# Micropropagation of some Egyptian date palm dry cultivars 1- Maturation of somatic embryos

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## ABSTRACT

*This study aimed to investigate the effect of adding different combinations of thiamine (5, 10, and 20 mg/l) and biotein (0, 1 and 2 mg/l) to culture medium on maturation of somatic embryos. Furthermore, the effect of MS-salt strength (¼, ½, ¾ and full strength) on maturation of embryos was investigated. Embryonic calli were obtained from shoot tips and primordial leaves of three dry date palm cultivars (Bartamoda, Sakkoty and Malkaby). Among the different treatments , a high frequency of mature embryos was proliferate from embryonic calli when cultured on ¾ MS + 10 mg/l 2,4-D + 3 mg/l 2iP + 10 mg/l thiamine HCL + 1mg/l biotein. This treatment gave 85.7 %, 92.5 % and 95.7% embryos with embryonic callus derived from shoot tip of Bartamoda, Malkaby and Sakkoty cultivars, respectively. However it was 58.7, 67.3 and 74.6 with those derived from primordial leaf explants of the three cultivars ,respectively. Then, the obtained embryos of the three cultivars were successfully developed to shoots when recultured onto hormone free of ¾ MS- salt medium.*

***Key words**: Date palm, somatic embryos, thiamine, biotein.*

## INTRODUCTION

Date palm tree is one of the most important horticultural crops cultivated in arid regions. The vegetative propagation in date palm is very limited due to their dioecious nature. Its propagation through seeds does not ensure true to type palms. Thus, the development of an *in vitro* rapid mass propagation system is a unique solution and a great advantage for quick clonal multiplication of superior date palm genotypes, to face the shortage of offshoots required for expansion of palm cultivated area (Ammar and Benbadis, 1977; Tisserat, 1979; Kackar *et al.*, 1989; Sudhersan *et al*. 1993 and Taha *et al.*, 2003). Plant tissue culture techniques are a very powerful tool, not only for quick clonal multiplication of superior cultivars, but also for elimination of disease transmission (Tisserat, 1979, Saker *et al.*, 1998; Bekheet and Saker, 1998; El-Kazzaz and El-Bahr, 2001; Bekheet *et al*., 2001 and Taha *et al*., 2003). Micropropagation of date palm through callus somatic embryogenesis pathways had been disclosed by several authors (Tisserat, 1979; Sharma *et al.*, 1984; Daquin and Letouze, 1988; Sudhersan *et al*., 1993 and Letouze *et al.*, 2000).

Plant cells in culture have requirements for vitamins; especially thiamine (B1), nicotinic acid, pyridoxine pantothenate, folate and biotein (H) (Gamborg and Shyluk, 1981).

Vitamins are required in trace amounts in plant tissue culture to enhance their growth (Torres, 1989). Thiamine is an important cofactor in carbohydrate metabolism, and biotein is important in carboxylation reaction (Al-Khayri, 2001). Thiamine and biotein biosynthesis pathways utilize the transfer of sulfur from cysteine to cofactor precursors (Begley, 1999).

This study aimed to investigate some factors affecting the *in vitro* maturation and multiplication of three Egyptian date palm dry cultivars.

## MATERIALS AND METHODS

### Plant materials

Female date palm *(Phoenix dactylifera* L.) offshoots of the three cultivars (Sakkoty, Malkaby and Bartamoda) were secured from Aswan in Upper Egypt. This region is considered as the best sources for excellent qualitative and good parameters of dry cultivars of date palm trees. Offshoots of the different identified cultivars were separated during fruiting stage from mother plants. The parameters of the offshoots were 100-125 cm in height, 30 cm in diameter and 30- 50 Kg in weight. These offshoots were used as mother plant material.

### Sterilization

After careful elimination of all leaves and adjourning tissues, the shoot tips and primordial leaf explants were excised from the offshoots. These explants were surface disinfected in 70 % ethanol for 1 min followed by immersion in 3 % sodium hypochlorite containing a few drops of Tween-20 with continuous stirring for 20 min. Then, these tissues were rinsed off using sterile distilled water four times and cultured individually on a culture containing initiation medium.

### Embryonic callus induction

Basal Murashige and Skoog (1962) medium (MS) supplemented with sucrose as energy source (30 g / l); phytagel (2.2 g/l) ; activated charcoal (3 g/l) ; myo-inositol (100 mg/l) ; 2,4-D (10 mg/l) ; 2iP (3 mg/l) ; $NaH_2Po_4$ (170 mg/l) and glutamine (200 mg/l) was used as a callus induction medium . Cultures were incubated in darkness and recultured onto the same fresh medium every six weeks. After three subcultures, white nodular embryonic calli were observed on the explants.

### Embryos formation

Obtained embryonic calli were transferred to MS basal medium containing 2iP (1.5 mg/l); 6-benzylaminopurine (BAP) (3 mg/l) and adenine sulfate (40 mg/l), and then incubated under cool light conditions (3000 Lux).

### Embryos maturation

To study the effect of thiamine and biotein, as additional vitamins, on maturation of the obtained embryos, different combinations of thiamine (5,10, and 20 mg/l) and biotein (0, 1 and 2 mg/l ) were added to the culture medium. The cultures were maintained for 12 weeks under light conditions (3000 Lux), and subcultured at the end of the 4 $^{th}$ week.

### *Shoots formation*

To investigate the effect of MS salt strength on shoot formation, hormone-free, 1/4, 1/2, 3/4 and full strength of MS-nutrient medium was used.

### Data analysis

These experiments were designed in a completely randomized design. The recorded data (fresh and dry weight of embryonic callus formation (%), no of embryos, embryo fresh and dry weight and embryo length (mm) as

well as number of shoots formed) were statistically analyzed using standard error (SE) according to the method described by Snedecor and Cochran (1980).

## RESULTS

### Embryonic callus induction

Effect of MS medium supplemented with sucrose as energy source (30 g/l), phytagel (2.2 g/l); activated charcoal (3 g/l) and myo-inositol (100 mg/l); 2,4-D (10 mg/l) ; 2iP (3 mg/l); $NaH_2Po_4$ (170 mg/l) and glutamine (200 mg/l) on callus production and its quality, from shoot tip and primordial leaf explants of the three cultivars are shown in Table (1). Responses of the two types of explants obviously varied depending on the cultivar. It was found that the percentage of callus production from shoot tip explants (98 % , 87 % and 64 %) were recorded with Sakkoty, Malkaby and Bartamoda cultivars, respectively (Fig.1). However, these values were 85%, 76 % and 55 % in case of primordial leaf explants with the three cultivars. On other hand, it was observed that Sakkoty cultivar gave the best quality characteristics of callus growth as compared with the other two cultivars.

***Table (1): Effect of MS medium supplemented with 2,4-D (10 mg/ l ) ; 2iP (3 mg/l) ;$NaH_2Po_4$ (170 mg/ l) and glutamine ( 200mg/ l) on callus production(%) and their quality from shoot tip and primordial leaves of Bartamoda, Malkaby and Sakkoty cultivars, after 120 days of cultivation.***

| Callus production (%) and their quality | Egyptian date palm cultivars | | | | | |
|---|---|---|---|---|---|---|
| | Bartamoda | | Malkaby | | Sakkoty | |
| | S.T. | P.L | S.T. | P.L | S.T. | P.L |
| Callus production | 64 ± 3.5 | 55±4.1 | 87±6. 8 | 76±4. 5 | 98±5.8 | 85±8.3 |
| Quality of callus | ++ | + | ++ | ++ | +++ | ++ |

Each treatment is the average of 10 replicates ± SE , +++ High quality ++ Medium quality + Low quality S.T. = Shoot tip, P. L. = Primordial leaves.

### *Embryos formation*

Date presented in Table (2) show the effect of transferring the obtained nodular calli on MS basal medium containing 2iP (1.5 mg/l); BAP (3 mg/l) and adenine sulfate (40 mg/l) on the percentages of embryo formation and its quality characteristics during three subcultures. The maximum percentage of embryo formation was recorded with embryonic calli derived from of Sakkoty, Malkaby and Bartamoda cultivars, respectively. This result was observed with callus of the two types of explants during the three subcultures. Embryonic calli derived from the shoot tip explants of the different date palm cultivars recorded high percentages of embryo formation as compared with primordial leaf explants. The descending order of the embryo formation percentages was 36 %, 27 % and 24 % after the first subculture and 58 %, 42 % and 35 % after the second subculture, while it was 74 %, 55% and 42 % after the third subculture for shoot tip explants of Sakkoty, Malkaby and Bartamoda cultivars, respectively. In case of the primordial leaf calli, it was 25%, 17 % and 12 % after the first subculture and 40 %, 29 % and 23 % after the second subculture. However, it was 61 %, 45 % and 40 % after the third subculture with Sakkoty, Malkaby and Bartamoda cultivars, respectively. Concerning the quality charact-eristics of the obtained embryos, they were observed with calli derived from shoot tip explants of Sakkoty, Malkaby and Bartamoda, respectively as compared with those embryos derived from primordial leaf explants.

***Table (2):Effect of MS-medium supplemented with 2iP (1.5 mg/ l); BAP(3mg/l) and adenine sulfate (40 mg / l) on percentage of embryo's formation and their quality of embryonic calli derived from shoot tip and primordial leaves explants of Bartamoda ,Malkaby and Sakkoty cultivars during three subcultures.***

| Embryos formation (%) and their quality | Egyptian date palm cultivars | | | | | |
|---|---|---|---|---|---|---|
| | Bartamoda | | Malkaby | | Sakkoty | |
| | S.T | P.L | S.T | P.L | S.T | P.L |
| | A. After 1 st subculture | | | | | |
| Embryos formation (%) | 24±2.15 | 12±1.95 | 27±2.33 | 17±1.19 | 36±1.85 | 25±2.15 |
| Quality | + | + | ++ | + | +++ | ++ |
| | B. After 2 nd subculture | | | | | |
| Embryos formation (%) | 35±2.18 | 23±3.18 | 42±5.13 | 29±2.30 | 58±3.20 | 40±4.52 |
| Quality | ++ | + | ++ | ++ | +++ | ++ |
| | C. After 3 rd subculture | | | | | |
| Embryos formation (%) | 42±3.22 | 40±3.35 | 54±2.18 | 45±3.15 | 74±5.63 | 61±4.28 |
| Quality | ++ | ++ | ++ | ++ | +++ | ++ |

Each treatment is the average of 10 replicates ± SE: +++ High quality ++ Medium quality + Low quality S.T. = Shoot tip , P. L. = Primordial leaves.

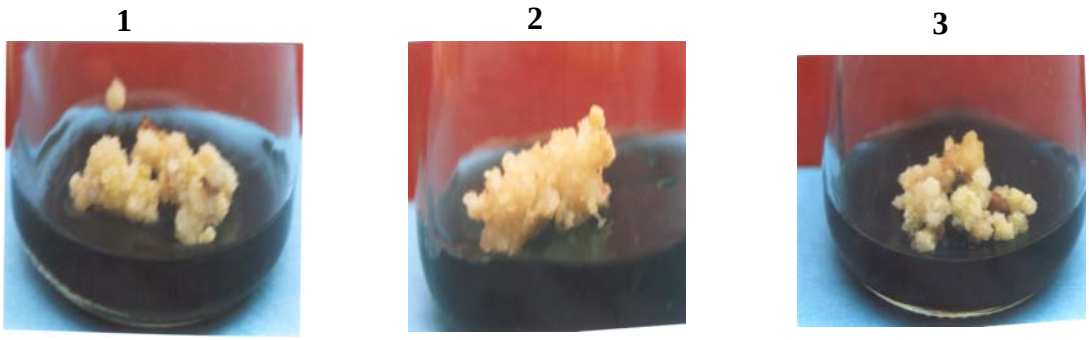


***Fig.(1):Embryonic calli derived from shoot tip explants of Sakkoty (1), Malkaby(2) and Bartamoda (3) cultivars, after 120 days of cultivation.***

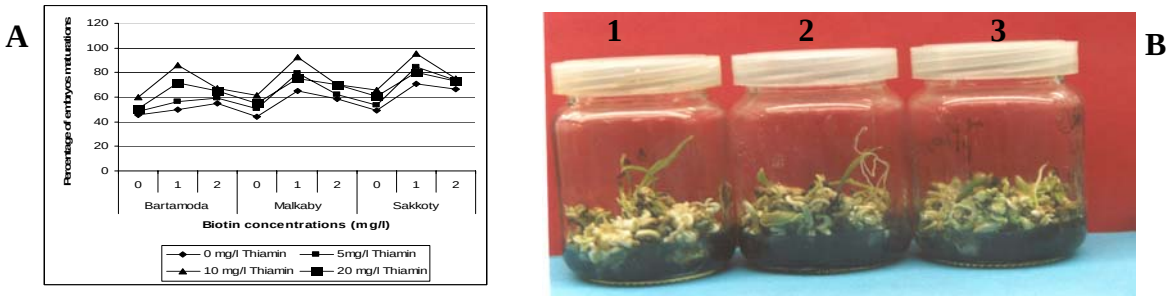


***Fig. (2-A).:Effect of thiamine (5, 10, and 20 mg/ l ) and biotein (0, 1 and 2 mg/ l) on the percentage of embryos maturation derived from shot tip explants of Bartamoda (1), Malkaby (2) and Sakkoty (3) cultivars.***

***Fig. (2-B):Embryos maturation derived from shoot tip explants of Bartamoda (1), Malkaby (2) and Sakkoty (3) cultivars.***

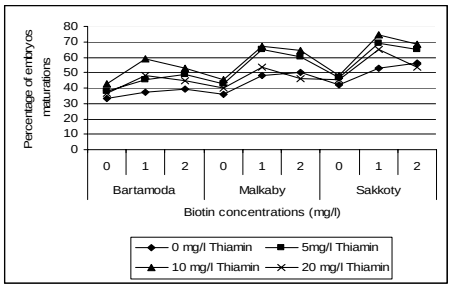


***Fig. (3): Effect of thiamine (5,10, and 20 mg/ l) and biotein (0, 1 and 2 mg/l) on percentage of embryos maturation derived from primordial leaf explants of Bartamoda (1), Malkaby (2) and Sakkoty (3) cultivars.***

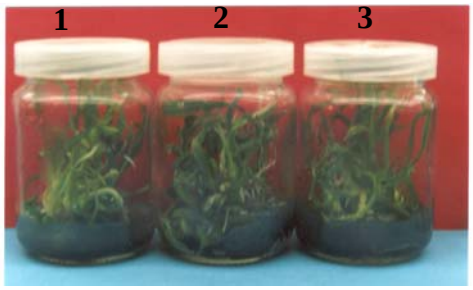


***Fig. (4): Shoots formation from embryo derived form shoot tip explants of Bartamoda Malkaby and Sakkoty (from lift to right) cultivars.***

### *Embryos maturation*

Data illustrated in Figs. (2 A, B and 3) show the effect of addition of different combinations of thiamine (5,10, and 20 mg/ l) and biotein (0, 1 and 2 mg/l) to culture medium on percentage of embryo maturation derived from shot tip explants (Figs 2-A and 4) and primordial leaf explants (Fig. 3) of Bartamoda, Malkaby and Sakkoty date palm cultivars. It was noticed that a high frequency of mature embryos was proliferated from embryonic calli when MS medium provided with 10 mg/l thiamine HCL + 1 mg/l biotein This treatment gave 85.7 %, 92.5 % and 95.7% with embryos derived from shoot tip explants of Bartamoda, Malkaby and Sakkoty cultivars, respectively. However, it was 58.7, 67.3 and 74.6 with those embryos derived from primordial leaf explants of Bartamoda, Malkaby and Sakkoty, respectively.

### *Shoot formation*

Data in Table (3) and graphically illustrated in Fig. (4) show that 3/4 MS-salt strength medium gave the highest percentage of shoot formation 95 %, 90 % and 83 % derived from shoot tip explants of Sakkoty , Malkaby and Bartamoda date palm cultivars , respectively. However, it was 82 %, 75 % and 70 % of shoots derived from primordial leaf explants of the three date palm cultivars, respectively.

***Table (3):Effect of 1/4 , 1/2, 3/4 and full strength of MS-medium on percentage of shoot formation from embryo derived from shoot tips and primordial leaf explants of Bartamoda, Malkaby and Sakkoty cultivars.***

| MS-salt medium | Percentage of shoot formation | | | | | |
|---|---|---|---|---|---|---|
| | Bartamoda | | Malkaby | | Sakkoty | |
| | S.T | P.L | S.T | P.L | S.T | P.L |
| ¼ Strength | 48 | 44 | 54 | 52 | 63 | 54 |
| 1/2 Strength | 60 | 55 | 65 | 58 | 72 | 67 |
| 3/4 Strength | 83 | 70 | 90 | 75 | 95 | 82 |
| Full Strength | 76 | 64 | 81 | 72 | 84 | 75 |

Each treatment is the average of 10replicates ± SE S.T. = Shoot tip, P. L. = Primordial leaves

## DISCUSSION

From the present results, it is clear that embryonic calli and embryos formation were obtained form the two types of explants. However, shoot tip explants gave the highest values of both embryonic calli and embryos formation, compared with the primordial leaves of the different date palm cultivars. This may be due to their meristematic tissues. In this respect, shoot tip explants of date palm were successfully used for either embryonic callus induction or embryos formation by several authors (Tissert, 1979; Zaid and Tisserat, 1983; Sharma *et al.,* 1984; El-Kazzaz and El-Bahr, 2001 and Taha *et al*., 2003). Concerning the effect of vitamins on embryos maturation, it was reported that the exogenous vitamins such as thiamine and biotein are essential for both callus initiation and induction (Gamborg and Shyluk 1981; Kumra and Chopra, 1982 and Al-Khayri, 2001).The date palm was found to be capable of plantlets regeneration from several tissues. In this investigation, it was found that 3/4 of MS-salt stress medium gave the best results. The obtained results are in accordance with those reported by many authors (Sharma *et al*., 1984; Kackar *et al*., 1989; Veramendi and Navarro, 1996; Bekheet *et al*., 2001 and Taha *et al*.,2003).

In conclusion, this investigation highlighted the *in vitro* propagation of Egyptian date palm dry cultivars Bartamoda, Sakkoty and Malkaby. It is important to clarify the effect of vitamins (thiamine and biotein) supplementation to the MS-salt strength medium on enhancement of mature embryos formation and elongation of the plantlets. It was found that ¾ MS-medium + 2,4-D (10 mg/l) + 2iP (3 mg/l) + thiamine HCL (10 mg/l) + biotein (1 mg/l) is the most suitable medium for embryos maturation and shoot proliferation. Sakkoty cultivar gave the best results than Bartamoda and Malkaby date palm cultivars.

## REFERENCES

**Al-Khayri, J. M. (2001).** Optimization of biotein and thiamine requirements for somatic embryogenesis of date palm (*Phoenix dactylifera* L.). In vitro Cell. Dev. Biol., Plant., 37: 453-456 .

**Ammar, S. and Benbadis, A. (1977).** Vegetative propagation of date palm (*Phoenix dactylifera L.*) by tissue culture of young plants derived from seeds. Compte Rendu Hebolomadaires de l'Acad. des Sci., D.28:1787.

**Begley, T. P.; Kinsland, J. Xi, C.; Taylor, S. and MeLafferty, F. (1999).** The enzyme-ology of sulphur activation during thiamine and biotein biosynthesis. Curr., Opinion Chem., Biol., 3, 623-629.

**Bekheet, S.A. and Saker, M.M. (1998).** *In vitro* propagation of Egyptian date palm: II-Direct and indirect shoot proliferation from shoot tip explants of *Phoenix dactylifera* L. cv. Zaghloul. The First International Congress on Date Palms, Al-Ain, United Arab Emirates, March 8-10.

**Bekheet, S. A.; Saker, M.M.; Taha, H. S. and Moursy, H.A. (2001).**Plant regene-ration *via* somatic embryogenesis in date palm (*Phoenix dactylifera* L.). Arab J. Biotech., 4 (1): 111-118.

**Daquin, F. and Letouze, R. (1988).** Regeneration of date palm (*Phoenix dactylifera* L.) by somatic embryogenesis, improved effectiveness by dipping in a stirred liquid medium. Fruits. 43:191-194.

**El-Kazzaz, A.A. and El-Bahr, M.K. (2001).** A method for *in vitro* propagation of the Egyptian date palm cultivar Samany Arab J. Biotech., 4 (2) 285-292.

**Gamborg, O. L. and Shyluk, J. P.(1981).** Nutrition, media and characteristic of plant cell and tissue culture. In: " Plant Tissue Culture: Methods and Applications in Agriculture". (T.A.Thorpe, ed), pp 21-44. Academic Press, New York.

**Kackar, L.; Solanki, R. and Joshi, P. (1989).** Micropropagation of date palm cv.Khadrawy using tissue culture technique. Indian Annals of Arid-Zone, 28: 1-2, 137-141.

**Kumra, P.K. and Chopra, R.N. (1982).** Effect of some growth substances vitamins and ultraviolet radiation on callus induction in the moss *Bryum coronatum* Schwagr. *Z.* Pflanzenphysiol. 108:143-150.

**Letouze, R.; DAquin, F.; Hamama, L.; Paquier, K.; Marionner, F. and Javouhey, M. (2000).** Mass propagation of date palm (*Phoenix dactylifera* L.) through somatic embryogenesis. Hisological study of embryo formation and cultivar identify-cation by RAPD markers. In: Proceeding of the date palm international symposium , Windhoek, Namibia. pp55-64.

**Murashige, T. and Skoog, F. (1962).** A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol., Plant, 15:473-497.

**Saker, M. M.; Moursy, H.A. and Bekheet, S. A. (1998).** *In vitro* propagation of Egyptian date palm: I Morphogenic responses of immature embryos. Bull.Fac.Agric., Univ. Cairo 49:203-214.

**Sharma, D.R.; Dawra, S. and Chowdury, J. B. (1984).** Somatic embryogenesis and plant regenartion in date palm (*Phoenix dactylifera* L. C.v khadrawi) through tissue culture. Indian Journal of Experiments Biology, 22: 596-598.

**Snedecor, G. W. and Cochran, W.G. (1980):** "Statistical Methods", 7th ed. Iowa State Univ. Press, P. 504.

**Sudhersan, C.; Abo El-Nil, M. and Al-Baiz, A. (1993).** Occurrence of direct somatic embryogenesis on the sword leaf of *in vitro* plantlets of *Phoenix dactylifera* L. cultivar Barhee. Current Science, 65: 887-888.

**Taha, H. S.; Bekheet, S.A. and El-Bahr, M.K. (2003).** Alternative approach for micropropagation of the date palm (*Phoenix dactylifera* L. c.v. Zaghlool). Arab J. Biotech., 6 (1): 103-112.

**Tisserat, B. (1979).** Propagation of date palm (*Phoenix dactylifera* L.) *in vitro*. J. Experimental Botany, 30: 1275-1283.

**Torres, K.C. (1989).** Tissue culture media-composition and preparation. In: Tissue Culture Techniques for Horticulture Crops. Torres, K.C. (ed.), pp. 26-51. An AVI Book, New York.

**Veramendi, J. and Navarro, L. (1996).** Influence of physical conditions of nutrient medium and sucrose on somatic embryo-genesis of date palm. Plant Cell Tiss., Organ Cult., 45, 159-161

**Zaid, A. and Tisserat, B.(1983).** Morpho-genetic responses obtained from a variety of somatic explants tissues of date palm. Botanical Magazine, 96:67- 73.

## الملخص العربي

# الإكثار المعملي لثلاثة أصناف جافة من نخيل البلح المصري: ١- إنضاج الأجنة الجسدية

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تهدف هذه الدراسة الى بحث تاثير إضافة تركيزات مختلفة من الثيامين(٥ و١٠ و٢٠ ملليجرام/ لتر) و البيوتين (صفر ، ١ و ٢ ملليجرام/ لتر) إلى البيئة المغذية على إنضاج الأجنة الجسدية . و درس كذلك تأثير تركيز أملاح بيئة موراشيج و سكوج المغذية (٤/١ ، ٢/١، ٤/٣ و المكتملة ) على إنضاج الأجنة الجسدية . تم الحصول على الكالوس الجنينى من القمم النامية و الأوراق الأولية لثلاثة أصناف جافة لنخيل البلح المصري هى البرتمودا ، السكوتى و الملكابى. تم الحصول على أعلى نسبة من الأجنة الجسدية الناضجة من أجنة الكالوس الجنينى عندما تم زراعتها على ¾ من قوة الأملاح لبيئة موراشيج و سكوج المغذية + ١٠ ملليجرام/ لتر داى كلورو فينوكسى حمض الخليك + ٣ ملليجرام/ لتر ٦,٦ الفا ميثيل امينو بيورين + ١٠ ملليجرام/ لترثيامين + ١ ملليجرام/ لتر بيوتين ، حيث أعطت هذه المعاملة نسبة ٨٥,٧ % ، ٩٢,٥ % و ٩٥,٧% من الأجنة الجسدية من الكالوس الجنينى الناتج من القمم المرستيمية من أصناف البرتمودا ، الملكابى و السكوتى على الترتيب ، بينما كانت النسبة ٥٨,٧ %، ٦٧,٣ % و ٧٤,٦ من تلك الناتجة من الأوراق الأولية من أصناف البرتمودا ، الملكابى و السكوتى على الترتيب. و تطورت الأجنة الجسدية الناتجة إلى نموات خضرية عندما زرعت على ¾ تركيز لأملاح بيئة موراشيج- سكوج المغذية بدون إضافات هرمونية. و استنتج من هذه الدراسة إمكانية الإكثار الخضري الدقيق (*in vitro*) لأصناف البلح المصري الجاف و كذلك أهمية إضافة بعض الفيتامينات للحصول على نتائج ناجحة.