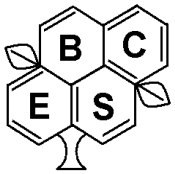

Journal

INFLUENCE OF LIGHT CONDITIONS AND GROWTH REGULATORS ON SOMATIC EMBRYOGENESIS OF DATE PALM

Gomaa, Amina,H.[1]; M. H. Abd Elzahr[1] and Fadia A. Hussein[2]

[1-]Faculty of Agiculture, Cairo University, Egypt.
[2-]Central Laboratory of Date Palm Research and Development, Agricultural Research Center, Giza, Egypt.

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ABSTRACT

Somatic embryogenesis was induced in callus cultures derived from shoot tip of date palm cv .Haiane. Callus was obtained from shoot tip after 9 months (1.5 month interval) of culture on Murashige and Skoog (MS) basal medium supplemented with 170 mg $NaH_2PO_4.2H_2O$, 200 mg/l glutamine; 40 mg/l adenine sulfate, 0.4mg/l thiamine-HCl and 3g/l activated charcoal, 30 g/l Sucrose, 6 g/l agar. This callus gave rise to an embryogenic mass after 2 subculture 4 weeks in between on medium containing 0.1 mg/l of NAA (naphthalene acetic acid). Various morphological abnormalities were recorded on some plants produced by somatic embryogenesis caused by somaclonal variations which restrict the utilization of commercial plant tissue culture propagation for date palm varieties. BA (benzyl adenine) or 2iP (2isopentenyl adenine) at 0.0, 0.1, 0.5 and 1.0 mg/l combined with NAA at the same concentration in separate experiments under different light conditions were used. Incubation of cultures on light enhanced normal shape of somatic embryo and germination percentage while dark condition induced abnormalities and embryogenic callus formation. Different effects of growth regulators at different concentrations and combination were also discussed in this concern (on this paper).

INTRODUCTION

Date palm is a dioecious, perennial monocot plant that is commercially important in Middle East and North Africa. The entire tree of date palm is utilized to provide food, shelter, fiber, clothing, furniture and many other-products. Moreover, date palm successfully tolerates extremely adverse environmental conditions, including drought, high temperature and salinity, which are the peculiar criteria of desert lands. It makes a significant contribution toward the creation of equable microclimates within the fragile oasis ecosystems, thus enabling sustainable agricultural development in many drought and saline affected regions (Barreveld, 1993).

The date palm, *Phoenix dactylifera* L., is one of the most economically important perennial plants in arid areas of the Middle-East and North Africa (Awad, 2007). Conventionally, this palm is propagated from offshoots, which are limited in number (Othmani *et al.,* 2009).

Plant tissue culture technique is known as a promising method for the mass-propagation of date palm. Two major methods, organogenesis and somatic embryogenesis were used to produce large number of identical plants. However, various morphological abnormalities were recorded on some plants produced by somatic embryogenesis caused by somaclonal variations which restrict the utilization of commercial plant tissue culture propagation for elite date palm varieties. Ibrahim (1999) noted that embryo stage had the highest frequency of abnormalities among the other stages; he also observed that the normally grown embryos varied as well as their vegetative characteristics. Tendency of the haustorial end to fold around it self few turns was a good sign of successful and continuous normal germination of embryiods. Moreover, Abo-El-Soaud (1999) reported that several morphological forms of date palm embryos were initiated and these have different potentials for regeneration. Along the way, abnormal structures would also be formed. Abd El-Baky (2001) showed that the effect of callus source, media and subculture on mean numbers and percentages of abnormal somatic embryos of date palm, the least percentage of abnormality in embryo formation was resulted in shoot tip callus (10.5%) followed by axillary bud callus (12.8%) and leaf primordial callus (18.7%) with significant differences between these averages.

This study was aimed to know some factors (plant growth regulators and light conditions) affecting these abnormalities and tried to overcome it in order to develop methods to control and early detection of this phenomenon and eventually increase plant tissue culture efficiency to produce plants true to type.

MATERIALS AND METHODS

These experiments were performed at the Center Laboratory for Date Palm researches and Development by using somatic embryogenesis of cv. Haiane which was obtained from Rashid, Beheira Government, Egypt shoot was sterilized as described by (Zaid, 2003) (1-2 cm in length) of date palm cultivar. Shoot apex were sliced longitudinally into 4 pieces and then cultured on Murashige and Skoog (MS) basal nutrient medium (1962) supplemented with 170 mg $NaH_2PO_4.2H_2O$, 200 mg/l glutamine, 40 mg/l adenine sulfate; 0.4 mg/l thiamine-HCl, 3 g/l activated charcoal, 30 g/l sucrose, 6 g/l agar and 10 mg/l 2,4-D + 3 mg/l 2iP as described by Mater (1986). The pH off all culture media were adjusted to 5.8±0.1 prior to the addition of agar, and then 35 ml of medium was dispensed into small jars (150 ml), jars were autoclaved at 121ºC and 1.1 kg/cm^2 for 20 min.

After the formation of embryonic callus (for six subculture, six week interval), cultures were transferred onto differentiated nutrient medium which consists of MS basal nutrient medium for two subcultures (4weeks in between) supplemented with 0.1 mg/l NAA in jars (150 ml) to form somatic embryos. All cultured jars were incubated in a temperature-controlled room at 25 ±2°C under 16 hrs daily exposure to low light intensity about 1000 lux illumination. Somatic embryo clusters containing (3-4 embryos) were used as explants material during this investigation.

Effect of BA, NAA and their combinations on date palm somatic embryo types under light conditions

BA and NAA at 0.0, 0.1, 0.5 and 1 mg/l and their different combinations were used to study their effect on types of Haiane somatic embryo formation. Clusters of somatic embryo were recultured on the same treatment for two subculture (4 weeks in between).

After this period normal embryos percentage, abnormal embryos percentage, number of germinated embryos and new embryonic callus were recorded.

Effect of 2iP, NAA and their combinations on date palm somatic embryo types under light conditions

Clusters of somatic embryo were cultured on MS media supplemented with 2iP and NAA in different concentrations and combinations at 0.0, 0.1, 0.5 and 1 mg/l to study their effects on types of Haiane somatic embryo formation. Normal embryos percentage, abnormal embryos percentage, number of germinated embryos and new clones were recorded after two subculture (4weeks inbetween) as affected by previous treatments. New embryonic callus in previous experiments were recorded visually as scores according to Pottino (1981)

Negative result (-) = 1

Below average results (+) = 2

Average results (++) = 3

Good results (+++) = 4

The obtained data were statistically analyzed using general models procedure adapted by SPSS (2004) for user guide with one-way ANOVA. Duncan test within program SPSS was done to determine the degree of significance between the means.

RESULTS AND DISCUSSION

Effect of BA, NAA and their combinations on date palm somatic embryo types under light conditions

Data presented in Table (1) clearly showed that somatic embryo were cultured on medium containing 0.0, 0.1, 0.5, 1.0 mg/l BA or NAA and their combination under light conditions for 8 weeks throughout two recultures to study their effects on normal and abnormal shape percentage.

Table (1). Effect of BA, NAA and their combinations on normal and abnormal somatic embryo types of date palm under light conditions

Treatments (mg/l)	Normal embryos percentage				Abnormal embryos percentage			
	Dark	Light	Mean	p-value	Dark	Light	Mean	p-value
Control	51.00^{a}	55.00^{a}	53.00^{a}	0.657	20.00^{d}	7.00^{g}	13.50^{g}	0.015
0.1 BA	46.00^{abc}	50.00a^{b}	48.00^{abc}	0.574	21.00^{d}	9.00^{efg}	15.00^{fg}	0.027
0.5 BA	37.00^{bcd}	43.00^{abcd}	40.00^{cde}	0326	27.00^{bcd}	14.00^{cdef}	20.50^{defg}	0.059
1.0 BA	30.00^{de}	34.00^{cdef}	32.00^{ef}	0.404	34.00^{abc}	24.00^{ab}	29.00^{abcd}	0.087
0.1 NAA	48.00^{ab}	50.00^{ab}	49.00^{ab}	0.777	20.00^{d}	8.00^{fg}	14.00g	0.022
0.5 NAA	37.00^{bcd}	44.00^{abc}	40.50^{bcde}	0.262	23.00cd	11.00^{defg}	12.50^{efg}	0.022
1.0 NAA	35.00^{cde}	36.00^{bcdef}	35.50^{de}	0.851	24.00cd	18.00^{bcd}	21.00^{cdefg}	0.168
0.1BA+0.1NAA	40.00^{bcd}	48.00^{abc}	44.00^{bcd}	0.051	21.00d	9.00^{efg}	15.00^{fg}	0.027
0.1BA+0.5NAA	34.00^{ce}	40.00^{bcde}	37.00^{de}	0.390	24.00cd	13.00^{a}	18.50^{efg}	0.029
0.1BA+1.0NAA	29.00^{de}	34.00^{cdef}	31.00^{ef}	0.312	27.00^{bcd}	20.00^{bc}	23.50^{cdef}	0.247
0.5BA+0.1NAA	36.00^{cd}	40.00^{bcde}	38.00^{de}	0.532	28.00^{bcd}	15.00^{cde}	21.50^{cdefg}	0.041
0.5BA+0.5NAA	30.00^{de}	36.00^{bcdef}	33.00^{ef}	0.265	31.00^{abcd}	19.00^{bc}	25.00^{bcde}	0.059
0.5BA+1.0NAA	24.00^{ef}	30.00^{defg}	27.00^{fg}	0.290	34.00^{abc}	24.00^{ab}	29.00^{abcd}	0.044
1.0BA+0.1NAA	26.00^{def}	28.00^{efg}	27.00^{fg}	0.753	36.00^{ab}	27.00^{ab}	31.50^{abc}	0.139
1.0BA+0.5NAA	19.00^{fg}	25.00^{fg}	22.00^{gh}	0.202	38.00^{ab}	31.00^{a}	34.50^{ab}	0.218
1.0BA+1.0NAA	13.00^{g}	20.00^{g}	16.50^{h}	0.090	41.00^{a}	33.00^{a}	37.00^{a}	0.173
Mean	33.44	38.31		0.028	28.06	17.63		0.000

Small letters in the same column with different superscripts differ at 5% level.

The normal embryo percentage

The percentage of normal shape of somatic embryo was increased by incubated somatic embryo cultures under light condition. In this concern, Calero (1989) found that red light (655±20nm) enhanced the initiation of somatic embryogenesis in cotyledonary sheaths of date palm cultured on modified Schenk and Hildebrandt medium containing 1.0 mg/l of 2,4-D and 0.1 mg/l of BA compared with white or blue (420±12nm) light or darkness. Regarding the effect of different concentrations of BA or NAA and their combinations, data revealed that using culture media without growth regulators or

that containing 0.1 mg/l NAA or 0.1 mg/l of BA produced the highest percentage of normal shape respectively without significant differences among them. While the lowest percentages were observed by using media containing 1 mg/l BA + 1 mg/l NAA and 1 mg/l BA+ 0.5 mg/l NAA. In red light 0.1 mg/l BA increased the number of embryoids of Tunisia date palm produced compared with the other BA concentrations (Calero *et al.*, 1990). The presence of BA was necessary for the normal production of plantlets. Interactions between light condition and different treatment showed that the highest values of normal shape percentage were observed by using control, 0.1 mg/l of BA, and NAA, 0.5 mg/l of NAA and BA, and 0.1 mg/l BA+ 0.1 mg/l NAA. Under dark condition the highest values were recorded with control, 1 mg/l NAA and 0.1 mg/l BA respectively without significant differences among them. (Eke *et al*., 2005) found that somatic embryos of date palm transferred to MS medium without hormones under light matured after about two subcultures and developed into shoots.

Abnormal embryos

Data in Table (1) clearly showed that the effect of different BA, NAA concentrations and their combinations under light and dark conditions. The darkness gave abnormalities embryos more than light condition; the mean value about different treatments effect showed that culture media with 1.0 mg/l BA + 1.0 mg/l NAA gave the highest result of abnormal shape of somatic embryos (37%). It was followed by MS medium supplemented with 1.0 mg/l BA + 0.5 mg/l NAA (34.50%). However, culture medium with 0.1 mg/l NAA gave the lowest percentage of abnormal shape of somatic embryos after control (14.00% and 13.50%) respectively. Culture MS medium supplemented with 0.1 mg/l BA or 0.1mg/l BA + 0.1 mg/l NAA gave the same percentage of abnormal embryo (15.0%). Also culture MS medium supplemented with 1.0 mg/l BA or 0.5 mg/l BA + 1.0 mg/l NAA showed the same percentage of abnormal embryo (29.00%).

Germination percentage

Data in Table (2) showed that germinated embryos percentage affected by BA, NAA in different concentrations and their combinations and light condition. Germination %was increased by incubated somatic embryo cultures under light conditions. Samosir *et*

al., (1998) found that plantlet regeneration resulted from coconut palm somatic embryos required illumination.

Table (2). Effect of BA, NAA and their combinations on date palm somatic embryo germination and embryonic callus formation under light conditions

Treatment mg\l	Germination percentage				Embryonic callus percentage			
	Dark	Light	Mean	p-value	Dark	Light	Mean	p-value
Control	29.00^{d}	43.00	36.00^{b}	0.076	2.00	1.66^{ab}	1.83^{abc}	0.374
0.1 BA	33.00^{bcd}	41.00	37.00^{ab}	0.136	1.66	1.66^{ab}	1.66^{bc}	1.000
0.5 BA	36.00^{abcd}	43.00	39.50^{ab}	0.207	1.33	1.33^{ab}	1.33^{c}	1.000
1.0 BA	36.00^{abcd}	43.00	39.00^{ab}	0.149	1.33	1.33^{ab}	1.33^{c}	1.000
0.1 NAA	32.00^{cd}	42.00	37.00^{ab}	0.080	2.33	1.66^{ab}	1.99^{abc}	0.519
0.5 NAA	40.00^{abc}	45.00	42.50^{ab}	0.359	3.00	2.33^{ab}	2.66^{ab}	0.492
1.0 NAA	41.00^{ab}	46.00	43.50^{ab}	0.315	3.33	2.66^{a}	2.99^{a}	0.768
0.1BA+0.1NAA	39.00^{abc}	43.00	41.00^{ab}	0.456	2.00	1.66^{ab}	1.83^{abc}	0.374
0.1BA+0.5NAA	43.0^{a}	45.00	44.00^{ab}	0.713	3.00	2.33^{ab}	2.66^{ab}	0.116
0.1BA+1.0NAA	44.00^{a}	46.00	45.00^{a}	0.712	3.33	2.66^{a}	2.99^{a}	0.643
0.5BA+0.1NAA	36.00^{abcd}	43.00	39.50^{ab}	0.186	2.00	1.00^{b}	1.50^{c}	-
0.5BA+0.5NAA	36.00^{abcd}	44.00	40.00^{ab}	0.152	2.33	1.33^{ab}	1.83^{abc}	0.349
0.5BA+1.0NAA	42.00^{a}	46.00	44.00^{ab}	0.392	2.66	1.66^{ab}	2.16^{abc}	0.349
1.0BA+0.1NAA	36.00^{abcd}	43.00	39.50^{ab}	0.207	1.33	1.00^{b}	1.16^{c}	0.374
1.0BA+0.5NAA	38.00^{abc}	44.00	41.00^{ab}	0.282	2.00	1.00^{b}	1.50^{c}	-
1.0BA+1.0NAA	40.00^{abc}	45.00	42.50^{ab}	0.344	2.33	1.33^{ab}	1.83^{abc}	0.349
Mean	37.56	43.88		0.00	2.25	1.66		0.003

Small letters in the same column with different superscripts differ at 5%level.

It's obviously from Table (2), that the addition of BA or NAA at different concentrations and combinations increased the percentage of germinated embryos without significant differences among them compared with control. The supply of 0.05 mg/l BAP (BA) on the germination medium of date palm could be useful in terms of germination percentage of somatic embryos (Zouine and El-Hadrami, 2007). However the highest percentage was noticed by using culture medium with 0.1 mg/l BA + 1.0 mg/l NAA (45%). Under dark condition, the highest values of germinated embryos observed by

using 0.1mg/l BA+0.5mg/lNAA, 0.1 mg/l BA+ 0.1mg/l NAA and 0.5mg/l BA+1.0mg/l NAA. An exogenous supply of BA has been found to improve somatic embryo development and germination in banana (Dheda *et al.,* 1991) and rubber tree (Montoro *et al*., 1992). In coconut palm somatic embryogenesis, the lowering of the 2,4-D concentration in the medium followed by the addition of BA, was found to be essential for the complete bipolar differentiation of the embryo (Verdeil *et al*., 1994). Under light condition, no significant difference could be observed among all treatments. However, using 1.0mg/l NAA, 0.1mg/l BA+1.0mg/l NAA and 0.5mg/l BA+1.0mg/l NAA, gave the highest value (46.0%)

Embryogenic callus

Embryonic callus described by Tisserat (1982) as white nodular callus with globular structures which translated into somatic embryos was formed at the base of cluster of somatic embryos.

Embryogenic callus has been explored in Table (2). Embryogenic callus was significant under dark compared with light condition. Data revealed that culture media containing 0.1mg/l BA+1.0mg/l NAA and 1.0mg/l NAA produced the highest mean values of embryogenic callus (2.66%) under light condition, while no significant differences could be observed among all treatments under dark condition. Significantly new embryonic callus of date palm formed on the surface of culture medium as the residual effect of 1.0 mg/l ABA+ 0.1mg/l BA (Zaid, 2003).

Effect of 2iP, NAA and their combinations on date palm somatic embryo types under light conditions

Data in Table (3) clearly showed that somatic embryos were cultured on medium containing 0.0, 0.1, 0.5, 1mg/l 2iP or NAA and their combinations under light conditions for 8 weekes through two recultures to study their effects on normal and abnormal shape percentage.

The normal percentage

Data in Table (3) clearly showed that, media without growth regulator (control), medium with 0.1 mg/l NAA, 0.1 mg/l 2iP and medium with 0.1 mg/l 2iP + 0.1mg/l NAA gave the highest results of normal shape of somatic embryos. It was followed by culture medium supplemented with 0.5mg/l 2iP+0.5mg/l NAA, while the effect of 1

mg/l 2iP + 1mg/l NAA recorded the lowest significant percentage of normal shapes. Interaction between treatments and light conditions showed that, control medium, media supplemented with 0.1 mg/l 2iP, 0.5 mg/l 2iP, 0.1 mg/l NAA, 0.5 mg/l NAA and 0.1 mg/l 2iP + 0.1 mg/l NAA produced the highest values of normal embryos under both light or dark conditions.

Table (3). Effect of 2iP, NAA and their combinations on normal and abnormal somatic embryo types of date palm under light conditions.

Treatments mg/l	% normal embryos				% abnormal embryos			
	Dark	Light	Mean	p-value	Dark	Light	Mean	p-value
Control	62.00^{a}	65.00^{a}	63.50^{a}	0.653	8.00^{g}	3.00^{h}	5.50^{h}	0.015
0.1 2iP	58.00^{ab}	60.00^{ab}	59.00^{ab}	0.797	10.00^{fg}	6.00^{fgh}	8.00^{fgh}	0.118
0.5 2ip	50.00^{abcd}	53.00^{abcd}	51.50^{bc}	0.705	12.00^{efg}	7.00^{defgh}	9.50^{efgh}	0.150
1.0 2ip	38.00^{def}	41.00^{def}	39.50^{de}	0.637	20.00^{abcd}	12.00^{bcd}	16.00^{bcd}	0.072
0.1 NAA	59.00^{ab}	60.00^{ab}	59.50^{ab}	0.874	8.00^{g}	3.00^{h}	5.50^{h}	0.015
0.5 NAA	52.00^{abcd}	54.00^{abcd}	53.00^{bc}	0.751	10.00^{fg}	4.00^{gh}	7.00^{gh}	0.085
1.0 NAA	40.00^{cdef}	42.00^{cdef}	41.00^{de}	0.818	15.00^{cdef}	8.00^{defg}	11.50^{defg}	0.65
0.1 2iP+0.1NAA	55.00^{abc}	57.00^{abc}	56.00^{abc}	0.751	10.00^{fg}	4.00^{gh}	7.00^{gh}	0.085
0.1 2iP+0.5NAA	44.00^{bcde}	49.00^{bcde}	46.00^{cd}	0.571	13.00^{defg}	6.00^{efgh}	9.50^{efgh}	0.052
0.1 2iP+1.0NAA	36.00^{def}	40.00^{def}	38.00^{def}	0.572	18.00^{bcde}	10.00^{cdef}	14.00^{cde}	0.033
0.5 2iP+0.1NAA	45.0^{bcde}	48.00^{bcde}	46.50^{cd}	0.669	16.00^{cdef}	9.00^{cdef}	12.5^{cdef}	0.049
0.5 2iP+0.5NAA	39.00^{cdef}	43.00^{cdef}	41.50^{de}	0.572	17.00^{cde}	11.00^{cde}	14.00^{cde}	0.124
0.5 2 iP+1.0NAA	32.00^{ef}	36.00^{ef}	34.00^{ef}	0.571	20.00^{abcd}	13.00^{abcd}	11.50^{abcd}	0.048
1.0 2iP+0.1NAA	36.00^{def}	39.00^{def}	37.00^{def}	0.668	22.00^{abc}	15.00^{abc}	18.50^{abc}	0.090
1.0 2iP+0.5NAA	31.00^{ef}	35.00^{ef}	33.00^{ef}	0.571	25.00^{ab}	18.00^{ab}	21.50^{ab}	0.127
1.0 2 iP+1.0NAA	27.00^{f}	31.00^{f}	29.00^{f}	0.571	27.00^{a}	20.00^{a}	23.50^{a}	0.127
Mean	44.00	47.06		0.221	15.68	9.31		0.000

Small letters in the same column with different superscripts differ at 5%level.

Abnormal percentage:

Generally, the percentage of abnormal shape of somatic embryos was increased by incubated somatic embryo cultures under dark condition. Effect of 1.0 mg/l 2iP + 1.0mg/l NAA recorded the highest percentage of abnormal shapes of embryos (23.50%), while control treatment recorded the lowest percentage of abnormal shapes of embryos (5.50%). Data also revealed that media with 0.5 mg/l 2iP + 1.0 mg/l NAA, 1.0 mg/l 2iP + 0.1mg/l NAA, 1.0 mg/l 2iP + 0.5 mg/l NAA and 1.0 mg/l 2iP + 1mg/l NAA gave the highest values of abnormal shape under both light and dark conditions.

Germination percentage

Data in Table (4) showed that no significant differences were observed between percentages of germinated embryos under light or dark condition. However, light condition produced the highest value in this concern (43.69%). Eke *et al*., (2005) reported that induction of somatic embryos of date palm using medium with 0.5 NAA and 1 mg/l 2iP was done either in the light or in the dark. Using culture medium with 0.5 mg/l 2iP + 1 mg/l NAA gave the highest percentage of germination (49.50%), while control (MS medium without plant growth regulators medium produced the lowest value (31.0%) compared with other treatments. Interaction between culture media and light conditions showed that, the lowest values of germination % were observed by using control followed by media supplemented with (0.1 mg/l 2iP) under dark or light condition. The highest values of germination% were observed by using media containing 0.5 mg/l 2iP + 1.0 mg/l NAA (51.0%), 1.0 mg/l NAA, 0.1 mg/l 2iP + 1.0 mg/l NAA (50.0%) and 1 mg/l 2iP + 1.0 mg/l NAA (49%) under light, while 0.5mg/l 2iP+1.0mg/l NAA gave the highest value of germination% under dark condition Saker *et al*., (1998).

Saker *et al*. (1998) found that 2iP is more effective than either kinetin or BA in shoot proliferation of date palm after callus formation phase. These results agreement with Yogesh *et al*. (2003) which stated that on *Euphorbia pulcherrima* the induction of somatic embryogenesis in red pigmented callus was achieved on MS supplemented with 2iP (9.8 µM) and NAA (2.69 µM). Reduced level of NAA (0.54 µM) in the same medium caused maturation of somatic embryoids. The embryoids germinated successfully, turned into plantlets. Eshraghi *et al.* (2005) transferred embryogenic callus of date

palm cv. Khanizi to a medium containing NAA and 2iP developed plantlets with a shoot and root.

Table (4). Effect of 2iP, NAA and their combinations on germination and embryonic callus of date palm somatic embryo types under light conditions

Treatments	Germination percentage				Embryonic callus percentage			
mg/l	Dark	Light	Mean	p-value	Dark	Light	Mean	p-value
Control	3.00^{c}	32.00^{c}	31.00^{f}	0.775	2.00^{abc}	1.66^{bc}	1.83^{bcd}	0.643
0.1 2iP	32.00^{bc}	35.00^{bc}	33.50^{ef}	0.632	2.00^{abc}	1.00^{c}	1.50cd	-
0.5 2ip	38.00^{abc}	40.00^{abc}	39.00^{bcdef}	0.749	1.33^{c}	1.00^{c}	1.16^{d}	0.374
1.0 2iP	42.00^{abc}	46.00^{ab}	44.00^{abcd}	0.633	1.33^{c}	1.00^{c}	1.16^{d}	0.374
0.1 NAA	34.00^{abc}	37.00^{abc}	35.50^{def}	0.634	2.33^{abc}	1.66^{bc}	1.99^{bcd}	0.230
0.5 NAA	40.00^{abc}	43.00^{abc}	41.50^{abcde}	0.483	3.00^{ab}	2.33ab	2.66ab	0.561
1.0 NAA	45.00^{ab}	50.00^{a}	47.50^{ab}	0.530	3.33^{a}	2.66^{a}	2.99^{a}	0.230
0.1 2iP+0.1NAA	35.00^{abc}	39.00^{abc}	37.00^{cdef}	0.750	1.33^{c}	1.00^{c}	1.16^{d}	0.374
0.1 2iP+0.5NAA	43.00^{abc}	45.00^{abc}	44.00^{abcd}	0.473	2.00^{abc}	1.66^{bc}	1.83^{bcd}	0.634
0.1 2iP+1.0NAA	46.00^{ab}	50.00^{a}	48.00^{ab}	0.531	2.66^{abc}	2.00^{abc}	2.33^{abc}	0.374
0.5 2iP+0.1NAA	39.00^{abc}	43.00^{abc}	41.00^{abcde}	0.635	1.66^{bc}	1.00^{c}	1.33^{d}	0.116
0.5 2iP+0.5NAA	43.00^{abc}	46.00^{ab}	44.50^{abc}	0.598	1.66^{bc}	1.33^{c}	1.49^{cd}	0.519
0.5 2iP+1.0NAA	48.00^{a}	51.00^{a}	49.50^{a}	0.532	2.00^{abc}	1.33^{c}	1.66^{cd}	0.116
1.02iP+0.1NAA	42.00^{abc}	46.00^{ab}	44.00^{abcd}	0.616	1.33^{c}	1.00^{c}	1.16^{d}	0.374
1.0 2iP+0.5NAA	44.00^{ab}	47.00^{ab}	45.50^{abc}	0.578	1.66^{c}	1.00^{c}	1.33^{d}	0.116
1.0 2iP+1.0NAA	46.00^{ab}	49.00^{a}	47.50^{ab}	0.050	2.00^{abc}	1.25^{c}	1.50^{cd}	0.158
Mean	40.44	43.69			1.98	1.25		0.000

Small letters in the same column with different superscripts differ at 5%level.

Embrygenic callus

The highest significant value of embryonic callus formation was recorded when were incubated under dark condition compared with light.

Culture medium supplemented with 1.0 mg/l NAA produced the highest value of embryonic callus followed by 0.5 mg/l NAA and 0.1 mg/l 2iP + 1.0 mg/l NAA without significant differences among them.

Interactions between treatments and light conditions showed that under both dark and light conditions, medium supplemented with 1.0 mg/l NAA produced the highest mean value of embryonic callus (3.33 and 2.66, respectively).

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تأثير ظروف الاضاءة ومنظمات النمو على الأجنة الخضرية لنخيل البلح

أمينه جمعه * ؛ محمد حلمى * و فاديه عبد المرضى حسين **

* كلية الزراعة- جامعة القاهرة- مصر

** معمل بحوث النخيل- معهد بحوث البساتين- الجيزة- مصر

تم الحصول على مزارع كالس لنخيل البلح صنف حيانى بزراعة القمة النامية على بيئة موراشيجى وسكوج المحتوية على 170 ملليجرام/لتر $NaH_2PO_4.2HO$ ، 200 ملليجرام/لتر جلوتامين ، 40 ملليجرام/لتر كبريتات الأدنين ، 0.4 ملليجرام/لتر ثيامين هيدروكلوريد ، 3جرام/لتر فحم نباتى نشط ،30جرام/لتر سكروز ، 6جرام/لتر آجار لمدة 9 أشهر مع النقل كل 1.5 شهر لبيئة طازجة وقد تم الحصول على الأجنة الجسمية بزراعة الكالوس على بيئة تحتوى على 0.1 مجم/لتر نفثالين حامض الخليك لمدة 8 أسابيع مع النقل لبيئة طازجة كل 4 أسابيع ونظرا لأن الاكثار عن طريق استخدام الأجنة الجسمية يؤدى لظهور بعض الأشكال غير الطبيعية نتيجة لما يعرف بالاختلافات الجسدية والتى تحد من استخدام زراعة الأنسجة بهذه الطريقة لذلك تم دراسة تأثير منظمات النمو 2-ايزوبنتنيل أدنين أو بنزيل أدنين مع نفثالين حمض الخليك بتركيزات صفر، 0.1 ، 0.5 ،1 مجم/لتر وكذلك التحضين فى الاضاءة والاظلام لمعرفة تأثير هذه العوامل على هذه الظاهرة.

وقد أوضحت النتائج أن تحضين مزارع الأجنة الجسمية لنخيل البلح صنف حيانى فى ظروف الاضاءة الطبيعية يؤدى لزيادة نسبة الأجنة الطبيعية وكذلك زيادة نسبة تحولها الى نبيتات بينما ادى تحضين الزراعات فى الاظلام للحصول على أعلى نسبة من الأشكال الغير طبيعية وزيادة تكون الكالوس وقد وجد أن زيادة السيتوكينينات 2-ايزوبنتنيل أدنين أو بنزيل أدنين الى 1مجم/لتر بالاضافة لوجود نفثالين حمض الخليك بتركيزاته قيد الدراسة أدى لزيادة نسبة الأجنة غير الطبيعية.