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Inducing Date Palm Somatic Embryogenesis and Plantlets Growth Using Date Palm Pollen Grains Extract

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THIS STUDY aimed to evaluate the effect of date palm pollen grains extract contents on date palm (*Phoenix dactylifera*) cv. Sewi growing *in vitro*. This experiment was done during somatic embryos germination stage and the elongation of complete plantlets. The somatic embryos and complete plantlets were cultured on Murashige and Skoog basal medium. The treatments were, T0: MS + 0.1 mg l^{-1}NAA and 0.5 mg l^{-1} BA, T1: T0 + 200 ppm date palm pollen grains extract, T2: T0 + 400 ppm date palm pollen grains extract. T3: MS + 200 ppm date palm pollen grains extract and T4: MS +400 ppm date palm pollen grains extract. The obtained results showed that, treatment with date palm pollen grains extract at 400 ppm (T4) significantly increased germination percentage up to 70%, followed by T3 treatment (50%). While, the secondary embryos formation percentage was enhanced withT2 treatment. Concerning the average number of leaves and roots it was significantly increased with T1. The highest value of shoot length was recorded with T4. Also, there was recurrent secondary embryos formation during the elongation stage, T2 medium gave significant increases in number of secondary embryos. Chemical analyses of samples that were taken from tissues during this study refer that, newly formed secondary somatic embryos contained the highest level of protein when originally somatic embryos were cultured on T2 treatment. Complete plantlets cultured on T2 treatment contained the highest levels of total indoles, chlorophyll a, b. While the carotenoids concentrations were increased within plantlets cultured on T4 treatment. These results cleared the stimulants effects of palm pollen grains extract on the morphological and chemical estimations of date palm growing *in vitro*.

Keywords: Date palm, Plantlets, Secondary embryogenesis, Palm pollen grains.

Date palm (*Phoenix dactylifera* L.) tissue culture recently was the important tools to production much more numbers date palm offshoots which described as true to type in the short time. Somatic embryogenesis is the process by which haploid or diploid cells that are not naturally embryogenic are induced to form embryos. Development proceeds through characteristic embryological stages leading to the development of differentiated plantlets (William and Moheswaran, 1986). All obtained somatic embryos were bipolar structures. Embryo production

by recurrent or secondary embryogenesis is the step giving somatic embryogenesis a multiplicative potential for clonal mass propagation (Merkle, 1995). Some workers have noted enhanced embryogenesis and improved embryo growth when media have been supplemented with amino acids in addition to NO_3^- and NH_4^+. Walker and Sato (1981) stated that, embryogenesis were created from alfalfa callus in the absence of either ammonium or nitrate ions. It is accepted that the presence of some reduced nitrogen is necessary for somatic embryogenesis in cell and callus cultures (Halperin and Wetherell 1965). Margara, (1984) reported that the use of a solution enriched in Mn, Zn and Bo is generally promoting organogenesis. The mineral composition of the $N_{30}K$ solution showed that, there is nitrogen-rich (30 mEql/l) which is consists of two-thirds in NO_3^- and potassium (15 mEql/l). This medium is also characterized by a high content of SO_4^{-2}, Ca^{2+} and an average content of total nitrogen, where the third is supplied as NH_4^+ from these contents Ben Ali and Lamarti (2014) showed that, the morphology of secondary embryos grown in the $N_{30}K$ medium exclusively showed the presence of three embryogenic stages: early cotyledonal with translucide aspect, white opaque, or green, and mature embryos, also, $N_{30}K$ medium presented better morphogenic potential, with different stages of embryogenic formation. Pollen grains are widely used in Egypt for many purposes. Pollens are male reproductive cells which rich in very important constituents *i.e.* phytochemicals and nutrients and are rich in carotenoids, flavonoids also they are good source of protein, amino acids, vitamins, dietary fiber, fatty acids, enzymes, hormones and minerals (Basim *et al*., 2006 and Kroyer & Hegedus 2001). Palm pollen grains from different Egyptian cultivars rich in different amino acids from 0.147 to 83.64 mg g^{-1}, B1 ranged from 11 to 60 mg g^{-1}, B2 ranged from 15 to 260 mg g^{-1} and B12 from 14 to 2316 mg g^{-1}, rich in different macro and micro elements N, P, K, C, Mg, Ca, Fe, Zn, Mn, S, and Na (Bishr and Samar 2012). Palm pollen grains contents of moisture 28.8 %, ash 4.57 %, fibers 1.37 %, fat 20.74 %, protein 31.11 % carbohydrates 13.41 %, pollen grains are considered a good source of micro elements B, Se, Mo Co, Cu and Ni. Palm pollen grains have glycosides, lake volatile substances, several steroids, brassinosteroid (Hassan 2011 and Zaki *et al*., 1993). The rate of *in vitro* multiplication can be increased by supplementing various natural additives. *In vitro* growth and regeneration of the plant can be improved by adding a small amount of organic nutrients to the medium (Molnar *et al*., 2011). Use of other natural supplements like tomato juice, orange juice, malt extract, yeast extract, casein hydrolysate in the media for promoting multiplication and development of *in vitro* cultures have been demonstrated by many authors (Carimi *et al*., 1998, on Citrus Rahman *et al*., 2004 and Aktar *et al*., 2007 on Orchid). Almeida-Muradian *et al*. (2005) reported that, chemical analysis of pollen grains has revealed the presence of a wide range of biochemically and nutritionally

important substances, minerals, trace elements, a wide range of carbohydrates, organic acids, lipids, sterols, nucleic acids, proteins, free amino acids as well as water and lipid soluble vitamins, as well as cover 100 different kinds of enzymes and cofactors. Basuny *et al*. (2013) found that olive and palm pollen grains contained phenolic compounds (422 and 220 ppm, respectively) and flavonoids (121.50 and 61.30 ppm, respectively) and they also found that, the high scavenging activities for the free radical 2, 2 diphenyl-1-picryl-hydrozyl (DPPH). The high antioxidant and antibacterial activities may probably be due to the highest contents of phenolic compounds growth and regeneration of plants from *in vitro* tissue cultures, which can be improved by small amounts of some organic nutrients. The reason of organic additives are added into culture medium besides being a natural source of carbon is because they contain natural vitamins, phenols, fiber, hormones and also proteins (Gnasekaran *et al*., 2010). Many of these amendments can be a source of amino acids, peptides, fatty acids, carbohydrates, vitamins and plant growth substances in different concentrations. auxins and cytokinins is not enough to regenerate the plant with high efficiency. This type of cultures in some cases may be improved by incorporation of additives in the media due to their growth and development promoting activities, coconut milk and casein hydrolysate increased shoot elongation of *Oroxylum indicum* L. (Thorpe *et al*., 2008 and Bansal and Gokhale, 2012). It is the intention of this research to investigate the effect of natural extract as palm pollen to enhance somatic embryogenesis and plantlets growth of date palm.

Material and Methods

This work was conducted during 2013 – 2014 in the Central Laboratory for Development and Research of Date Palm (ARC) to evaluate the effect of date palm pollen extract as plant growth substances on the germination of somatic embryos (SE) and the growth of complete plantlets of date palm cv. Sewi.

Plant material and nutrient medium

The mature somatic embryos derived from date palm embryogenic callus cv. Sewi and complete plantlets were cultured on the basal nutrient MS (Murashige and Skoog 1962) medium supplemented with 170 mg l^{-1} NaH_2PO_4. H_2O, 100 mg l^{-1} myo-inositol, 0.04 mg l^{-1} adenine sulphate, 0.4 mg l^{-1} thiamine. HCl, 200 mg l^{-1} glutamine, 30 g l^{-1} sucrose and 6 g l^{-1} agar. Five treatments were used during this study:

(T0): MS medium + 0.1 mg l^{-1} NAA+0.05 mg l^{-1}BA
(T1):T0 + 200 ppm pollen extract
(T2):T0 + 400 ppm palm pollen extract
(T3): MS medium free from hormone + 200 ppm palm pollen extract
(T4): MS medium free from hormone + 400 ppm palm pollen

The acidity of the final medium was adjusted to pH 5.7- 5.8 prior to the addition of agar. Media were dispensed into culture jars (200 ml) containing 40 ml/jar and capped with polypropylene closures and autoclaved at 121 °C and 1.5 kg/cm^2 for 20 min.

Culture Conditions

Cultures were incubated 16-h photoperiod day/night condition at 27 ± 2°C for two sub-cultures (6 weeks for each of them). The following estimations were measured

Somatic embryos germination percentage, average number of embryos, embryo length (cm), leaves numbers, shoot length (cm), roots numbers, root length (cm) and average number of newly formed secondary embryos.

Chemical estimations

1-Total indoles (mg g^{-1} f. wt.) 2- chlorophyll a, b and carotenoids (mg g^{-1} f. wt.) 3-Total proteins contents mg/g (f.w.)

Total indoles

The fresh leaves samples in methanolic extract, were calorimetrically estimated at the wavelength of 530 nm, using Para dimethyl amino benzaldhyde test as adopted by Larsen *et al*. (1962) and Salim *et al*. (1978) and the concentration was calculated as indole acetic acid (mg g^{-1} f. wt.).

Chlorophyll a and b : As described by Lichtentaler and Wellburn (1985).

Total soluble proteins: Total soluble protein levels were measured by using BIO-RAD protein assay dye reagent by the method of Bradford (1976).

Pollen grains : Pollen grains of Egyptian date palm (*Phoenix dactylifera* L., cvs) were collected at the end of March and the extract of grains contents are shown in Table 1 as Hassan (2011) and Table 2 as Hassan *et al*. (2008).

Preparation of Palm Pollen Extracts : Extract was prepared by using ethanol (0.1 g pollen and 10.0 ml ethanol), the solvent was removed from the obtained extract by evaporation. The residue was re dissolved in the same volume of (10.0 ml) DW (Nagai *et al*., 2002)

Experimental design : Complete randomized design with three replicates and three plantlets for each replicate, two growth seasons. Data were analyzed by analysis of variances (ANOVA) and the means were compared following t- test using LSD values at 5 % level (Snedecor and Chocran, 1990).

TABLE 1. contents of date palm pollen extract.

Palm pollen grains contents								
Elements		**Chemical composition % g/100 g d.w.**		**Amino acids %**		**Fatty acids %**		
						Saturated fatty acid		
Carbon C	27.8 %	Moisture%	28.8	Isoleucine	1.49	Capric acid	0.46	
Nitrogen N	54.1 %	Ash %	4.57	Leucine	3.34	Lauric acid	4.82	
Magnesium Mg	0.12%	Fiber %	1.37	Lysine	2.95	Myristic acid	13.33	
Phosphorus P	0.66%	Fat	20.74	Phenylalanine	1.63	Palmitic acid	34.45	
Sulfur S	0.69%	Proteins %	31.11	Threonine	1.72	Stearic acid	2.04	
Potassium K	5.5%	Carbohydrates %	13.41	Valine	1.81	Arachidic acid	7.32	
Calcium Ca	7.0%	Vitamins		Hisitidine	1.61	Mono saturated fatty acid		
Zinc Zn	281.0 mg/100g	A (IU/100g)	7708.33	Methionine	0.11	Palmitoleic acid	7.07	
						Oleic acid	7.19	
Iron Fe	241.0 mg/100g	E (IU/100g)	3030.92	Alanine	2.61	Polyunsaturated fatty acid		
Manganese Mn	284.0 mg/100g	C (mg/100g)	89.09	Arginine	1.61	Linoleic acid	14.24	
Sodium Na	0.22 mg/100g			Aspartic acid	3.55	Arachidonic acid	4.57	
Boron B	309.4 mg/100g			Glutamic acid	1.74	Eicosapentaenoic acid	0.52	
Nickel Ni	302.4 mg/100g			Glycine	2.24			
Cobalt Co	305.4 mg/100g			Serine	1.89			
Copper Cu	319.6 mg/100g			Cysteine	0.42			
Molybdenum Mo	302.2 mg/100g			Tyrosine	1.55			
				Proline	0.28			

TABLE 2. Total indole contents of pollen extracts (mg/g pollen).

Pollen extract	Indole content
Water extract	10±0.34
Ethanol extract	9±0.21

Results and Discussion

Somatic embryos conversion %

Data presented in Table 3 showed that, the germination percent of somatic embryos conversion % (SE) significantly increased when the culture media supplemented with palm pollen extract at (T4) 400 and (T3) 200 ppm (70 and 50 %,

respectively (Fig. 1 e and d) without supplementation of growth regulators in comparable to (T0) 30% for control treatment (Fig. 1 a), meanwhile T2 media gave the lowest germination percent of somatic embryos (20%) (Fig. 1 c).

TABLE 3. Effect of palm pollen extract on the conversion of somatic embryos into complete plantlets and recurrent secondary embryogenesis during germination stage

Treatments	Somatic embryos conversion %	Average number of secondary embryos	Embryo length
T0	30cd	10c	1.5
T1	40bc	12bc	2.3
T2	20 d	30 a	1.5
T3	50 b	15 b	2.0
T4	70 a	12bc	2.0
LSD	17.86	3.036	NS

Average number of secondary embryos (SE)

The average number of SE (Table 3, Fig. 1 c) was increased (30 embryos/ cluster) when the culture medium was supplemented with 0.1mg^{-1} NAA, 0.05 mg^{-1} BA and 400 ppm pollen extracts (T2) compared with T0 medium (10 embryos/cluster). In this respect, Alkhateeb (2008) found that, the highest number of somatic embryos and longest shoot produced by date syrup at concentration of 6%, syrup locally known as 'Dibs' to the culture medium of date palm at concentration of 6% can be used totally as a replacement of sucrose which was the normally sugar used in most of plant tissue culture medium.

Embryo length

Data on the length of embryos (Table 3) showed no significant difference in embryo length among the all tested media. The highest length was obtained from (T1 Fig. 1 b) graduated by T3 and T4 (2.3, 2.0 and 2.0 respectively). In this respect, somatic embryogenesis is frequently regarded as the best system for propagation of superior genotypes, mostly, because both root and shoot meristems are present simultaneously (Kim 2000). Pinto *et al*. (2002) reported that, the repetitive somatic embryogenesis represents a continuing source of embryogenic material. Also, they found that all converted plants obtained were derived from morphologically normal secondary embryos. From the previous studies of Asemota *et al*. (2007) they found that callus of date palm explants was initiated at 5 mg^{-1} NAA from leaf explant when cultured in the Eeuwen's medium supplemented with NAA from 5-20 mg^{-1} . Data presented here showed that, conversion of original somatic embryos enhanced strongly by added pollen palm extract without addition of NAA and BA (T4 and T3 treatments) this data insures the previous data obtained by Chen *et al*. (2005) and Amo-Marco and Picazo (1994) who proved that adding plant extracts juice of coconut, tomato, potato, onion, banana, orange, apple, pineapple and yeast to the culture medium enhanced the growth of tissues, furthermore addition of corn (maize) syrup to basic culture media improves embryogenesis of wild carrot (Kinnersley and Henderson 1988). Use of other natural supplements like tomato juice, orange juice,

malt extract, yeast extract, casein hydrolysate in the media for promoting multiplication and development of *in vitro* cultures have been demonstrated by many authors (Carimi *et al*., 1998 on Citrus, Rahman *et al*., 2004 and Aktar *et al*., 2007 on Orchid). Embryogenic structure induction was enhanced by increasing the concentration of picloram in the induction medium of *Manihot esculenta* Crantz Cassava cvs Ubalua and Mbanaso (2014).

Average number of leaves/cluster

Table 4 and Fig. 1 f-j demonstrated that the complete plantlets which were cultured on the media added with palm pollen to test its effect of their growth, the treatment T1 and T3 increased the number of the leaves (17.33 and 16.67 leaves/culture) followed by T2 14.33 leaves/culture, insignificant differences were found between T4 and control treatment.

Shoot Length (cm)

Shoot length (Table 4) exhibited highly significant increase (5.3cm) when plantlets were cultured in T4 treatment (Fig. 1 j). However, T0 medium gave the lowest value of shoot length (3.0 cm).

Average number of roots/cluster

The highly significant average number of roots/cluster (Table 4 Fig. 1 g) was found in T1 treatment (15.33) and T3 (11.67) but lowest numbers of roots were obtained by control treatment (6.33).

TABLE 4. Effect of palm pollen on the growth of complete date palm plantlets during elongation period.

Treatments	Average number of leaves/cluster	Shoot Length cm	Average number of roots/ cluster	Root length cm	Average number of secondary embryos
T0	9.33c	3.0c	6.33d	2.2	4.0c
T1	17.33a	4.0bc	15.33a	2.5	6.0ab
T2	14.33b	3.7bc	8.67c	2.7	7.0a
T3	16.67a	4.6ab	11.67b	3.2	5.0bc
T4	9.67c	5.30a	7.33d	3.5	5.0bc
LSD	2.063	0.483	1.786	NS	1.785

Root length (cm)

Concerning to root length (Table 4) that enhancing from control treatment 2.2 cm to T1 2.5 cm, T2 (2.7 cm) and T3, T4 produced the longest roots 3.2 and 3.5 cm respectively.

Average number of secondary somatic embryos

Significant differences were found among treatments, 7 and 6 embryos/ cluster were produced respectively when palm pollen extract were added to the culture media at 400 and 200 ppm in addition to NAA at 0.1 mg^{-1} and BA at 0.05 mg^{-1} compared to control treatment 4.0 embryos/ cluster. NAA improve the

development of embryos of *Boscia senegalensis* (Pers.) Lam. Poir to seedlings. Maximum number of shoots/explant (14.8 ± 0.6) was obtained on MS medium supplemented with 3.0 mg^{-1} BA. 67.0% of excised shoots rooted either on 1/2MS medium augmented with or without 0.25 mg^{-1} IBA. The highest number of roots (1.2 ± 0.4) and root length (0.5 ± 0.2 cm) was produced on 0.25 mg^{-1} IBA-containing medium (Daffalla *et al*., 2011). 200 and 400 ppm of palm pollen extract significantly increased shoot and root length and number of shoot and roots of *Musa spp* Banana *in vitro* (Hassan *et al*., 2008 and El-Assar *et al*., 2004 on date palm cv. Sewi). In a comparison of the effects of adding coconut water (Nambiar *et al*., 2012 on *Dendrobium hybrid* known as D. Alya Pink (DAP)), banana extract, pineapple juice or tomato juice to the Nitsch medium. Tomato juice followed by coconut water had the best effect on growth and differentiation of *Cymbidium longifolium* (Siddique and Paswan,1998). The lemon juice and conducted milk are used in the vegetative production for potato plantlets *in vitro* (Hassen, 2011). Other scientists stated that *in vitro* lemon juice concentration 2 mg^{-1} increased plant height and leaves numbers, nodes and branches of potato (*Solanum tuberosum* L.) and root length and number (Ali *et al*., 2014).The use of pollen extracts, licorice and lemon juice in media had effects on plant growth (Al-Kaaby and Hussein 2001). Also the shoot and root formation was better in this species in absence of plant growth regulators. Probably this response is due to the endogenous hormone levels in the tissues of *Cyrtochilum loxense* an endangered orchid (González and Cueva, 2014).

Average number of secondary embryos

From obtained data, when palm pollen were added to the culture media at 200 and 400 ppm without the addition of any growth substances and re-cultured the converted embryos again on its same concentrations, there was a high formation of secondary somatic embryos (un-shown data). Also the leaf width increased in the medium added with 400 ppm on a free hormone medium. In this respect, secondary somatic embryogenesis was also observed when primary somatic embryos were sub-cultured on the same somatic embryo induction medium (Swamy *et al*., 2005). Embryo origin is especially relevant to the genetic uniformity of regenerated plants, as a multicellular origin may result in the formation of genetically variable plants, a uni-cellular origin is the desired pathway for practical applications of embryo cloning such as genetic transformation. In the cork oak system, secondary embryos mainly originate by meristematic budding from a compact mass of proliferation (Puigderrajols *et al*., 2001). Fernández-Guijarro *et al*. (1995) showed that, induced somatic embryogenesis in cultures of leaves from young seedlings of *Quercus suber* L. two-stage process, in which benzyladenine and naphthaleneacetic acid were added first at high and then at low concentrations, was required to initiate the process. Somatic embryos arose when the explants were subsequently placed on medium lacking plant growth regulators. The embryogenic lines remained productive by means of secondary embryogenesis, on medium without growth regulators. 1 mg^{-1} of both IBA+NAA and 1 mg^{-1} of both IAA+IBA resulted in adequate rooting of chickpea (*Cicer arietinum* L.) after six week of culturing (Islam *et al*., 2005).

TABLE 5. The levels of protein within the secondary embryos, indoles, carotenoids and chlorophyll a, b within complete plantlets of date palm cv. Sewi cultured on media supplemented with palm pollen with or without growth regulators.

Treatments	Protein level in secondary embryos	Complete plantlets			
		Indoles	Carotenoids	The levels of chlorophyll	
				Ch. a	Ch. b
	mg g^{-1} f. wt.				
T0	1.00c	4.85c	0.02	0.31b	0.06
T1	1.50ab	6.95b	0.06	0.40a	0.09
T2	1.55a	11.09a	0.02	0.42a	0.13
T3	1.00c	5.55bc	0.067	0.41a	0.11
T4	1.15bc	7.10b	0.097	0.40a	0.10
LSD	0.037	1.599	NS	0.0595	NS

Protein level in secondary embryos

Data illustrated in Table 5 showed the different levels of proteins within the secondary embryos formed during germination period on culture media supplemented with palm pollen at 200 and 400 ppm with or without growth regulators (0.1 mg l^{-1} NAA and 0.05 mg l^{-1}BA). Culture media added with growth regulators and palm pollen at 200 and 400 ppm (T1 and T2) increased the levels of protein 1.55 and 1.50 mg g^{-1} f. wt., respectively. However, added culture media with palm pollen at 200 ppm without growth regulators gave low levels equal to control (1mg g^{-1} f. wt.). Plant hormones play an essential roles in plant metabolism and can influence cell cycle proteins (Sanchez *et al*., 2005).

Indoles

Concerning to Indole level (Table 5) which increased and reached to its highest level (11.09 mg g^{-1} f. wt.) when the plantlets were cultured on media added with 200 ppm and 0.1 mg l^{-1} NAA and 0.05 mg l^{-1}BA (T1). Control medium (T0) that contained 0.1 mg l^{-1}NAA and 0.05 mg l^{-1} BA without any supplementation of the two concentrations of palm pollen was responsible for reducing the indole concentration (4.85 mg g^{-1} f. wt.) within the cultured plantlet.

Carotenoids and chlorophyll a, b mg g^{-1} f. wt.

Looking to the levels of carotenoids and chlorophyll a, b (Table 5) data showed that, chlorophyll a increased 0.42 for T2, graduated by 0.41 for T3 and 0.40 and 40 respectively for T1 and T4, moreover chlorophyll b and carotenoides exhibited insignificant differences between treatments in compared to control treatment. On the respect of useful effect of date palm pollen as a natural substance on the growth and chemical contents (Swamy *et al*., 2014) stated that, use of 10% tomato extract, 20% banana extract, 10% carrot extract, and 10% papaya extract in MS medium have efficiently increased multiple shoots of *Pogostemon cablin* Benth, shoot length, and fresh weight of the shoots. The natural supplements also effectively increased the chlorophyll content, total protein, and total carbohydrate content in the plant.

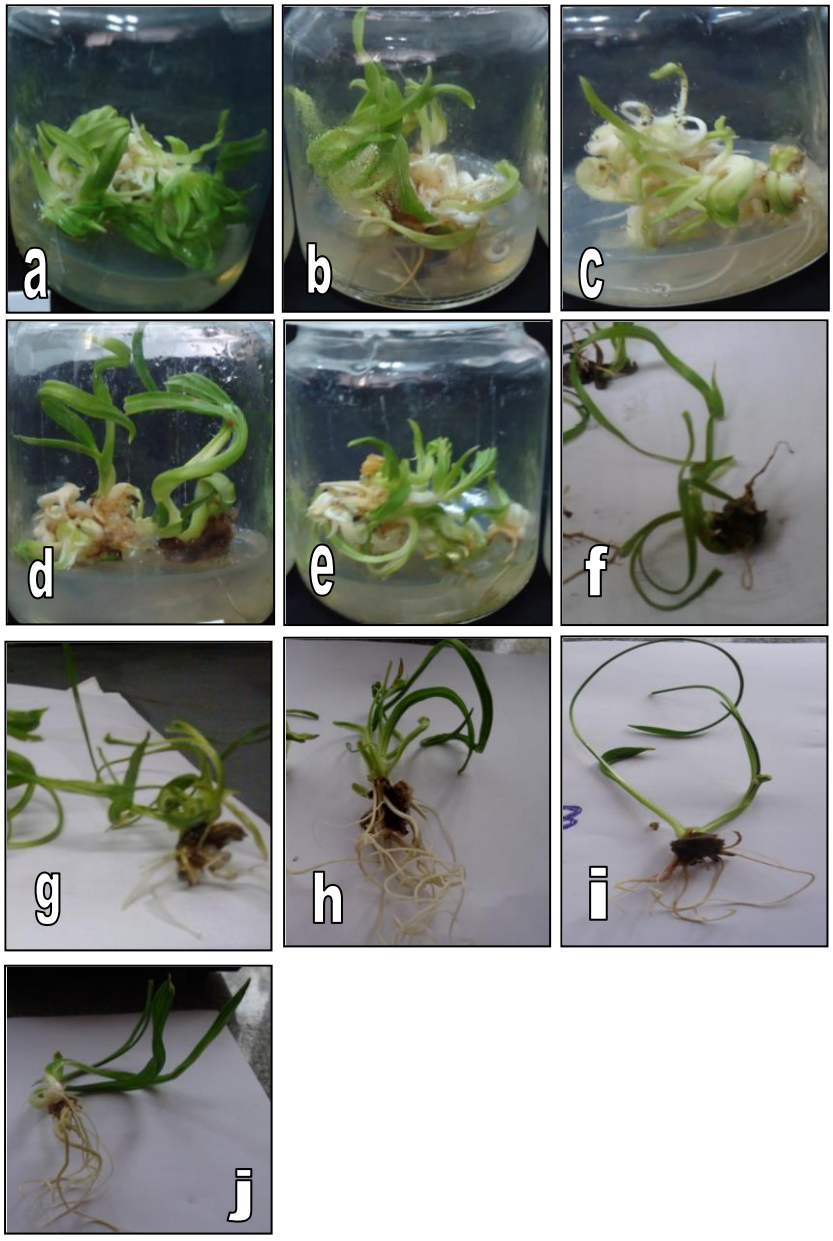

Fig.1. Germination of somatic embryos and elongation of complete plantlets of date palm cv. Sewi on different culture media.

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تشجيع تكون الاجنة الثانوية ونمو نبيتات نخيل البلح عن طريق استخدام مستخلص حبوب لقاح نخيل البلح

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المعمل المركزي لأبحاث وتطوير نخيل البلح –مركز البحوث الزراعية – القاهرة – مصر.

تم تقييم تأثير استخدام مستخلص حبوب لقاح نخيل البلح على مرحلة انبات الأجنة ومرحلة استطالة النباتات الكاملة لنبات نخيل البلح صنف سيوى معمليا. حيث تم زراعة الأجنة الجسدية و النباتات الكاملة على بيئة موراشيجى وسكوج مع او بدون نفتالين حامض الخليك والبنزيل ادنين مضاف إليهم مستخلص حبوب لقاح نخيل البلح. حيث المعاملة (.T) تحتوى على بيئة موراشيجى وسكوج فى وجود ٠,١ ملجم/لتر نفتالين حامض الخليك بالاضافة الى ٠,٠٥. ملجم/لتر بنزايل ادينين . المعاملة (T1) تحتوى على (.T) مضاف اليها ٢٠٠جزء فى المليون مستخلص حبوب لقاح نخيل البلح. المعاملة (T2) تحتوى على (.T) مضاف اليها ٤٠٠جزء فى المليون مستخلص حبوب لقاح نخيل البلح . المعاملة (T3) تم اضافة ٢٠٠جزء فى المليون مستخلص حبوب لقاح نخيل البلح لبيئة موراشيجى وسكوج. (T4) تم اضافة ٤٠٠جزء فى المليون مستخلص حبوب لقاح نخيل البلح لبيئة موراشيجى وسكوج. وأوضحت النتائج أن استخدام المستخلص بتركيز ٤٠٠ جزء فى المليون(T4) مع الاجنة الجسدية ادى الى زيادة النسبة المئوية للانبات (٧٠٪) يليها المعاملة (T3) . المعاملة (T2) أدت الى زيادة النسبة المئوية لتكوين الأجنة الثانوية. كما أوضحت النتائج أن زراعة النباتات الكاملة على المعاملة (T1) أدى لزيادة متوسط عدد الاوراق و الجذور. بينما أظهرت المعاملة (T4) أعلى قيمة لطول الأفرع.كما لوحظ ان فى مرحلة الأستطالة تم تكوين أجنة ثانوية بصورة متكررة. أما المعاملة (T2) أدى للحصول على أعلى قيمة معنوية فى عدد الأجنة الثانوية. كما أظهرت نتائج التحاليل الكيميائية أن المعاملة (T2) مع الأجنة الجسدية اعطت أعلى مستوى للبروتين فى الأجنة الثانوية المتكونة. وأعطت مع النباتات الكاملة أعلى مستوى من الاندول والكلوروفيل a,b. المعاملة (T4) أدت الى زيادة تركيز الكاروتنيد داخل النباتات.

الكلمات الدالة : نخيل البلح، نبيتات ، أجنة ثانوية ، مستخلص حبوب لقاح نخيل البلح.