USING COMPLEX ORGANIC ADDITIVES IN TISSUE CULTURE MEDIUM OF DATE PALM

ZEINAB E. ZAID[1], REHAB A. SIDKY[1] AND M. A. EL-SHAMY[2]

1. *The Central Laboratory for Date Palm Researches and Development, ARC, Giza*
2. *Horticulture Research Institute, ARC, Giza*
E-mail: zemmz2005@yahoo.com

(Manuscript received 30 June 2008)

Abstract

Coconut milk, Casein hydrolysate and Malt extract were used as a complex organic additives to investigate callus growth and somatic embryos production in *Phoenix dactylifera* L. cv. Bartamuda. The addition of complex organic additives with different concentrations to callus formation medium was effective compared with control medium. Casein hydrolysate and malt extract were recorded the lowest value of browning degree without significant differences. While control medium (Free- complex organic additives medium) recorded the highest value of browning degree. Coconut milk gave the best result to callus production compared with casein hydrolysate and malt extract. The same and the best results were observed when added 300 mg/l coconut milk or 500 mg/l casein hydrolysate through callus formation stage. However, These callus after continuous growth, white embryogenic nodular callus was formed. The second stage, mature nodules were transferred to germinated medium containing of 0.1 mg/l NAA+0.05 mg/l BA and complex organic additives which were used in these study to improve somatic embryos production. Embryoids mature and germinant were observed after 6 weeks. All concentrations of coconut milk and malt extract were effective for improvement and increasing of secondary somatic embryos, while casein hydrolysate with various concentrations was ineffective. The data recorded the highest average number of shoot when added coconut milk to germination medium. We observed that, the strongest plantlets raise on the medium supplemented with malt extract. Malt extract was superior as compared to coconut milk or casein hydrolysate to encourage length of shoots. A liner increase in both fresh weight and dry weight was observed in various concentrations of complex organic additives as using compared with control medium. Generally, Adding complex organic additives to culture medium date palm *in vitro* enhanced callus formation and accelerated growth and development of somatic embryos.

Abbreviation: MS – Murashige and Skoog; COA- Complex organic additives; CM – Coconut milk; CH – Casein-hydrolysate; ME – Malt extract; BA – 6-benzyladenine; NAA – naphthalene acetic acid; FW – Fresh weight; DW – Dry weight

INTRODUCTION

The effects of using yeast and plant extracts *in vitro* culture have been investigated by number of workers. In really media undefined components such as fruit juices, yeast extracts and protein hydrolysates, were frequently used in place of defined vitamins or amino acids, or even as further supplements. Overbeek *et al.* (1941) succeeded in growing immature *Datura* embryos in culture by including the liquid endosperm of *Cocos nucifera* (coconut milk) in their culture medium. Coconut milk was also shown to stimulate cell division in other cultured tissues and its use as a supplement was adopted in many laboratories. Other complex plant juices and liquid endosperms have been shown to possess stimulatory properties more or less similar to those of coconut milk. These include liquid endosperm from immature corn, tomato juice, immature fruits and seeds (Steward and Shantz, 1959), orange juice, malt extract, yeast extract, casein hydrolysate, leaf extracts, sap from a number of plants and tumour extracts. Similarly, (Straus 1960) has shown that tomato juice, yeast extract or casein hydrolysate function by supplying a form of organic nitrogen (mixture of amino acids) while malt provide an auxin, kinetin minositol, urea and arginine to *in vitro* cultured explants.The objective of this study was to encourage callus production and improve somatic embryogenesis by using complex organic additives such as coconut milk, casein hydrolysate and malt extract.

MATERIALS AND METHODS

This study was carried out at the Central Laboratory for date palm research and development throughout the period from 2004 to 2006.

Plant material:

The healthy offshoots of Phoenix dactylifera L. cv. Bartamuda were selected from mothers date palm trees grownin Aswan (2 – 4) years; ranging in weight from 5 - 7 kg and about 50 - 80 cm in length.

Explants preparation and sterilization:

The selected young offshoots were carefully transferred to the laboratory after separation from mother tree. Removing leaves from offshoots were continued until the white soft leaves nearer the apical meristem had appeared. The apical meristem plus few leaf primordial was used as explants material. Explants were soaked in running tap water for 1 - 2 hrs, and soaked in sterile anti-oxidant solution of ascorbic (100 mg/L) and citric acid (150 mg/L) for 25 - 30 min to avoid culture browning.

Explants were surface sterilized under aseptic conditions by immersion at 0.5 g/L mercuric chloride ($HgCl_2$) for 5 min and then rinsed one-time with sterile distilled water and transferred to double surface sterilization by commercial Clorox (5.25%) sodium hypochlorite $(NaOCl)_2$ supplemented with two drops of Tween-20 per 100 ml solution, the first one by 40% chlorox for 15 min and thoroughly washed with sterilized distilled water for one time and the second one by 60% chlorox for 25 min and then washed with sterilized distilled water for three times. Outer soft leaves were removed to obtain a shoot-tip. Shoot-tip 5 - 10 mm in length, composed of the apical meristem and (4 – 6) leaf primordial, cut longitudinally into 4 sections and inoculated onto culture medium.

Culture medium and culture condition:

The basal nutrient medium employed throughout this study contained Murashige and Skoog (MS) inorganic salts (1962), 30 gm/L sucrose and 6.0 g/l agar. The pH was adjusted to 5.7 ± 0.1 before the addition of agar. Media were dispensed into culture vessel All culture vessels were capped with polypropylene closures and then autoclaved for 20 min at 121°C 1.5 kg/cm² pressure.

Experiment 1: Effect of coconut milk, casein hydrolysate and malt extract on browning and callus formation

This experiment was designed to study the effect of different concentrations of complex organic additives (coconut milk, Casein hydrolysate and Malt extract) to induce maximum callus formation and established embryogenic callus derived from shoot tip explants.

Shoot apex explants (sliced longitudinally into 4 pieces) were cultured on MS basal medium supplemented with 170 mg $NaH_2PO_4.2H_2O$; 200 mg glutamine ; 40 mg adenine sulfate; 0.9 thiamine-HCl; 0.5 nicotinic acid; 0.5 pyridoxine HCl; 10 mg 2,4-D; 3 mg 2ip; 3 g activated charcoal / liter and various concentrations different complex organic additives as follows:-

- Control medium (Free - complex organic additives medium)
- 100, 300 and 500 mg/l coconut milk
- 100, 300 and 500 mg/l casein hydrolysate
- 100, 300 and 500 mg/l malt extract

The nutrient medium of each treatment was dispensed into small jars (150 ml). Each treatment = 3 replicates and each replicate = 3 jars each jar contained one piece of sterilized shoot tip explants which was placed over the solid surface of medium. All culture jars were maintained in the complete darkness at 27±1°C. The explants were repeatedly subcultured for 3 subcultures at 6 weeks intervals into corresponding fresh callus initiation media. Data were calculated in every treatment at

the end of each subculture for at least 3 subcultures (6 weeks for each one) . Data were taken as the average per explant as follows:

1. Browning degree.
2. Callus formation degree.

The average of browning degree and the average of callus formation were scored visually as follows: (according to Pottino, 1981).

Negative results = 1
Below average results = 2
Average results = 3
Above average growth = 4
Excellent growth = 5

Harvested callus from different treatments was transferred onto an intermediate medium consisted of basal medium supplemented with 10 mg/l 2,4-D and 1 g/l activated charcoal (Abul-Soad *et al.*, 2002) to obtain white embryogenic nodular callus, and then the free nodular callus collected and were cultured on germination medium supplemented with 0.1 mg/l NAA for month to obtain embryoids.

Experiment 2: Effect of coconut milk, casein hydrolysate and malt extract on growth and development of somatic embryos

Repeated Embryo used in this experiment as a explants (Cluster of 3-4 embryo arose repetitive, which are usually of normal morphology) described by George, 1993, and Zaid *et al.*, 2004. The explants were cultured on MS basal medium supplemented with 0.05 mg/l BA + 0.1 mg/l NAA as a control medium and various concentrations of different complex organic additive to improve and develop of somatic embryos production as follows:-

- Control medium (free - COA medium)
- 50, 100 and 150 mg/l coconut milk
- 50, 100 and 150 mg/l casein hydrolysate
- 50, 100 and 150 mg/l malt extract

Data were calculated in every treatment at the end of each subculture for 2 subcultures (4 weeks for each one) . Data were taken as the average per explant as follows:

1. Average secondary embryo number
2. Average individual shoot number
3. Average shoot length (cm)
4. Growth value (Final fresh weight – initial fresh weight) as described by Ziv,1992

Initial fresh weight

5. Fresh weight and dry weight (g)

Statistical analysis

One factor randomized complete block design was used and data were subjected to analysis of variance separation of means among treatments was determined using L.S.D. test at 5% according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Effect of complex organic additives on Browning

Adding coconut milk, casein hydrolysate and malt extract to culture medium conduct to redaction browning degree significantly compared with control medium (Free- complex organic additives medium). Casein hydrolysate and malt extract were recorded the lowest value of browning degree without significant differences in between (1.99 and 1.77 respectively). While control medium (Free- complex organic additives medium) recorded the highest value of browning degree (3.66). Data in (Table 1) clearly showed that, there is no significant differences among varies concentrations of complex organic additives of browning degree.

Effect of complex organic additives on callus production

Regarding the effect of complex organic additives on callus formation, data reveled that the highest significant value of callus initiation was recorded from culture medium supplemented with both coconut milk and casein hydrolysate without difference significant in between (3.66 and 3.55 respectively). whereas, adding malt extract to culture medium was not effect for callus stimulation. There are distinctive significant differences among different concentration of COA under investigation. It was appeared from data in (Table 2) that adding 300 mg/l to culture medium was more effective for stimulating callus production (3.99) followed by 500 mg/l and 100 mg/l (3.22 and 2.66 respectively). Generally, the result showed that using complex organic additives in culture medium is greatly influenced for callus formation and vigorous growth.

Coconut milk was also shown to stimulate cell division in other cultured tissues and its use as a supplement was adopted in many laboratories. Casein hydrolysate can be a source of calcium, phosphate, several microelements, vitamins and most importantly, a mixture of up to 18 amino acid. It produces an improvement in the growth of *Cardamine pratensis* and *Silene alba* suspensions, only if medium deficient in phosphorus (Bister-Miel *et al.,* 1985). Generally, complex organic additives were frequently used in place of defined vitamins or amino acids.

Using complex organic additives for growth and development of somatic embryo

The repeated embryo of *phoenix dactylifera* cv. bartamuda were cultured on MS basal medium supplemented with 0.05 mg/l BA + 0.1 mg/l NAA and various concentrations of different COA to determined secondary somatic embryo, individual shoot, shoot length and growth vigor of somatic embryo produced, data recorded in (Table 3, 4, 5 and 6).

Vegetative somatic embryo

The effect of different concentration of COA on average number of secondary somatic embryo/repeated embryo show at Table 3. Adding CM to proliferation medium was more effective for secondary embryo production (9.33 secondary embryo/repeated embryo), followed by ME (6.88 secondary embryo/repeated embryo) with different significant differences in between, while using CH to proliferation medium was ineffective for secondary embryo production (4.10 secondary embryo/repeated embryo). No significant differences between CH or control medium which give 4.33 secondary embryo/repeated embryo.

Referring the effect of different complex organic additives concentrations (50.0, 100 and 150 mg/l) on secondary embryo production, data clearly showed that added 150.0 mg/l COA to proliferation medium recorded the greatest significant value of secondary embryo production (7.10 secondary embryo/repeated embryo). There was no significant differences between the average value of secondary embryo number/repeated embryo from 50.0 or 100.0 mg/l of COA concentrations (6.66 and 6.55 respectively). The interaction between COA and their concentrations was significant, the best result recorded when add 150.0 mg/l CM to culture medium (12.0 secondary embryo/repeated embryo). These results aggregated with Khalil, *et al.*, 2002 who declared that transfer explants of banana cultivar Dwarf Brazilian to MS medium supplemented with 10% coconut water produced rapidly proliferating embryogenic callus that developed into secondary somatic embryos. Khalil, *et al.*, 2002 reveled that, primary somatic embryos were produced when explants of immature male flower buds were cultured on MS basal medium plus 100.0 mg/l Malt extract and then transfer to basal medium. On the other hand, Mohamed and Mohd 2005 reported that, addition CH (100.0 mg/l) to the shoot induction medium enhanced the growth of regenerates and those results disaggregate with our results

Individual shoot

Individual shoots were appeared after two months from culturing on proliferation medium supplemented with different concentrations COA (Table 4). The presence of CM in culture medium was more effective for stimulating the individual

shoot number. Whereas, the average individual shoot number/repeated embryo was 3.77, while control medium recorded the lowest value of average individual shoot number/repeated embryo (2.66). However, a significant differences were observed among the effect of different concentrations of COA on average individual shoots number. The largest number of individual shoot produced when added 150.0 mg/l CM to culture medium (6.00 individual shoot/repeated embryo). The same results was decided by Devinder-Prakash, *et al.,* 2002 of *Anthurium andraeanum*, they stated that, the highest number of seedling with first and third leaves was recorded for medium supplemented with coconut water.

Shoot length (cm)

It was appeared from data in (Table 5) that mean values showed significant differences among the average shoot length (cm) as affected by different COA. The medium containing malt extract gave the highest significant shoot length (1.50 cm), it was followed by 1.20 cm of adding coconut milk to culture medium. While control medium recorded the lowest value of shoot length (0.80 cm). Concerning the differences between shoots length of different concentrations COA under investigation data reveled that, increasing COA concentration from 50.0 to 150.0 mg/l decrease shoots length from 1.33 to 1.00 cm. With regard the interaction between COA and their concentrations on shoot length data indicated that, the tallest shoot noted when added 50.0 ME to culture medium (2.0 cm). El-Assar *et al.,* 2004 studied the effect of coconut water, date palm meristimatic tissues extract and casein hydrolysate on date palm cv. sewi tissues grown *in vitro*. They reported that, the presence of date palm meristimatic tissues extract in the media composition significantly increased the mean shoot length.

Growth value

It was appeared from data in (Table 6) that, adding coconut milk and malt extract to culture medium was more forcible to increased growth value of developed shoots compared with the growth value of shoots developed on control medium (5.24, 4.88 and 2.69 respectively). While, adding casein hydrolysate to culture medium decreased significantly the growth value of developed shoots compared with the growth value of shoots developed on control medium (1.96 and 2.69 respectively). Media supplemented with 100.0 and 150.0 mg/l of complex organic additives showed the highest significant of growth value (4.44 and 4.03 respectively) without significant difference. While adding 50.0 mg/l of complex organic additives decreased significantly the growth value (3.62). The best significant growth value(6.51) noticed by adding 150.0 mg/l malt extract to culture medium.

Table 1. Effect of coconut milk, casein hydrolysate and malt extract on browning of *Phoenix dactylifera* L. cv. Bartamuda after 18 week from culturing

(A) Complex organic additives	(B) Treatment mg/l			Mean (A)
	100	300	500	
Control(0.0)				3.66a
Coconut milk	2.33	2.33	2.33	2.33b
Casein hydrol	2.33	2.33	1.33	1.99
Malt extract	2.00	1.33	2.00	1.77
Mean (B)	2.00a	1.99a	1.88a	

Table 2. Effect of coconut milk, casein hydrolysate and malt extract on callus formation of *Phoenix dactylifera* L. cv. Bartamuda after 18 week from culturing

(A) Complex organic additives	(B) Treatment mg/l			Mean (A)
	100	300	500	
Control(0.0)				2.33b
Coconut milk	3.66b	4.66a	2.66c	3.66a
Casein hydrol	2.33c	3.66b	4.66a	3.55a
Malt extract	2.00c	3.66b	2.33c	2.55
Mean (B)	2.66c	3.99a	3.22b	

Table 3. Effect of coconut milk, casein hydrolysate and malt extract on vegetative embryos number of *Phoenix dactylifera* L. cv. Bartamuda after 2 months from culturing

(A) Complex organic additives	(B) Treatment mg/l			Mean (A)
	100	300	500	
Control(0.0)				4.33c
Coconut milk	8.00b	8.00b	12.00a	9.33a
Casein hydrol	5.33cd	3.33e	3.66e	4.10c
Malt extract	6.66c	8.33b	5.66cd	6.88b
Mean (B)	6.66b	6.55b	7.10a	

Table 4. Effect of coconut milk, casein hydrolysate and malt extract on individual shoot number of *Phoenix dactylifera* L. cv. Bartamuda after 2 months from culturing

(A) Complex organic additives	(B) Treatment mg/l			Mean (A)
	100	300	500	
Control(0.0)				2.66c
Coconut milk	2.00c	3.33b	6.00a	3.77a
Casein hydrol	0.00	1.33cd	0.66e	0.66d
Malt extract	3.66b	4.00b	1.66cd	3.10a
Mean (B)	1.88	2.88	2.77	

Table 5. Effect of coconut milk, casein hydrolysate and malt extract on shoot length (cm) of *Phoenix dactylifera* L. cv. Bartamuda after 2 months from culturing

(A) Complex organic additives	(B) Treatment mg/l			Mean (A)
	100	300	500	
Control(0.0)				0.80d
Coconut milk	1.00d	1.40c	1.40c	1.20b
Casein hydrol	1.00d	0.80e	0.80e	0.86c
Malt extract	2.00a	1.50b	1.00d	1.50a
Mean (B)	1.33a	1.23b	1.06c	

Table 6. Effect of coconut milk, casein hydrolysate and malt extract on growth value of *Phoenix dactylifera* L. cv. Bartamuda after 2 months from culturing

(A) Complex organic additives	(B) Treatment mg/l			Mean (A)
	100	300	500	
Control(0.0)				2.69c
Coconut milk	4.85d	5.04c	5.85b	5.24a
Casein hydrol	2.91e	2.01f	0.96g	1.96d
Malt extract	3.10e	5.05c	6.51a	4.88b
Mean (B)	3.62b	4.03a	4.44a	

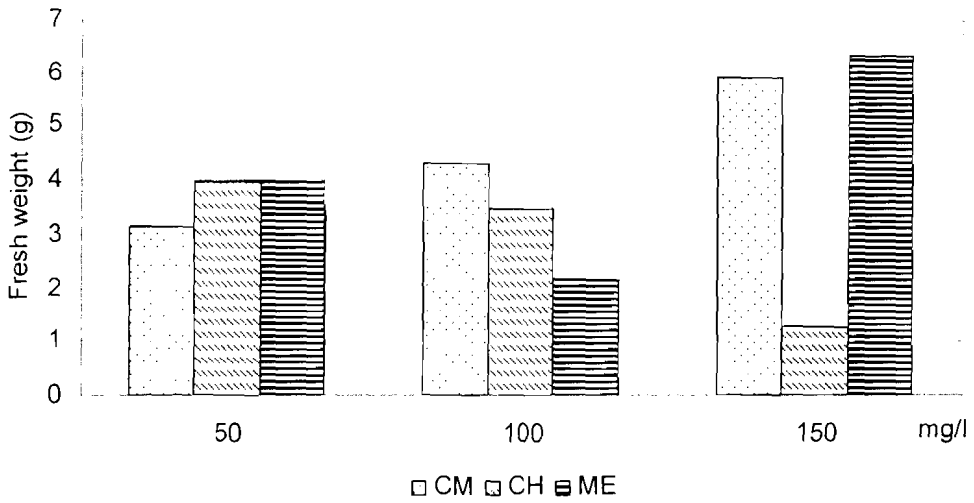

Figure 1. Fresh weight of somatic embryos after 2 months of culture on basal medium supplemented with various concentrations of different complex organic additives
CM = Coconut milk; CH = Casein hydrolysate; ME =Malt extract

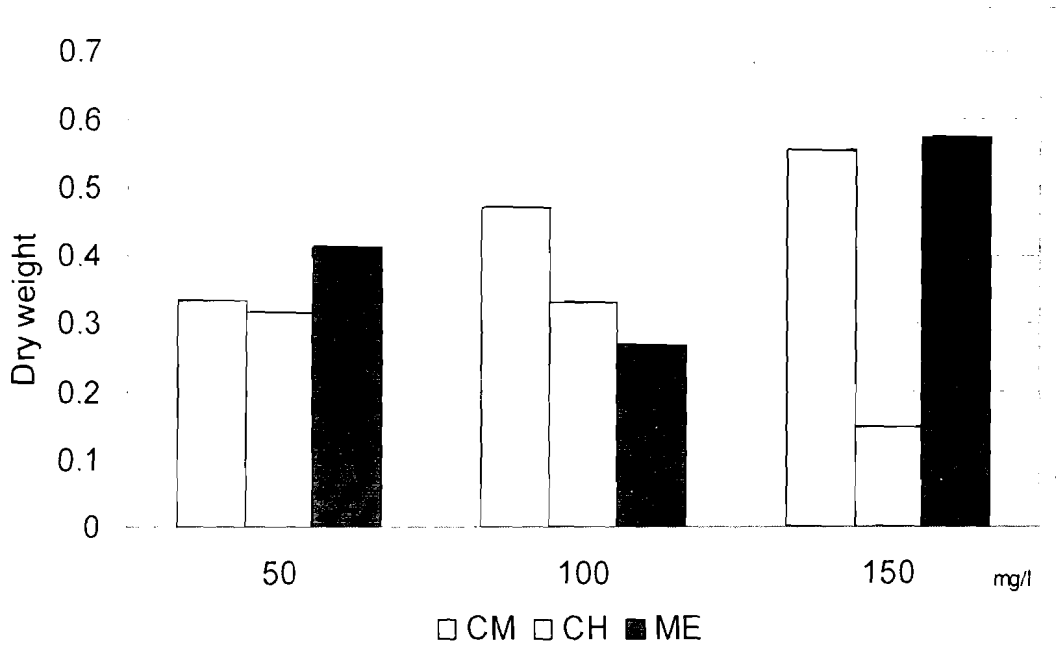

Figure 2. Dry weight of somatic embryos after 2 months of culture on basal medium supplemented with various concentrations of different complex organic additives
CM = Coconut milk; CH = Casein hydrolysate; ME =Malt extract

Fresh weight and dry weight (g)

Data in (fig1 and 2) show that, increasing COA concentration from 50.0 to 150.0 mg/l increased fresh weight and dry weight, reaching maximum value of fresh and dry weight recorded when added 150.0 mg/l malt extracts to culture medium (6.31 and 0.572 g respectively). Casein hydrolysate with different concentrations recorded the lowest values of fresh weight and dry weight.

Generally, Adding complex organic additives to culture medium date palm *in vitro* enhanced callus formation and accelerated growth and development of somatic embryos. On the other hand, COA caused increasing in diameter of shoots and the strongest plantlets raise when were cultured on 50.0 or 100.0 mg/l malt extract (data untabulated). A liner increase in both fresh weight and dry weight was observed in various concentrations of complex organic additives as using compared with control medium.

ACKNOWLEDGEMENT

This study was supported by The Central Laboratory for Date Palm Researches and development. The authors are very grateful to Dr. Abdel-Moneem El-Bana for providing plant material and all the facilities throughout this study and Dr. Amina Gomaa for critical reading of the manuscript.

REFERENCES

1. Abul-Soaud, A.A., Z.E. Zaid, A. Salah, R. A. Sidky. 2002. Tissue culture of date palm (*Phoenix dactylifera L.*). The Third Scientific Agricultural Sciences Conference. Faculty of Agriculture- Assiut Universty.
2. Bister-Miel, F., J.L. Guignard, C. Agier. 1985. Glutamine as an active component of casein hydrolysate: its balancing effect on plant cells cultured in phosphorus deficient medium. Plant Cell Reports. 4, 161-163.
3. Butenko, R.G. 1968. In Tissue Culture and Plant Morphgenesis, Israel Program for Scientific Translation, Jerusalem, pp. 40-45.
4. De Ropp, R.S., J.C. Vitucci, B.L. Hutchings, J.M. Williams. 1952. Effect of coconut fractions on growth of carrot tissues. Proceeding of Society of Experimental Biology 81: 704-709.
5. Devinder-Prakash., M. B. Angadi, G. Shankarmurthy. 2002. Effect of different adjuvants on in vitro embryo culture of Anthurium andraenum. Journal of Ornamental Horticulture New Series 5 (2): 67-68.
6. El-Assar, A.M., W.M. El-Messeih, M. R. El-Shenawi. 2004. Applying of some natural extracts and growth regulators to culture media and their effects on Sewi

cv. date palm tissues growth in vitro. Assiut-Journal-of Agricultural-Sciences, 35 (4) : 155-168.

7. Khalil, S.M., K.T. Cheah., E.A Perez, D.A. Gaskill, J.S. Hu. 2002. Regeneration of banana (Musa spp. AAB cv. Dwarf Brazilian) via secondary somatic embryogenesis. Plant-Cell-Reports. 20 (12) : 1128-1134.
8. Mohamed-Anis., Mohd-Faisal. 2005. In vitro regeneration and multiplication of Psoralea corylifolia-an endangered medicinal plant. Indian- Journal- of – Biotechnology. 4 (2): 261-264.
9. Murashige, T., F.A. Skoog. 1962. A revised medium for rapid growth and biooassays with tobacco tissue cultures. Plant Physiol., 15: 473-479.
10. Pottino, B. G. 1981. Methods in plant tissue culture. Dept. of Hort., Agric. College, Maryland Univ., College Park, Maryland, USA, pp. 8-29.
11. Overbeek, J.V., M. E. Conklin, A.F. Blakeslee. 1941. Factors in coconut milk essential for growth and development of very young Datura embryos. Science 94: 350-352.
12. Steel, R. G., J. H Torrie. 1980. Principles and Procedures of Statistics, a Biomerical Approach. Mc Grow-Hill Book Company, New York, 469-517.
13. Ziv, M. 1992. The use of growth retardants for the regulation and acclimatization of in vitro plants. Karssen, C. M.; Loon, L. C. Van; Vregdenhil, D.(eds), Progress in Plant Growth Regulators, 809 - 817. Kluwer Academic publishers. Printed in the Netherlands.
14. Steward, F.C., E.M. Shantz. 1959. The chemical regulation of growth (some substances and extracts which induce growth and morphogenesis). Ann. Rev. Plant. Physiol. 10: 379-386.
15. Straus, J. 1960. Maize endosperm tissue grown in vitro : Development of a synthetic medium. Am. J Bot. 47: 641-646.
16. Zaid, Z.E., A. H. Gomaa., I.A. Ibrahim. 2004. In vitro growth and development of different somatic embryo shapes of some date palm genotype.The Second Intrnational Conference on Date Palm. 6 – 8 October, El-Arish, Egypt, 223 -242.

أستخدام المستخلصات الطبيعية فى مزارع أنسجة نخيل البلح

زينب السيد زايد[١] ، رحاب أحمد صدقى[١]، ممدوح أحمد الشامى[٢]

١. *المعمل المركزى للأبحاث وتطوير نخيل البلح – مركز البحوث الزراعية – جيزة*

٢. *معهد بحوث البساتين – مركز البحوث الزراعية – جيزة*

درست تأثير استخدام بعض المستخلصات الطبيعية مثل لبن جوز الهند و كازين هيدروليزات ومستخلص المولت على النمو فى المعمل لنباتات نخيل البلح صنف البرتمودا وذلك من خلال مرحلتين هى تكوين الكالس وإنتاج الأجنة الجسمية. زرعت القمة النامية على بيئة موراشيجى وسكوج المعدلة ومحتوية 1.5g/l A.C+3.0mg/l 2ip +10.0 mg/l 2,4-D ومضاف إليها مختلف تركيزات المستخلصات الطبيعية المستخدمة فى الدراسة التي تتراوح بين (١٠٠,٠و ٣٠٠,٠و ٥٠٠,٠ ملجم/لتر). تم تحضين الأجزاء النباتية فى الإظلام على درجة حرارة٢٧±١ لمدة ١٨ أسبوع وجد أن إضافة المستخلصات الطبيعية إلى بيئة إنتاج الكالس كان مؤثر جداً مقارنة بالبيئة الخالية منها. سجلت أقل قيمة لتلون الأجزاء النباتية باللون البنى عند استخدام كازين هيدروليزات أو مستخلص المولت (١,٧٧و ١,٩٩ على التوالى) بينما سجلت أعلى قيمة للتلون البنى فى البيئة الكنترول. لوحظ خلال مرحلة إنتاج الكالس أن إضافة لبن جوز الهند إلى البيئة أعطى أعلى نتيجة (٣,٦٦) مقارنة بإضافة كازين هيدروليزات ومستخلص المولت الذين سجلوا (٣,٥٥و ٢,٥٥ على التوالى). تم تسجيل أحسن ونفس النتيجة عندما أضيف ٣٠٠,٠ مج/لتر لبن جوز الهند أو ٥٠٠,٠ مج/لتر كازين هيدروليزات إلى بيئة إنتاج الكالس (٤,٦٦). أستمر الكالس فى النمو حتى تكونت حبيبات الكالس الجنينى ثم تم نقلها إلى بيئة الإنبات التى تحتوى على ٠,١ ملجم/لتر نفثالين أسيتك أسيد + ٠,٠٥ ملجم/لتر بنزيل أدينين و كذلك تركيزات المستخلصات الطبيعية موضوع الدراسة واستخدمها لتحسين إنتاج الأجنة الجسمية. بدء ظهور الأجنة الناضجة والمنبتة بعد حوالى ٦ أسابيع، حيث إن إضافة ١٠٠,٠ أو ١٥٠,٠ مج/لتر لبن جوز الهند إلى البيئة كان مؤثر فى زيادة و تحسين إنتاج الأجنة الجسمية الثانوية، أما أستخدام الكازين هيدروليزات بمختلف تركيزا ته كان غير مؤثر. تفوقت كل تركيزات مستخلص المولت فى أنتاج النبيتات الأكثر طولاً مقارنة بالتركيزات المستخدمة من لبن جوز الهند و الكازين هيدروليزات. حدثت زيادة فى الوزن الرطب وكذلك الوزن الجاف فى بيئات المستخلصات الطبيعية عنه فى البيئة الخالية منه. عموما إضافة المستخلصات الطبيعية إلى بيئة زراعة نخيل البلح معمليا تعمل على تحسين انتاج الكالس وسرعة النمو والتطور للأجنة الجسمية