	3	25	4	33.33	8	66.66	2.58
4	10 2,4-D + 3 2ip	12	1	8.33	4	33.33	5	41.66	10	83.33	3.00
5	1.0 NAA	12	2	16.66	1	8.33	0	000	3	25.00	1.33
6	10 NAA	12	0	0.0	4	33.33	3	25.00	7	58.33	2.42
7	10 NAA + 3 2ip	12	1	8.33	4	33.33	4	33.33	9	75.00	2.75
	L.S.D. 5%.									3.107	0.857

1< =+ = Small value 2 < = ++ = Moderate value 3 < = +++ = High value

Table (6) Effect of different concentrations of Auxin (2,4-D or NAA) and 3.0 mg/L 2ip on Embryogenic callus of date palm (*Phoenix dactylifera* L.) Sewi cultivar after 6 weeks.

Treatments		No. of Explant	Embryogenic callus								
			+		++		+++		Total		Means of callus value
No.	Concentrations (mg/l)		No.	%	No.	%	No.	%	No.	%	
1.	Control	12	2	16.66	0	0.0	0	0.0	2	16.66	1.17
2	1.0 2,4-D	12	3	25.0	2	16.66	1	8.3	6	50.0	1.83
3	10 2,4-D	12	0	0.0	3	25.0	4	33.33	7	58.33	2.50
4	10 2,4-D + 3 2ip	12	0	0.0	4	33.33	5	41.66	9	75.0	2.92
5	1.0 NAA	12	1	8.33	2	16.66	0	0.0	3	25.0	1.42
6	10 NAA	12	1	8.33	3	25.0	4	33.33	8	66.66	2.85
7	10 NAA + 3 2ip	12	2	16.66	3	25.0	6	50	11	91.66	3.17
	L.S.D. 5%									3.053	0.872

1< =+ = Small value 2 < = ++ = Moderate value 3 < = +++ = High value

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