EFFECT OF BASAL SALTS AND SUCROSE CONCENTRATIONS ON MORPHOGENESIS IN TEST TUBES OF FEMALE INFLORESCENCE OF DATE PALM (*PHOENIX DACTYLIFERA* L.) ZAGHLOUL CV

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Abstract

Date palm organized tissues (vegetative buds and somatic embryos) were formed directly from female inflorescence. In this study, some of induction factors like carbon source and salt strength were examined for establishing organized structures. Different growth stages for the spikelet explants (2.5 and 7.0) which derived from the female inflorescence of date palm were used based on the initial length of spikelet explants. Therefore, the combined effect of different sucrose concentrations (20, 30, 40 and 50 g l-1) and varies powers of Murashige and Skoog (MS) ¼, ½, ¾ and full strength were utilized as well. The obtained results revealed that the percentage of shoot formation of intermediate date palm spikelets (7.0 cm in length) was increased when the basal nutrient medium included 50.0 gm l-1 sucrose as a source of carbon instead of the usual concentration (30.0 gm l-1) and the full concentration of MS basal salt which approved that it is the suitable salt strength more than other dilutions of MS. Subsequently, the shoot formation percentage was 33.0 %. Regarding induction of embryos whether directly or indirectly, the success percentage was increased from 53.0 % to 73.0 % when the basal nutrient medium of date palm spikelets at early stage (2.5 cm in length) was involved ¾MS and 40.0 gm l-1 sucrose. Finally, using inflorescence explants in tissue culture technique of date palm is solving many problems encounter such technique.

Key words: Tissue culture, inflorescence, organogenesis, date palm, and flower.

INTRODUCTION

Biodiversity in date palm plants all over the world produced so many individuals. These individuals in sometimes were amazing. Mostly there are no offshoots beside these superior lines. The need to maintain these superiors lead us to looking for an alternative method instead of offshoots.

The micropropagation of date palm by an inflorescence as a valuable explant is very important. It would produce new plants without destroying the mother plant. Particularly, mother plants can't produce offshoots when they are in fruiting stage. As well as, the new lines of date palm that produced from culturing the seeds and at the same time have characteristics more desirable than cultivated palms.

Moreover, many research considered with selection and evaluation programs for male palms. The success of these programs was restricted to present offshoots

around the trunk, for subsequent traditional propagation. But most of the vigorous males were individual. Inflorescence explant was the best solution to micropropagate these males.

Utilizing inflorescence explants as a source for tissue culture technique are very promising. Regarding benefits related to in vitro culture, bacterial and fungal contaminations were greatly reduced. Because of the enclosed sheath of the inflorescence (spathe cover) protected it. Secondly, the abundance of meristematic tissues within the inflorescence is existed. These meristematic tissues have morphogenetic potential to differentiation. Thirdly, production organized tissues whether somatic embryos or vegetative buds directly form the explants was occurred in a short time. After 4.5 months in culture as recorded by Abo-El-Soad *et al.*, 2004. Some researches concern with inflorescence of date palm. But a few of these investigation has been succeeded. Since 1973, several workers attempted to culture palm inflorescences. Explants of female and male oil palm inflorescences were cultured on a variety of media and usually developed somewhat normally, but callus was not obtained (Smith and Thomas, 1973). Date palm inflorescence culture was also largely investigated by Drira and Benbadis (1985). Morphogenetic responses were found dependent on the origin and physiological stage of the explant.

Bhaskaran and Smith (1992, 95), reported that immature floral buds showed various responses. Some enlarged in size and produced root-like structures from the carpels. Others turned tan and died. But several of them enlarged and formed callus from the carpels which subsequently produced embryogenic callus indistinguishable from the embryogenic callus from the shoot tip. The response of cultures in the light was not significantly different from those in the dark in terms of callus development. Embryogenic callus was white, had a granular appearance and was friable.

Meristematic capabilities of inflorescence spikelets of date palm mainly depend on the age of explant. In other wise, the developmental stage of spikelet meristems plays an important role in organogenesis. Abo-El-Soad *et al.*, (2004) reported that inflorescence meristems of female date palm produced directly vegetative buds. However, about 53 % of explants were able to alternate from floral case to vegetative case. Also, Abo-El-Soad *et al.*, (2005) stated that most of the florets meristems induced somatic embryos. In some cases, somatic embryos formation associated with minimal callus formation.

There are some factors could be affect the process of vegetative buds or somatic embryos formation. This paper aimed to study the effect of basal salt strength and sucrose concentration in the nutrient media on morphogenesis of date palm florets.

MATERIALS AND METHODS

The cloned material of *Phoenix dactylifera* L. (Zaghloul cv.) was secured from female palms grown at the western farm of Agriculture Faculty, Cairo University. This cultivar is commonly known as the one of the important soft cultivars in Egypt.All explants were taken from adult female trees of approximately 4-5 meter in height. Only the outer inflorescence of adult female tree was used.

The mature leaves (fronds) were removed acropetally with the aid of a hatchet and tapestry knife. When the first inflorescence was appeared in the axil of the leaf, it was carefully removed with its protective sheath (spathe) intact with a clean knife and placed in an antioxidant solution made up of 150 mg citric acid and 100 mg ascorbic acid per liter of sterilized water.

Inflorescence was taken immediately in the antioxidant solution to the laboratory and washed with detergent powder (Rabso) under running tap water for 15 minutes. After rinsing the spathe with running tap water, the spathe was sterilized with mercuric chloride ($HgCl_2$) at 0.1% for 5 min (Abo-El-Soad *et al.*, 2004). under free contaminants conditions and then rinsed three times with sterilized distilled water.

The inflorescence, after sterilizing the spathe, was removed from the sheath and the spikeletlets were cut into 1-2 cm long pieces or distinctly cultured if the spikeletlets are less than 2 cm in length. Spikelets were used in two ages (2.5 and 7 cm in length). Each spikelet carries many florets (the initials of female flowers). All explants were cultured onto the different treatments of experiments conducted through starting stage.

Media preparation:

The basal nutrient medium employed throughout this study contained Murashige & Skoog (MS) inorganic salts (1962) supplemented with (in mg l^{-1}):

0.5 nicotinic acid; 0.5 pyridoxine-HCl; 1.0 thiamine-HCl; 100.0 myo-inositol; 2.0 glycine; 6 000.0 agar (win-lap.) and 30 000.0 sucrose (except sucrose experiment). After preparation of the medium, the pH was adjusted to 5.7 ± 0.1 before the autoclaving. Media were dispensed into small culture vessels (150 ml-small jar) in aliquots of 35 ml per jar and were capped with polypropylene closures. Media were then autoclaved for 20 minutes at 1.11 Kg/Cm^2 and 121°C.

Culture conditions:

Ambient conditions of growing mature date palm tress in the groove were recorded through February and March months of the first year of study.

Average-temperature degrees (high and low °C, respectively) and relative humidity (high and low %, respectively) were measured during 6-period intervals (60 days) of season 2000, according to Dokky Station of Meteorological (area where the inflorescence source is grown).

Month / Element	February			March		
	1-10	11-20	21-29	1-10	11-20	21-31
Temperature	20 ; 9	21 ; 9	20 ; 9	22 ; 8	26 ; 11	27 ;13
Humidity	91 ; 45	90 ; 66	91 ;37	75 ; 21	75 ; 21	74 ;17

In vitro cultures were incubated under darkness in a temperature-controlled room at 27 ± 2 °C. Data collection and re-culturing were performed at 6 weeks intervals. During this stage, many observations were taken and contaminated explants or ceased growths were discarded. Those that were viable and showed indications of response were selected for further subculture.

Effect of MS salt strength and sucrose concentrations on growth and development of date palm spikelets of Zaghloul cultivar was studied. Different combinations of MS salt strength (¼, ½, ¾, and 1 full strength) in combination with varies sucrose concentrations (20, 30, 40 and 50 gm l^{-1}), in addition to the suitable PGRs concentration [2.5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} IBA + 0.2 mg l^{-1} 2ip] and 6 g l^{-1} agar (win-lap). All cultures were incubated for 4.5 months (through three subcultures) in growth room under the previous culture conditions with the effect of PGRs. Also, the same data were taken, after the third subculture.

RESULTS AND DISCUSSION

The Influence of MS salt strength and sucrose concentrations on shoot and somatic embryo formation percentages of date palm spikelets cv. Zaghloul (2.5 cm in length), after 18 weeks in culture (throughout 3 subcultures) is shown in Table 1. Data showed that increasing the salt strength steadily increased the mean value of shoot formation percentage. Hence, they were 5.0; 5.0; 13.3 and 20.1 (%) when MS concentrations were ¼; ½; ¾ and full strength of MS, respectively. On the other hand, using 50 gm l^{-1} sucrose in the basal nutrient medium encouraged the shoot formation, where the mean value of shoot formation percentage was 18.3 %. The interaction effect between MS salt strength and sucrose concentrations gave a significant difference. The best result was obtained when the basal medium included full strength of MS and 50 gm l^{-1} sucrose, where the shoot formation percentage was 27.5 %.

The concentration of dissolved substances, especially macro- and micronutrients and sucrose determines the osmotic potential of the culture medium of date palm spikelets. It is considered an important factor known to influence growth and morphogenesis of spikelet explants. Selection of an appropriate concentration of salts and sugar can be important and differs between different genotypes, sometimes even between those which are closely related. Shoots of spikelet explants formed rapidly when cultured onto nutrient medium containing full strength of MS (20.1%). This result is in agreement with Pierik *et al.* (1988), who reported that single node

cultures of *Syringa vulgaris* elongated and multiplied most rapidly when grown on a medium with MS salts or 1.25 × MS salts. On contrary, this result is disagree with Mekers (1977) who reported that shoot cultures of some Bromeliads showed increased survival in Stage I and an improved rate of multiplication in Stage II when MS salts were used at a quarter or half strength. With respect to sugar concentration Bergmann (1967) declared that only a limited number of plant cell lines have been isolated which are autotrophic when cultured *in vitro*. Autotrophic cells are capable of fully supplying their own carbohydrate needs by carbon dioxide assimilation during photosynthesis. The optimum concentration of sucrose of spikelets explants to induce morphogenesis or growth is necessary. Addition 50 gm l^{-1} sucrose promoted the soot formation of spikelets when compared to usual concentration (30 mg l^{-1}).

With respects to embryo formation, these data ensure the previous data were obtained with the effect of inflorescence age. Subsequently, the embryo formation was better than shoot formation in this age of inflorescence. So, 73.0 % of date palm spikelets at length 2.5 cm formed embryos when the basal nutrient medium containing ¾MS and 40 gm l^{-1} sucrose, as an interaction effect. Most of these embryos formed through indirect somatic embryogenesis. Where, the spikelets in this early age tend to form white friable callus (Fig. 1) after 18 weeks in culture. Regarding the effect of MS strength, data explored that utilizing ¾MS was the best result when compared to other MS strengths. Also, increasing the sucrose concentration in the basal nutrient medium from 20 to 40 (gm l^{-1}) rising the embryo formation percentage from 35.1 to 53.3 (%). Elevating the sucrose concentration more than 40 g l^{-1} reduced the embryo formation. From the previous results, the best concentration of sucrose of shoot formation (50 gm l^{-1}) differed about the suitable

concentration of sucrose appropriate to induce somatic embryos (40 gm l^{-1}). This result is in harmony with George (1993) who reported that sucrose levels in culture media which result in good callus growth manner, not is optimal for morphogenesis and either lower or higher levels may be more effective. However, Asaka *et al.* (1994) produced a lot of ginseng (*Panax ginseng*) embryoids by culturing the embryogenic tissue on 100 gm l^{-1} sucrose; it was about ten times of that produced by culturing on 30 gm l^{-1} sucrose.

Also, it was noticed presence of direct pro-embryos (Fig. 2) at this age on the most combinations of MS and sucrose concentrations.

The Influence of MS salt strength and sucrose concentrations on shoot and somatic embryo formation percentages of date palm spikelets cv. Zaghloul (7.0 cm in length), after 18 weeks in culture (throughout 3 subcultures) is displayed (Table 2). Data show that, shoot formation percentage was increased by increasing the MS salt strength, where the mean values of shoot formation were 8.3; 10.0; 14.8 and 25.1 (%) for ¼; ½; ¾ and full strength of MS salts, respectively. On the other side, there are significant differences among various sucrose concentrations, and the mean value of shoot formation was increased by increasing the sucrose concentration in the basal

nutrient medium. In addition, the interaction effect between salt strength and sucrose showed significant differences. Hence, the highest shoot formation was gained with inclusion of full strength of MS salt and 50 gm l^{-1} sucrose in the nutrient medium. Where, the shoot formation percentage was 33.0 %. It is observable that, the shoot formation in this age was increased than the early stage of spikelets growth and development.

Regarding embryo formation, reducing the MS salt strength lower than ¾MS reduced the induction of pro-embryos. Where, the mean value of embryo formation decreased from 41.5 % when the basal nutrient medium supplemented with ¾MS to 26.8 and 16.5 (%) when the basal nutrient medium was involved ½MS and ¼MS salts, respectively. It is noticed that, the reverse of shoot formation was found with embryo formation, since the embryo formation was relatively decreased in this age compared to embryo formation in the formerly age (spikelets at length 2.5 cm). But most of spikelets in this middle age of growth and development (7.0 cm) incline to induce pro-embryos directly. Also, direct conversion of reproductive meristems into distinct somatic embryos that proceed their growth and germinated into a green shoots (Fig. 3). On the other hand, the embryo formation was increased by increasing the sucrose concentration in the nutrient medium from 20 gm l^{-1} to 40 gm l^{-1}. However, there was a significant difference among different combination of MS salt strength and sucrose concentration. The highest percentage was occurred onto the nutrient medium containing ¾MS with 40 gm l^{-1} sucrose where the percentage of embryo formation as a total was 53.0 %. From the previous results the combined effect between salts concentration and sucrose concentration simultaneously in the nutrient medium is important. Where, Gamborg *et al.* (1974) reported that the uptake of inorganic ions can be dependent on sugar concentration and the benefit of adding increased quantities of nutrients to a medium may not be apparent unless the amount of sugar is increased at the same time.

Generally, when we compare the results of basal salts and sucrose concentration to the obtained results of the age of date palm spikelets, it was found that determination the optimal concentration of the main constituents of the basal nutrient medium is important and the same time increased the total percentage of success. Since, the only 20.0 % of date palm spikelets at length 7.0 cm were able to induce shoot formation (Table 2). This percentage was increased when the basal nutrient medium included 50.0 gm l^{-1} sucrose as a source of carbon instead of the usual concentration (30.0 gm l^{-1}) and the full concentration of MS basal salt which approved that it is the suitable salt strength than other dilutions of MS. Subsequently, the shoot formation percentage was 33.0 % (Table 2). Therefore, if we investigate more related factors of date palm-inflorescence micropropagation, we could increase the success percentage.

Regarding induction of embryo formation whether directly or indirectly, the success percentage was increased from 53.0 % to 73.0 % (Table 1) when the basal

nutrient medium of date-palm spikelets at length 2.5 cm was composed of ¾MS and 40.0 gm l^{-1} sucrose.

At the final, using floral parts of date palm tree as explants is wonderful and promising on the commercial level. Depending on spikelet explants which derived from date palm inflorescence considered as a breakthrough in micropropagation of date palm. Because of it overcame the most obstacles which encounter such technique in vitro. For instance, bacterial contamination and long time of starting stage. In addition to it maintains the desirables of individual trees whether female or male plants. Consequently, increasing the number of high demand varieties. But the investigation needs to focus on histological aspects for more explanations about the direct somatic embryo development.

Table 1. Influence of MS salt strength and sucrose concentrations on % explants formed shoots and somatic embryos of date palm spikeletlets cv. Zaghloul (2.5 cm in length), after 18 weeks in culture (throughout 3 subcultures).

(A) Salt strength	(B) Sucrose (gm l^{-1})									
	Shoot formation (%)					Embryo formation (%)				
	20	30	40	50	Mean (A)	20	30	40	50	Mean (A)
¼MS	0.0	7.0	0.0	13.0	5.0 c	27.5	20.0	27.5	27.5	25.6 c
½MS	0.0	7.0	0.0	13.0	5.0 c	33.0	40.0	53.0	53.0	44.8 b
¾MS	7.0	13.0	13.0	20.0	13.3 b	40.0	53.0	73.0	60.0	56.5 a
MS	13.0	20.0	20.0	27.5	20.1 a	40.0	53.0	60.0	33.0	46.5 b
Mean (B)	5.0 d	11.8 b	8.3 c	18.3 a		35.1 c	41.5 b	53.3 a	43.4 b	

Each treatment contains 45 explants. Means followed by the same letters aren't significantly different. L.S.D at 0.05 AB 2.3 3.7

Table 2. Influence of MS salt strength and sucrose concentrations on % explants formed shoots and somatic embryos of date palm spikelets cv. Zaghloul (7.0 cm in length), after 18 weeks in culture (throughout 3 subcultures).

(A) Salt strength	(B) Sucrose (gm l^{-1})									
	Shoot formation (%)					Embryo formation (%)				
	20	30	40	50	Mean (A)	20	30	40	50	Mean (A)
¼MS	0	13	7	13	8.3 c	20	13	20	13	16.5 c
½MS	7	7	13	13	10.0 c	20	27.5	27.5	33	26.8 b
¾MS	13	13	13	20	14.8 b	33	40	53	40	41.5 a
MS	20	20	27.5	33	25.1 a	27.5	53	40	33	38.4 a
Mean (B)	8.3 c	10.0 c	14.8 b	25.1 a		25.1 c	33.4 a	34.9 a	29.8 b	

Each treatment contains 45 explants. Means followed by the same letters aren't significantly different. L.S.D at 0.05 AB 2.2 2.7

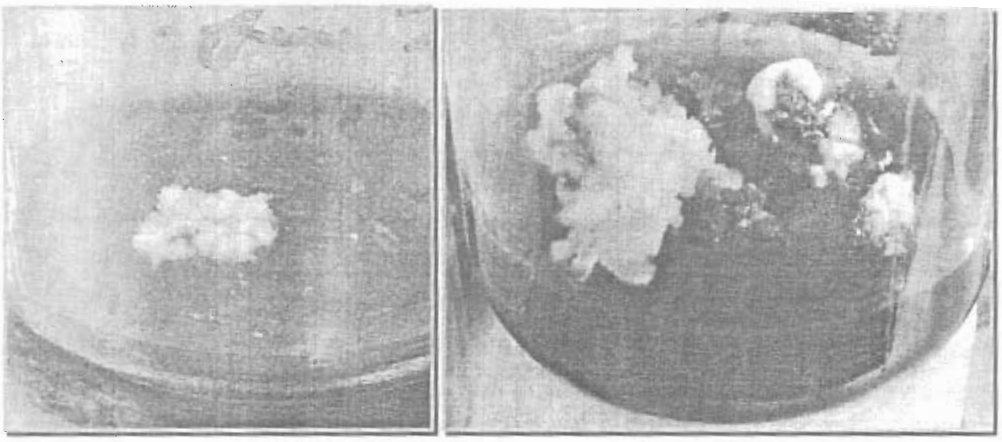

Fig. 1 White friable callus derived from spikelet explants of date palm after 4.5 months in culture

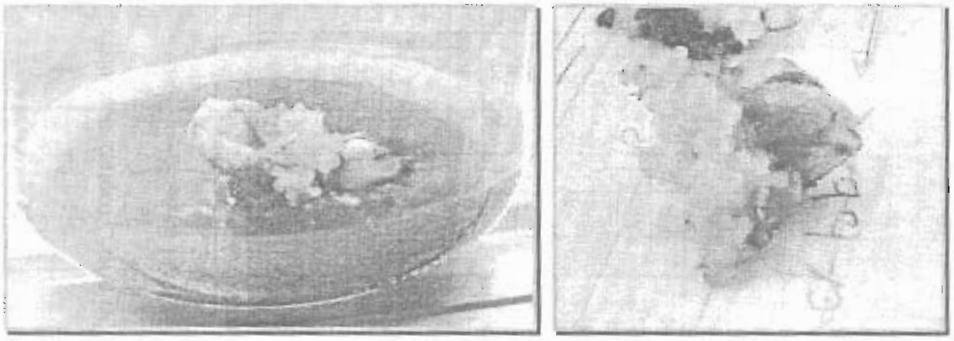

Fig. 2 Direct pro-embryos progenies from basal parts of spikelet explants of date palm (2.5 cm in length), after 4.5 months in culture.

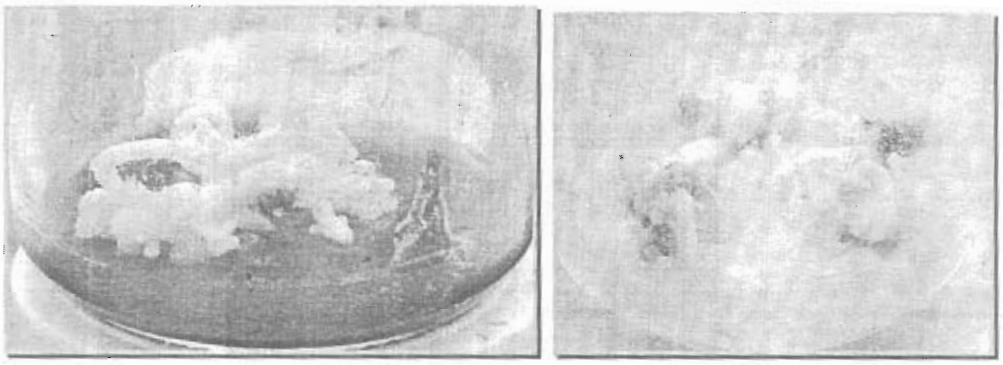

Fig. 3 Differentiated somatic embryos from date palm florets (Left photo), and green shoots on free-hormone medium (Right photo).

REFERENCES

1. Abo-El-Soaud, A. A., N. R. El-Sherbeny and E. I. Bakr. 2004. Organogenesis in female inflorescence of date palm *(Phoenix dactylifera* L. cv. Zaghloul*).*International Conference on Date Palm. 6-8 October, College of Agriculture and Environmental Sciences, Sues Canal University, North Sinai, Egypt. Pp 139-163
2. Abo-El-Soaud, A. A., N. R. El-Sherbeny and E. I. Bakr. 2005. Date palm (*Phoenix dactylifera* L. cv. Zaghloul) production using somatic embryogenesis of female inflorescence. The 3rd Conference Recent Technologies in Agriculture. 14-16 November, Faculty of Agriculture., Cairo University, Egypt.
3. Asaka, I., I. Li, M. Hirotani, Y. Asada, T. Yoshishikawa and T. Furuya. 1994. Mass production of ginseng (*Panax ginseng*) embryoids on media containing high concentration of sugar. Planta Medica 60:146.
4. Bergmann, L. 1967. Wachtum gruner suspension kulturen von *Nicotiana tabacum* var. Sansum mit CO_2 als kohlenstoffquelle. Planta 74:243.
5. Bhaskaran, S. and R.H. Smith. 1992. Somatic embryogenesis from shoot tip and immature inflorescence of *Phoenix dactylifera* L. cv. Barhee. Plant Cell Reports 12: 22-25.
6. Bhaskaran, S. and R.H. Smith. 1995. Somatic embryogenesis in date palm (*Phoenix dactylifera* L.). S. Jain, P. Gupta and R. Newton (eds.), Somatic Embryogenesis in Woody Plants Vol. (2) 461-470.
7. Drira, N. and A. Benbadis. 1985. Multiplication vegetative du palmier dattier (*Phoenix dactylifera* L.) par reversion en culture *in vitro* de'bauches florales de pieds femelles. Journal of Plant Physiological Vol. (119): 227-235.
8. Gamborg, O.L., F. Constable and J.P. Shyluk. 1974. Organogenesis in callus from shoot apices of *Pisum sativum.* Physiological Plantarum 30:125-128.
9. George, E.F. 1993. Plant Propagation by Tissue Culture. Exegetics Limited, Edington, Part 1, The Technology p. 23-28.
10. Mekers, O. 1977. *In vitro* propagation of some Tillansoideae (Bromeliaceae). Acta Horticulture 78:311-320.
11. Murashige, T. and F.A. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiological Plantarum 15:473-479.
12. Pierik, R.L.M., H.H.M. Steegamans, A.A. Ellas, O.T.J. Stiekema, and A.J. Van Der Velde. 1988. Vegetative propagation of *Syringa vulgaris* L. *in vitro.* Acta Horticulture 226:195-204.
13. Smith, W.k. and J.A. Thomas. 1973. The isolation and *in vitro* cultivation of cells of *Elaeis guineensis.* Oleagineaux 28:123-7.

تأثير تركيزات الأملاح الأساسية و السكروز علي التشكل داخل أنابيب الأختبار للنـــــــــورة المـــــؤنثـــة لنخيـــــل البلـــــح صنف الزغلول

عـــــادل أبـــو السعـــــود[١] ، نبيـــــل الشربينـــي[٢]، السيـــــد بكـــــــر[٢]

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تم تكوين انسجة متميزة لنخيل التمر (براعم خضرية و أجنة جسمية) مباشرة من النورة الزهرية المؤنثة. في هذه الدراسة، اختبرت بعض عوامل الدفع لتكوين تراكيب متميزة مثل مصدر الكربون و قوة الملح. تم استخدام الشماريخ الزهرية للنورة المؤنثة لنخيل التمر في مراحل نمو مختلفة(٢,٥ و ٧,٠سم)، اعتماداً علي طول الشماريخ الزهرية عند بداية الزراعة. كما تمت دراسة التأثير المشترك لتركيزات مختلفة من سكر السكروز (٢٠، ٣٠، ٤٠، ٥٠ جرام/لتر) و الأملاح الأساسية لموراشيجي و سكوج (MS) بتراكيز مختلفة (٠,٢٥ ، ٠,٥٠ ، ٠,٧٥ ، ١,٠ قوة أملاح). دلت النتـــائج علـــي ان النسبة المئوية لتكوين الأفرع الخضرية لشماريخ نخيل التمر الوسطية (٧ سم فـــي الطـــول) زادت عندما شمل الوسط الغذائي الأساسي ٥٠ جرام/لتر سكروز كمصدر للكربون بدلاً من التركيز المعتاد (٣٠ جرام/لتر)، و تركيز كامل من الأملاح الأساسية لموراشيجي و سكوج و التي ثبت انها أنســـب قوة ملح بالمقارنة بالتخفيفات الاخري لأملاح موراشيجي و سكوج. و بالتالي كانت النســـبة المئويـــة لتكوين الأفرع الخضرية هي ٣٣ %. فيما يتعلق باستحداث الأجنة سواء مباشرة او غيـــر مباشـــرة، زادت نسبة النجاح من ٥٣% الي ٧٣% عندما احتوي الوسط الغذائي الأساســـي لشـــماريخ نخيـــل التمر، حديثة العمر (٢,٥ سم في الطول) علي ٠,٧٥ من قوة املاح موراشـــيجي و ســـكوج و ٤٠ جرام/لتر سكروز. أخيراً ، يحل استخدام الأجزاء النباتية الناتجة من النورة ا لزهرية فـــي زراعـــة أنسجة نخيل التمر الكثير من المشاكل التي تعترض هذا التكنيك.