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In vitro PROPAGATION OF EGYPTIAN DRY DATE PALM 2-EFFECT OF NUTRIENT MEDIUM COMPONENTS ON SOMATIC EMBRYOGENESIS

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ABSTRACT

This study aimed to evaluate the effect of cytokinins (2ip, BA, kin. and BA- riboside) with or without NAA on embryo formation of Egyptian dry date palm (Sakkoty , Malakaby , Gondeila , Shamia and Bartamuda). The study also included the effect of glutamine (200 mg/l), thiamine HCl (10 mg/l) and Ca-panthothianic acid on somatic embryo germination and secondary embryo formation. Nutrient medium (MS) supplemented with 0.1 mg /l NAA (M1) increased the number of embryos followed by MS basal medium without plant growth regulators (M0) and 0.2 mg/l kinetin (M6) without significant differences.The Shamia cv. showed superiority of embryo numbers followed by Gondeila and Bartamuda cvs. MS nutrient medium without plant growth regulators (control medium) was superior in stimulating embryo length, while the lowest embryo length was observed by adding 0.1 mg /l kin. +0.1mg/l NAA. Shamia cv. recorded the highest embryo length followed by Bartamuda and Malakaby cvs. Germination percentage of somatic embryos was increased on MS basal medium containing 0.1mg/l NAA.

The highest significant value of secondary embryos was achieved with MS basal medium without plant growth regulators followed by MS medium containing 0.1mg/l NAA , 200 mg/l glutamine ,10 mg/l thiamine HCl and 5 mg/l Ca-panthothianic acid (MD). Malakaby and Bartamuda cvs. recorded the highest value of secondary embryos, while Shamia was the lowest .

Key words: *dry date palm, germination, in vitro, somatic embryogenesis.*

1. INTRODUCTION

Somatic embryogenesis is the process by which the somatic cells or tissues develop into differentiated embryos and when fully developed is capable of developing into a plantlet. Embryos can be obtained, either directly from cultured explants or indirectly from callus and isolated single cells in culture. The process of embryogenesis involve various stages of differentiation and development (Purohit, 1999). More recently, the embryos of date palm derived from somatic cells are being utilized in the preparation of artificial seeds which appear to be of great value in plant propagation program (Hamed, 2002).

Induction of an embryogenic state in differentiated explants often requires extensive proliferation through unorganized callus cycles , death or disruption of surrounding explant cells, and or high levels of synthetic auxin (100 mg/l 2,4- D for date palm). The somatic embryo nodules formed in callus when transferred to MS medium with 0.1mg/l GA3 and 0.1mg/l 2iP and the plantlets of date palm are developed directly from somatic embryos(Madhuri & Shanker, 1998). The aim of this work was to study the effect of plant growth regulators containing –medium on somatic embryo maturation and germination of Egyptian dry date palms namely Sakkoty Malakaby ,Gondeila, Shamia and Bartamuda. The study also included the effect of glutamine ,thiamineHCl and Ca-panthothianic acid on somatic embryo germination and secondary embryo formation through *in vitro* propagation of these distinguished cultivars, which are suitable to new Egyptian agricultural projects in Toshkay and Shark El- Aoinate.

2. MATERIALS AND METHODS

2.1. Maturation of somatic embryos

In this experiment , embryogenic callui resulting from date palm shoot tips from Egyptian dry cultivars (Sakkoty, Malakaby, Gondeila, Shamia and Bartamuda from Aswan Governnorate) and cultured on MS (Murashige and Skoog, 1962) basal medium supplemented with 100 mg/l 2,4–dichlorophenoxy acetic acid (2,4-D) + 3mg/l isopentyladenine (2iP) were used as a source of plant material (Khattab *et al.,* 2003). Embryogenic callui were divided into pieces of approximately (1x1 cm) and cultured on the following media: 0-MS (control) 1-MS+0.1mg/l naphthaleneacetic acid (NAA) 2-MS+0.1mg/l NAA+0.1 mg/l Kinetin (kin.). 3-MS+0.1mg/l NAA+ 0.05 mg/l benzyladenine (BA) 4-MS+0.1mg/l NAA+0.1 mg/l 2iP. 5-MS+0.1mg/l NAA + 0.1mg/l BA ribozide .6-MS+0.2mg/l Kin. 7-MS+0.4 mg/l 2iP and 8-MS+ 0.2 mg/l BA ribozide.

These media were selected after obtaining promising results from several preliminary experiments. For each cultivar under investigation each treatment included three replicates (small jars). Each jar contained about 1x1 cm. embryogenic callus (friable callus). All culture jars were incubated in total darkness at 27±2°C for three months with subculturing to corresponding fresh medium every month. The average number of embryos and length were recorded after three months.

2.2. Germination of somatic embryos

In this experiment, individual embryos (5mm in length) were separated from previous proliferating cultures and inoculated in the following media: A (MS) , B(MS+ 0.1mg/l NAA) , C (MS +200 mg/l glutamine +10 mg/l thiamine-HCl +5 mg/l Ca- panthothianic) and D (MS +0.1 mg/l NAA+200 mg/l glutamine + 10 mg/l thiamine HCl + 5mg/l Ca-panthothianic acid). All cultured jars were incubated at 27±2°C for three months in light provided by cool white fluorescent tubes giving about 3000 lux for 16 hours / day. Developed embryo explants were subcultured every four weeks for three months. Each treatment contained five replicates and each replicate contained one embryo. Percentage of embryo germination and average number of

secondary embryos were calculated after three months.

Data obtained were subjected to the analysis of variance of randomized complete design as recommended by Snedecor and Cochran (1980). LSD at 5% level of significance was used to compare between means according to Stell (1960).

3. RESULTS AND DISCUSSION

3.1. Effect of plant growth regulators on embryo formation

This experiment was done in order to evaluate the effect of different concentrations of cytokinins (2ip, BA, kin, BA-ribozide) with 0.1 mg/l NAA or without NAA on embryo formation when these supplies were added to MS basal medium.

3.1.1 Number of embryos

Data in Table (1) reveal the embryo formation as affected by date palm genotype and plant growth regulators containing nutrient media.

Concerning the effect of different media, data showed that MS basal medium supplemented with 0.1 mg/l NAA (M1) recorded superiority in the enhancement of stimulated number of embryos followed by (M0) control medium and M6 (0.2 mg/L kin) medium. The results were 6.3, 5.9 and 5.8 embryo /explant, respectively, without significant differences among them. No significant difference could be observed between M3, M4, M5, M7 or M8. The results were 4.6, 4.9, 4.7, 4.8 and 4.7 embryo / explant, respectively. The lowest significant value of embryo formation was observed by using culture medium with adding 0.1 mg/l kin + 0.1 mg/l NAA. The result was 3.13 embryos.

Regarding the effect of date palm genotype on the number of embryos, the data reveal that the Shamia cv. showed superiority of the embryo number followed by Gondeila cv. and Bartamuda cv. The results were 6.52, 5.70 and 4.70, respectively, with significant differences among them. Malakaby and Sakkoty cvs. recorded the lowest value (4.00) and (3.96), respectively, without significant differences in between.

Referring the interaction between different genotypes and

media supplemented with different plant growth regulators, data show that the highest significant values of embryo number were observed when Shamia cv. explants were cultured on a medium supplemented with 0.2 mg/l kinetin. (9.3),followed by Bartamuda and Shamia cvs. explants cultured on medium containing 0.1 mg/l NAA. The results were 8.3 and 7.7, respectively, without significant differences inbetween and Shamia cv. when explant cultured on medium containing 0.2 mg/l BA-ribozide (7.7), while the lowest values were observed by culturing Malakaby cv on a medium containing 0.1 mg/l NAA + 0.1 mg/l kin, culturing Sakkoty or Bartamuda cvs. on medium of the same composition and culturing Bartamuda cv. on medium containing 0.1 mg/l NAA + 0.05 mg/l BA, also by culturing Malakaby cv. on medium supplemented with 0.4 mg/l 2iP, and finally by culturing Sakkoty cv. on medium containing 0.2 mg/l BA-ribozide.

Table (1): Effect of media supplemented with different plant growth regulators on embryo formation (number of embryos) of date palm genotypes after 3 months.

Medium No.	MS basal media +(M)		Cultivars (C)					Mean
	Auxin (mg/L)	Cytokinin (mg/L)	Bartamuda	Shamia	Gondeila	Malakaby	Sakkoty	
M0	**0.00**	**0.00**	6.3	6.3	6.7	5.0	5.0	**5.9 A**
M1	**0.1NAA**	**0.00**	8.3	7.7	6.0	4.7	4.7	**6.3 A**
M2	**0.1NAA**	**0.1Kin**	3.0	3.3	4.3	2.0	3.0	**3.1 C**
M3	**0.1NAA**	**0.05BA**	3.0	6.0	6.3	4.0	3.7	**4.6 B**
M4	**0.1NAA**	**0.12iP**	4.3	6.0	6.0	4.3	4.0	**4.9 B**
M5	**0.1NAA**	**0.1BAr**	3.7	6.0	4.3	5.3	4.3	**4.7 B**
M6	**0.00**	**0.2Kin**	4.7	9.3	6.7	4.3	4.0	**5.8 A**
M7	**0.00**	**0.42iP**	4.3	6.3	6.3	3.0	4.0	**4.8 B**
M8	**0.00**	**0.2BAr**	4.7	7.7	4.7	3.3	3.0	**4.7 B**
Mean			4.70 c	6.52 a	5.70 b	4.00 d	3.96 d	

L.S.D at 0.05 % C = 0.0484 M = 0.0649 C*M = 0.1451

3.1.2. Length of embryos (cm)

Data presented in Table (2) show the effect of plant growth regulators containing - medium on embryo length of date palm

genotypes.

Regarding the effect of different media, data revealed that MS basal nutrient medium (control) recorded superiority of stimulated the embryo length than other media (0.65 cm), followed by (M7) medium supplemented with 0.4 mg/L 2ip and (M1) medium of adding 0.1 mg/L NAA, as the results were 0.54 and 0.55cm, respectively, without significant differences inbetween, while the lowest significant value of embryo length was observed by using (M2) medium as presented in Table (2) .

Concerning the effect of date palm genotypes on the embryo length,, data reveal that Shamia cv. recorded the highest significant value of embryo length(0.56 cm.) followed by Bartamuda and Malakaby cvs. as the mean values were 0.50 and 0.47, respectively, without significant differences inbetween. No significant difference could be observed between Gondeila or Malakaby cvs. The lowest significant value of embryo length was observed with Sakkoty cv. (0.44 cm).

Referring to the interaction between different genotypes and different media, data show that the highest value of embryo length was noticed when explants of Malakaby and Shamia cvs. were cultured on MS medium (free of cytokinin and auxin) .The results were 0.9 and 0.8 cm, respectively, without significant difference inbetween. The lowest significant value was observed when the explants of all cultivars under investigation were cultured on a medium supplemented with 0.1mg/l NAA+ 0.1 mg/l kin.

Maturation is the terminal event of embryogenesis which is characterized by the attainment of mature embryo morphology, accumulation of storage carbohydrates ,lipids and proteins , reduction in water content , and often a gradual decline or cessation of metabolism. Although complete maturation is not absolutely necessary in order to obtain plants from nonzygotic embryos , it is required to achieve high rates of plant recovery. As such , a number of culture medium components has been shown to promote maturation (Trigiano and Gray, 2000).

From the results mentioned in Table (2), it could be noticed that using MS medium+0.1mg/l NAA, MS medium-free from growth regulators and MS medium +0.2 mg/l kinetin, produced the highest

significant values of mature embryos. These results are confirmed by the work of Tisserat (1979 a & b, 1981a & b), Sharma *et al.* (1984), Kackar *et al.* (1989) and Shakib *et al.* (1994), who mentioned that somatic embryos and viable plantlets were induced on MS medium without growth regulators.

Table (2): Effect of media supplemented with different plant growth regulators on embryo length (cm) of date palm genotypes after 3 months.

Medium No.	MS basal media + (M)		Cultivars (C)					Mean
	Auxin (mg/l)	Cytokinin (mg/l)	Bartamuda	Shamia	Gondeila	Malakaby	Sakkoty	
M0	0.0	0.0	0.5	0.8	0.6	0.9	0.5	0.65 A
M1	0.1NAA	0.0	0.7	0.7	0.4	0.3	0.5	0.54 B
M2	0.1NAA	0.1 Kin	0.3	0.3	0.3	0.3	0.3	0.29 D
M3	0.1NAA	0.05 BA	0.4	0.6	0.3	0.5	0.4	0.44 C
M4	0.1NAA	0.1 2iP	0.6	0.5	0.4	0.4	0.4	0.47 C
M5	0.1NAA	0.1 Bar	0.4	0.4	0.5	0.5	0.3	0.44 C
M6	0.0	0.2 Kin	0.5	0.5	0.4	0.5	0.5	0.47 C
M7	0.0	0.4 2iP	0.6	0.5	0.6	0.5	0.5	0.55 B
M8	0.0	0.2 Bar	0.4	0.6	0.4	0.4	0.4	0.43 C
Mean			0.50 b	0.56 a	0.45 cd	0.47 bc	0.44 d	

L.S.D at 0.05 % C = 0.0484 M = 0.0649 C*M = 0.1451

The initiation of embyogenic cells requires *in vitro* culture of the appropriate explant or on a medium that contains specific plant growth regulators. Auxins induce the formation of embryogenic cells, possibly by initiating differential gene activation and also appear to promote an increase of embryogenic cell populations through repetitive cell division while simultaneously suppressing cell differentiation and growth embryos(Gray, 2000).

3.2.Effect of MS nutrient medium containing NAA or glutamine, thiamine HCl and Ca-panthothianic acid or both on somatic embryo germination and secondary embryo formation

3.2.1. Somatic embryo germination

Data in Table (3) show that germination percentage as affected by genotype and different media (MA , MB , MC , MD).:as

MA= MS basal nutrient medium only (MS + 0.0).

MB= MS + 0.1 mg/l NAA.

MC=MS+200mg/l glutamine +10mg/l thiamine-HCl +5mg/l Ca-panthothianic acid
MD = MC+ 0.1 mg/l NAA

Regarding the effect of genotype, our data show that the highest significant values of germination percentages were observed with Bartamuda, Malakaby and Sakkoty cvs. (23.75%) followed by Shamia cv. (22.5%), while the lowest significant value of germination percentage was obtained with Gondeila cv. (21.25%).

With respecting to the effect of different media on germination percentage, the data in Table (3) reveal that MD medium recorded superiority of stimulated germination percentage than other media (27%), followed by MB and MA as the values were 23 and 22%, respectively, with significant differences inbetween. The lowest significant value was observed with the medium MC (20%).

Table (3): Effect of different media on germination percentage of date palm genotypes after 3 months.

Medium (M)	Cultivars (C)					Mean
	Bartamuda	Shamia	Gondeila	Malakaby	Sakkoty	
MA	25.0	15.0	20.0	25.0	25.0	22 .0 C
MB	20.0	25.0	25.0	20.0	25.0	23.0 B
MC	20.0	25.0	15.0	20.0	20.0	20.0 D
MD	30.0	25.0	25.0	30.0	25.0	27.0 A
Mean	23.75 a	22.50 b	21.25 c	23.75 a	23.75 a	

L.S.D. at 0.05 C=0.517 M=0.462 C*M = 1.035

MA=MS MB=MS+0.1mg/l NAA
MC=MS+200mg/l glutamine+10mg/l thiamine-HCl+5mg/l Ca-panthothianic acid
MD=MS+0.1mg/lNAA+10mg/Lthiamine-HCl+200mg/lglutamine+5mg/lCa-panthothianic acid

Referring to the interaction between date palm genotypes and different media, our data indicated that the highest significant values of germination percentage were observed when Bartamuda and Malakaby cvs. explants were cultured on MD medium. The values were (30%) for both cultivars, without significant difference inbetween, while the lowest significant values were observed when Shamia cv. explants were cultured on MA medium (MS), and

Gondeila cv. explants cultured on MC medium (15%) as presented in Table (3) .

3.2.2. Number of secondary embryos

Data presented in Table (4) and Fig. (1) show the number of secondary embryos as affected by date palm genotypes (Malakaby, Gondeila, Bartamuda, Shamia and Sakkoty) and type of media used.

Concerning the effect of date palm genotypes on secondary embryo number, the data revealed that Malakaby and Bartamuda cvs. recorded the highest values of secondary embryos, followed by Sakkoty and Gondeila cvs. as the mean values were (8.25, 8.17, 7.67 and 7.67), respectively, without significant differences among them, while the lowest value was observed with Shamia cv. (7.33).

Regarding the effect of different media, our data showed that the highest significant value of the secondary embryos was observed with the control medium free from plant growth regulators, followed by MD and MC media, as the results were 10.1, 8.0 and 7.2, respectively, with significant differences among them, while the lowest significant value of the secondary embryos was recorded with MB medium containing 0.1 mg/L NAA.

Table (4): Effect of different media on the number of secondary embryos of date palm genotypes after 3 months.

Medium (M)	Cultivars (C)					Mean
	Bartamuda	**Shamia**	**Gondeila**	**Malakaby**	**Sakkoty**	
MA	10.67	9.33	9.67	9.67	11.00	**10.1 A**
MB	6.00	5.67	6.00	7.00	5.33	**6.0 D**
MC	7.67	7.00	7.33	7.67	6.33	**7.2 C**
MD	8.33	7.33	7.67	6.67	8.00	**8.0 B**
Mean	**8.17 a**	**7.33 b**	**7.67 ab**	**8.25 a**	**7.67 ab**	

L.S.D at 5% C=O.6332 M=0.5663 C*M=1.266

Concerning the interaction between date palm genotypes and different media, the data indicated that the highest value of the secondary embryo number was observed when Sakkoty explants were cultured on the control medium (11.00), followed by Bartamuda

cultivar on the same medium. The lowest significant values of secondary embryo numbers were observed when Sakkoty, Shamia, Gondeila and Bartamuda cvs. explants when cultured on MS medium containing 0.1mg/l NAA (MB) as the values were 5.33, 5.67, 6.0 and 6.0, respectively, without significant difference among them.

Results under discussion indicated that germination percentages ranged from 15 to 30 regardless the cultivar and nutrient medium used and the best medium was MS medium supplemented with 0.1 mg/l NAA + 10 mg/l Thiamine-HCl + 200 mg/l glutamine + 5 mg/l Ca-pantothenate which achieved the highest value (30%) with Bartamuda and Malakaby cultivars. In this respect, Mater (1986) reported mature nodules germinated within 2 months on medium containing NAA at 0.1 mg/l, and the second subculture of individual embryos (5 - 10 mm long) on the same medium was necessary to avoid competition and promote plantlet growth of date palm.

MS basal medium containing NAA, glutamine , thiamine HCl and Ca-panthothianate achieved 27% germination percentage for dry date palm somatic embryogenesis as compared with MS basal medium with NAA (23%) or glutamine , thiamine HCl, and Ca- panthothianic acid (20%) regardless cultivars used. It may be due to the interaction between NAA, amino acids and vitamins used (organic compounds). Organic acids have three roles in plant tissue culture media ; they may act as chelating agents , improving the ability of some micronutrients ,they can buffer the medium against pH change and they may act as nutrients (George , 1993).

Abo El-Soaud (1999) found that somatic embryos when isolated and cultured on fresh media produced numerous embryos and plantlets. The small embryos germinated into plantlets which produced adventitious roots with MS medium containing 0.1 mg/l NAA. The healthy rooted plantlets were transferred to soil through a series of acclimatization procedures.

In this respect, Purohit (1999) mentioned that at low auxin levels, shoot meristem formation is usually achieved early after the initiation of cotyledons, so that under inappropriate culture conditions, germination can occur prematurely to give weak or inviable plantlets. High auxin levels can inhibit development and growth of the shoot meristem if young pro-embryos are not transferred to a low-auxin or

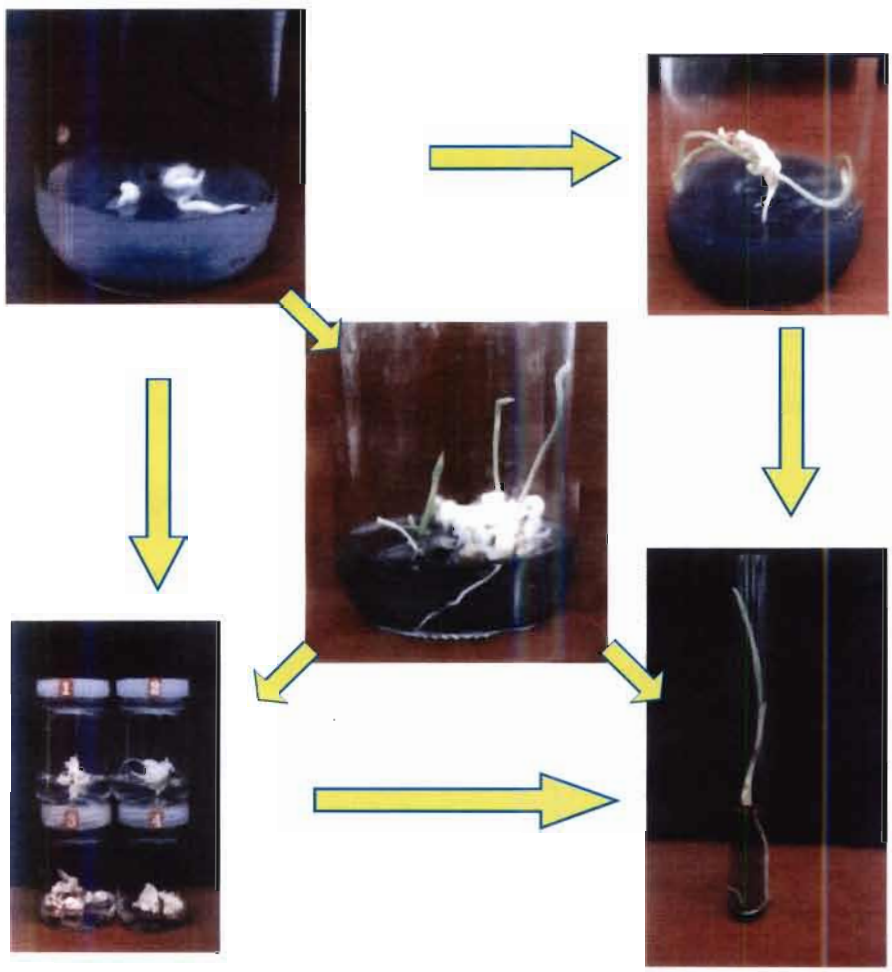

Fig. (1) : Maturation and Germination of(Malakaby cv.) somatic embryo.

zero-auxin medium after induction. It is essential that for germination an embryo must have functional shoot and root apices with the activity of meristematic growth.

Secondary embryos are also known as repetitive, accessory or proliferative. The power of embryo cloning techniques and their exploitation for mass propagation, metabolite production or genetic transformation have recurrent embryogenesis as their bases.It also occurs when primary somatic embryos fail to mature normally into plantlets and instead give rise to successive cycles of embryos, most commonly from superficial cells of the cotyledons or hypocotyls. The process is probably homologous with the proliferation of globular pro - embryos in standard embryogenic cultures, differing only with respect to the stage at which integrated control of development is lost. Expression of secondary embryogenesis may become a problem, if it can not be controlled when germination and normal growth are required (Purohit, 1999).

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إكثار الأصناف الجافة المصرية لنخيل البلح باستخدام الزراعة النسيجية
٢- تأثير مكونات الوسط الغذائى على تكوين الاجنة الجسدية

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ملخص

أجرى هذا البحث لدراسة تأثير التركيزات المختلفة للسيتوكينينات (الكينيتين Kin. -ايزوبنتينيل ادينين 2iP—بنزيل ادينين BA-بنزيل ادينين ريبوزييدBAr) بالاضافة الى٠,١ ملجم نفثالين حمض الخليك NAA أو عدم وجوده على تكوين ونضج الأجنة الجسدية لبعض أصناف نخيل البلح الجافة (سكوتى -ملكابى-جنديلة-شامية-برتمودا). كما شملت الدراسة تأثير الجلوتامين (٢٠٠ ملجم/ لتر) والثيامين (١٠ ملجم/ لتر) وكذلك بانتوثينات الكالسيوم (٥ ملجم /لتر) على انبات الاجنة وتكوين الاجنة الثانوية. ولقد اثبتت النتائج ان الوسط الغذائى المحتوى على بيئة MS والمضاف اليه ٠,١ ملجم/ لتر NAA شجعت تكوين الأجنة (متوسط عددالاجنة ٦,٣) يليها بيئة المقارنة (متوسط عدد الاجنة ٥,٩) حيث ان هذا الوسط الغذائى لايحتوى على منظمات النمو النباتية Mo) والبيئة M6 (MS+ ٠,٢ ملجم / لتركينيتين) حيث وصل متوسط عدد الاجنة الى ٥,٨ جنين بينما كانت أقل قيمة لتكوين الأجنة عند استعمال ٠,١ ملجم كينيتينن + ٠,١ ملجم/لتر نفثالين حمض الخليك NAA . سجل صنف الشامية تفوقا فى عدد الأجنة (متوسط عدد الاجنة ٦,٥٢) يليه صنفى الجنديلة (متوسط عدد الاجنة٥,٧) والبرتمودا (٤,٧) بينما لوحظت أقل قيمة من عدد الأجنة مع السكوتى (٤,٠) والملكابى (٣,٩).

وجد أن الوسط الغذائى الخالى من منظمات النمو النباتية (بيئة المقارنة) عمل علىزيادة طول الأجنة بينما أعطت البيئة التى تحتوى على ٠,١ ملجم/ لتر كينيتين + ٠,١ ملجم / لتر NAA أقل قيمة لطول الأجنة . سجل صنف الشامية أعلى قيمة لطول الأجنة يليه صنفى البرتمودا والملكابى بينما سجل صنف السكوتى أقل قيمة لطول الأجنة. ولقد سجلت- البيئةMD تفوقا فى نسبة الإنبات

(٢٧,٠%) يليها البيئة MB حيث كانت نسبة انبات الاجنة ٢٢ %. بينما سجلت البيئة MC أقل نسبة للإنبات٢٠%. سجلت الأصناف البرتمودا – الملكابى والسكوتى أعلى نسب للإنبات بينما سجل الصنف الجنديلة أقل نسبة للإنبات .

– وجد أن البيئة المغذية MA حققت أعلى قيمة فى عدد الأجنة الثانوية (حيث كان متوسط عدد الأجنة الثانوية ١٠,١) يليها البيئة MD (٨) بينما البيئةMB حققت أقل عدد من الأجنة الثانوية (٨). ولقد سجل صنفى الملكابى (٨,٢٥) والبرتمودا (٨,١٧) أعلى قيمة للأجنة الثانوية بينما سجل الصنف شامية أقل القيم (٧,٣٣) بغض النظر عن الوسط الغذائى المستخدم.