# A method for *in vitro* propagation of the Egyptian date palm cultivar Samany

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## ABSTRACT

*The Egyptian date palm cultivar Samany was used as a starting plant material for in vitro micropropagation. Different types of explants (shoot tips, lateral buds, primordial leaves and leaf middle ribs) excised from offshoots were used. The explants were surface sterilized by dipping in 70 % Ethanol for 90 Sec, followed by dipping in 100% Clorox for 30 min., then dipping in 1.5 g/l $HgCl_2$ for 5 min. Supplement of the culture medium with 80 or 100 mg/l 2,4-D gave the best results of callus proliferation only from leaf middle ribs. However, addition of 100 mg/l 2,4-D, 3 mg/l 2iP and 3 mg/l Kin gave the best results of callus improvement, quality characterization and appearing of many embryo-like structures. Transfer of the embryogenic calli on medium amended with 3 mg/l Kin, 3 mg/l 2iP and 3 mg/l NAA gave the best results for inducing date palm plantlets from Samany explants in vitro. Transfer of the embryogenic calli to medium without plant hormones gave shoots only. It was concluded that suitable sterilization of explants, type of explant and plant growth regulator play important roles in micropropagation of date palm in vitro.*

***Key words**: Micropropagation, In vitro, Tissue culture, Date palm, Phoenix dactylifera.*

## INTRODUCTION

Historically, date palm (*Phoenix dactylifera* L.) is a tropical and sub-tropical tree belonging to the family Palmae (Arecaceae) that represents about 200 genera and 2500 species of woody perennial monocotyledon plants (Moore, 1973). The date palms are cultivated primarily for fruit as a high energy food of high sugar content as well as a good source of iron and potassium. Moreover, the date palm trees are not only utilized to provide food, shelter, fiber, fuel, clothing, furniture but also used for folk medicine.

Palm tree can tolerate the environmental stresses such as salinity, drought, high temperature and high water content of the soil. It is an excellent tree for cultivation in various regions of the globe (Carpenter and Ream, 1976). It is known that propagation of date palm through seeds will give rise to unqualified variable fruits as well as half of the produced plants will produce male progenies, which cannot be ditinguished at least before four to six years after sowing. Moreover, propagation of the maternal homogeneous date palm trees from seeds is very limited due to their high degree of genetic heterozygosity and dioecious nature. Conventionally, the only source of obtaining maternal homogeneous date palm plant occurrs only when using the offshoots or suckers that arise from the base of the mother

plants. However, the availability of offshoots or suckers is very limited in number during the whole life of palm trees. So, it is not sufficient for commercial production of date palm trees (Moursy and Saker, 1996). Therefore, tissue culture micropropagation in date palm has been employed as a unique technique to aid in the propagation of numerous plants to overcome the previous problems (Shaheen, 1990). Several reports have been reported on the success of date palm cloning *via* tissue culture approaches (Tisserat, 1979, 1981 & 1984; Zaid and Tisserat, 1983; Abo El-Nil, 1986; Mater, 1986; Ibrahim and Hegazy, 1998 & 1999; Ibrahim, 1999; Al-Ghamdi, 2000). Therefore, using tissue culture as an alternative technique istead of seed propagation to produce large numbers of genetically uniform date palm trees seems advatageous. The present study reports a successful method of *in vitro* micropropagation of the famous Egyptain date palm cv.Samany.

## MATERIALS AND METHODS

### Plant material

Date palm (*Phoenix dactylifera* L.) offshoots of the Egytian cultivar Samany were secured from mother trees grown in Rashid region as the best source for growing excellent trees of this cultivar. The measurements of offshoots were 150 cm in height, 30 cm in diameter and 30-50 Kg in weight. The external leaves of offshoots were gradually acrobatically separated using hatchet and serrated knife. Lateral buds with the optimum size (0.5-1.5 cm) were carefully excised. After taking off the most hard leaves, the shoot tips and the soft basal leaves were excised and prepared for sterilization.

### Sterilization

This part of study included the determination of the efficiency of different materials such as Clorox, ethanol 70% and $HgCl_2$ on sterilizing different date palm explants.

### Pretreatment

Different explants i.e., shoot tips, lateral buds and primordial leaves excised from the offshoots and leaf middle ribs free from leaflets were surface sprayed using ethanol 70 % and washed with tap water and then with distilled water for 5-6 times. Suitable size of shoot tips, lateral buds, basal and primordial leaves and leaf middle ribs were kept until use in sterilized distilled water containing 1.5 g/l from each of the pre-filtered ascorbic acid and citric acid as antioxidants.

### Post-treatment

Shoot tips and other explants were surface sterilized under aseptic conditions using different sterilized materials as follows:

One part of the pretreated explants was transferred and immersed in 100% commercial Clorox for 15, 20, 30, 40 and 50 min as a first treatment. Secondly, another part of date palm explants was treated with 70 % ethanol for 90 sec and then immersed in 100% commercial Clorox for the same previous times as a second treatment. The remaining parts of the explants were also treated as the second treatment and followed by immersion in pre-filtered sterilized 1.5 g/l $HgCl_2$ for 5 min. The sterilized explants were then rinsed in sterilized distilled water 6 times. Sterilized explants were maintained in 150 mg/l pre-filtered sterilized mixture of ascorbic acid + Citric acid. Four shoot tips, 10 lateral buds, 10 leaf middle ribs and 10 primordial leaves were used per each clorox time treatment per experiment.

**Medium composition**

Sterilized shoot tips, lateral buds, primordial leaves and leaf middle ribs were cultured on modified solidified basic MS nutrient medium (Murashige and Skoog, 1962). The basic MS medium was supplemented with 170 mg/l Na $H_2PO_4.2H_2O$, 200 mg/l $KH_2PO_4$, 10 mg/l thiamin HCl, 200 mg/l glutamine, 0.15-0.30% activated charcoal, 20 mg/l adenine sulfate, 1.7 g/l phytagel and 30 g/l sucrose and was designated as MSm. The MSm medium was also amended with various plant growth regulators, i.e., cytokinins as 1-5 mg/l 2-isopentenyl adenine (2iP); 1-5 mg/l kinitin (Kin) or auxins as 60-200 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D) or 1- 4 mg/l naphthalene acetic acid (NAA) either solely or in combinations. The pH of the media was adjusted to 5.7 with 0.1 M NaOH or 0.1 M HCl. 150 ml of the used nutrient media were dispensed into jars of 400 ml in size. The media were then autoclaved at 121°C for 20 min. All cultures with the explants were maintained at a constant temperature of 28 ± 1°C in a growth chamber under conditions of 1500 Lux from cool white lamps for 16 hr light / 8 hr dark. The subculturs were renewed every 30 days and data were recorded every 60 days.

**Callus initiation**

For enhancing of callus induction, the explants were cultured on MSm medium supplemented with various concentrations (0, 60, 80, 100 and 200 mg/l) of 2,4-D. Data of this experiment were recorded every two months as a percentage of explants induced callus, viable non-callused explants and dead explants.

**Embryogenic callus proliferation**

For proliferation embryogenic calli and embryo-like structures, the initaited calli were transferred into MSm medium supplemented with various concentrations (1, 2, 3, 4 and 5 mg/l) of 2iP and (1, 2, 3, 4 and 5 mg/l) of Kin and 100 mg/l 2,4-D. The data were recorded every 30 days of subculture.

**Shoot induction and root initiation**

For enhancing the shoot and root formation, the embryogenic callus and the embryo-like structures were cultured on hormone-Free MSm medium or MSm medium supplemented with 1, 2, 3 and 4 mg/l 2ip, 1, 2, 3 and 4 mg/l) Kin and 1, 2, 3 and 4 mg/l NAA.

## RESULTS AND DISCUSSION

Data in Table (1) show that the best results of disinfected surviving explants were obtained from immersion of explants for 50 min in commercial Clorox 100% (5.25 gl/l) from the first treatment. Data showed also that using 70% ethanol as a pretreatment had increased survival of explants. It was found that the immersion of explants for 5 min in $HgCl_2$ after the treatment of explants with ethanol 70% followed by 100% Clorox for 30 min increased the percentages of the disinfected and surviving explants (third treatment). Moreover, increasing exposure time to Clorox followed by 5 min in $HgCl_2$ decrease the percentages of the survived explants but without any infection. However, date palm explants have particularly proven to be difficult to surface sterilization under sterile conditions (Tisserat, 1979&1981) because the plant material is taken from an open field growing for not less than three years under unprotected environmental conditions.

It is concluded that the best methodology for surface sterilization of the date palm explants excised from the cultivar Samany is the treatment with the formula 70 % ethanol for 90 Sec + 100% commercial Clorox for 40 min, followed by immersing the treated

***Table (1): Percentages of disinfected survived explants of Samany date palm as affected by various sterilization methods.***

| Time in min for Clorox | % disinfected survived explants | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Clorox 100% | | | | Ethanol 70% for 90 sec + Clorox 100% | | | | Ethanol 70% for 90 sec + Clorox 100% + $HgCl_2$ for 5 min | | | |
| | ST | LB | LMR | PL | ST | LB | LMR | PL | ST | LB | LMR | PL |
| 15 | 25 | 10 | 10 | 10 | 25 | 20 | 10 | 20 | 25 | 20 | 20 | 20 |
| 20 | 25 | 20 | 10 | 10 | 25 | 30 | 20 | 20 | 25 | 30 | 40 | 30 |
| 30 | 25 | 20 | 20 | 20 | 50 | 40 | 30 | 20 | 75 | 60 | 50 | 40 |
| 40 | 50 | 30 | 30 | 20 | 75 | 40 | 30 | 30 | 100 | 100 | 100 | 100 |
| 50 | 50 | 30 | 30 | 20 | 75 | 50 | 40 | 40 | 100 | 100 | 100 | 100 |

ST = shoot tip, LB = lateral bud, LMR = Leaf middle rib, PL = primordial leaf.

***Table (2): Percentages of callus induction from different explants of cultivar Samany of date palm at various periods of culturing on MSm medium supplemented with various concentrations of 2,4-D.***

| Plant growth Regulators and Days of subcultures | % callused (C ), non callused viable (NCV) and Dead (D) explants | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot tip | | | Lateral bud | | | Leaf middle rib | | | Primordial leaf | | |
| | C | NCV | D | C | NCV | D | C | NCV | D | C | NCV | D |
| 0 mg/l 2,4-D | | | | | | | | | | | | |
| 60 days | 0 | 83.33 | 16.67 | 0 | 91.66 | 8.34 | 0 | 88.88 | 11.12 | 0 | 80 | 20 |
| 120 days | 0 | 88.33 | 16.67 | 0 | 83.33 | 16.67 | 0 | 88.88 | 11.12 | 0 | 70 | 30 |
| 180 days | 0 | 66.66 | 33.34 | 0 | 75 | 25 | 0 | 66.67 | 33.33 | 0 | 60 | 40 |
| 240 days | 0 | 50 | 50 | 0 | 66.66 | 33.34 | 0 | 66.67 | 33.33 | 0 | 40 | 60 |
| 60 mg/l 2,4-D | | | | | | | | | | | | |
| 60 days | 0 | 66,67 | 33.33 | 0 | 75 | 25 | 0 | 88.88 | 11.12 | 0 | 80 | 20 |
| 120 days | 0 | 66.67 | 33.33 | 0 | 66.67 | 33.33 | 0 | 77.77 | 22.23 | 0 | 70 | 30 |
| 180 days | 0 | 50 | 50 | 0 | 58.33 | 41.67 | 0 | 77.77 | 22.23 | 0 | 50 | 50 |
| 240 days | 0 | 33.33 | 66.67 | 0 | 50 | 50 | 0 | 66.67 | 33.33 | 0 | 40 | 60 |
| 80 mg/l 2,4-D | | | | | | | | | | | | |
| 60 days | 0 | 66.67 | 33.33 | 0 | 75 | 25 | 0 | 77.78 | 22.22 | 0 | 70 | 30 |
| 120 days | 0 | 50 | 50 | 0 | 66.67 | 33.33 | 0 | 66.67 | 33.33 | 0 | 60 | 40 |
| 180 days | 0 | 50 | 50 | 0 | 50 | 50 | 22.22 | 44.45 | 33.33 | 0 | 50 | 50 |
| 240 days | 0 | 33.33 | 66.67 | 0 | 33.33 | 66.67 | 11.11 | 44.45 | 44.44 | 0 | 40 | 60 |
| 100 mg/l 2,4-D | | | | | | | | | | | | |
| 60 days | 0 | 50 | 50 | 0 | 50 | 50 | 0 | 77.78 | 22.22 | 0 | 60 | 40 |
| 120 days | 0 | 50 | 50 | 0 | 33.33 | 66.67 | 11.12 | 44.44 | 33.33 | 0 | 50 | 50 |
| 180 days | 0 | 33.33 | 66.67 | 0 | 25 | 75 | 22.22 | 33.34 | 44.44 | 0 | 30 | 70 |
| 240 days | 0 | 33.33 | 66.67 | 0 | 16.66 | 83.34 | 22.22 | 22.22 | 55.56 | 0 | 20 | 80 |
| 200 mg/l 2,4-D | | | | | | | | | | | | |
| 60 days | 0 | 25 | 75 | 0 | 16.67 | 83.33 | 0 | 44.44 | 55.56 | 0 | 20 | 80 |
| 120 days | 0 | 16.66 | 83.34 | 0 | 0 | 100 | 0 | 33.33 | 66.67 | 0 | 0 | 100 |
| 180 days | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 22.22 | 77.78 | 0 | 0 | 100 |
| 240 days | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 11.12 | 88.88 | 0 | 0 | 100 |

6 shoot tips, 12 lateral buds, 9 leaf middle ribs and 10 primordial leaves were used per each treatment

explants in $HgCl_2$ at the concentration of 1.5 g/l for 5 min. However, contamination by infectious forms of microorganisms is considered as a bottle neck in *in vitro* micropropagation. Therefore, the work with tissue cultures must be carried out under very complicated and effective aseptic conditions.

Data in Table (2) show no chance for the various explants to respond for callus induction on MSm medium free of the plant growth regulators (0 mg/l 2,4-D) or on medium supplemented with 60 mg/l or 200 mg/l 2,4-D until 240 days of incubation. However, addition of 80 mg/l and 100 mg/l of the 2,4-D to growth medium enhanced the emergence of the shoot tips (Fig. 1) but not lateral buds and also enhanced the callus formation only from 11.12 % and 22.22 % of the middle ribs explants (Figs. 2 and 3), respectively, giving the best record after 240 days of incubation. In this respect, the presented data are agreed with results of Ibrahim and Hegazy (1998), who reported that hormone-free medium inhibited the morphogenesis process in starting stage of cultivation of the date palm explants. However, Ibrahim (1999) found that the callus induction could be enhanced from shoot tips and primordial leaf explants on medium free of plant hormones. Moreover, Al-Ghamdi (2000) induced the callus from buds and leaf primordia but not from cork tissue on medium supplemented with 100 mg/l 2,4-D + 3 mg/l 2ip + 170 mg/l $Na\ H_2\ PO_4$ + 100 mg/l inositol. On the other hand, the present data agree with the work of Tisserat (1979, 1981), Abo El-Nil (1986) and Mater (1986), who found that addition of high amount of 2,4-D had a strong effect on callus production from shoot tip, bud and primordial leaf explants. However, in this work, the callus could be induced only from the middle rib explants but not from shoot tip, lateral bud or primordial leaf explants. This might be the first time to report callus induction from middle rib explants for *in vitro* micropropagation of date palm. The initiated callus from the middle rib explants was creamy white in colour with nodular structures. In this respect, it was reported that addition of 100 mg/l 2,4-D to callus inducing medium enhanced the formation of the embryogenic callus from other types of date palm explants (Tisserat, 1979; Zaid and Tisserat, 1983; and Ibrahim, 1999). It was noticed that increasing the amount of 2,4-D increased the callus mortality connected with increasing the period of incubation.

Data in Table (3) show that the addition of plant growth regulators (2ip and Kin) to the MSm medium containing 2,4-D was stimulative for increasing the percentage of the embryogenic calli rich with the embryo-like structures (Fig. 4). However, the best MSm medium for increasing callus mass, maintenance and giving the highest percentage of optimum callus with developed embryo-like structures (Fig 4) from the middle rib explants is that composed of MSm + 100 mg/l 2,4-D + 3 mg/l Kin +3 mg/l 2ip. The present results agreed with those of Ibrahim (1999) in date palm tissue culture. It is concluded that Kin plays an important role for induction of date palm somatic embroys *in vitro*.

Data in Table (4) show that MSm medium without plant growth regulators stimulated only the induction of 27 non-rooted shootlets (Fig. 5) out of 40 embryogenic callus cultures. Moreover, addition of plant growth regulators with low concentrations (1 or 2 mg/l) of Kin, 2ip and NAA was not helpful for inducing *in vitro* rooted plantlets but favored for non-rooted shootlets induction (Fig. 6) from the embryogenic callus cultures giving.

The concentration of 3 mg/l of each of Kin, 2ip and NAA was the only combination helpful for rooted plantlet induction (Fig. 7). Out of 50 callus cultures, 23 rooted plantlets could be obtained. On the other hand, it was

*Table (3): Influence of different concentrations of 2ip and Kin added to MSm medium supplemented with 100 mg/l 2,4-D for advancing the growth of the embryogenic callus and embryo-like structure production.*

| MSm with plant growth regulators ($mgl^{-1}$) | | | No. tested callus cultures | % embryongenic (E) and non embryongenic (N) callus cultures at 4 subculture periods | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 30 days | | 60 days | | 90 days | | 120 days | |
| 2,4-D | 2ip | Kin | | E | N | E | N | E | N | E | N |
| 100 | 0 | 0 | 10 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| 100 | 1 | 1 | 15 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| 100 | 2 | 2 | 14 | 14.2 | 85.8 | 21.4 | 78.6 | 28.6 | 71.4 | 28.6 | 71.4 |
| 100 | 3 | 3 | 20 | 45 | 55 | 60 | 40 | 75 | 25 | 75 | 25 |
| 100 | 4 | 4 | 20 | 15 | 85 | 20 | 80 | 25 | 75 | 25 | 75 |
| 100 | 5 | 5 | 20 | 5 | 95 | 10 | 90 | 10 | 90 | 10 | 90 |

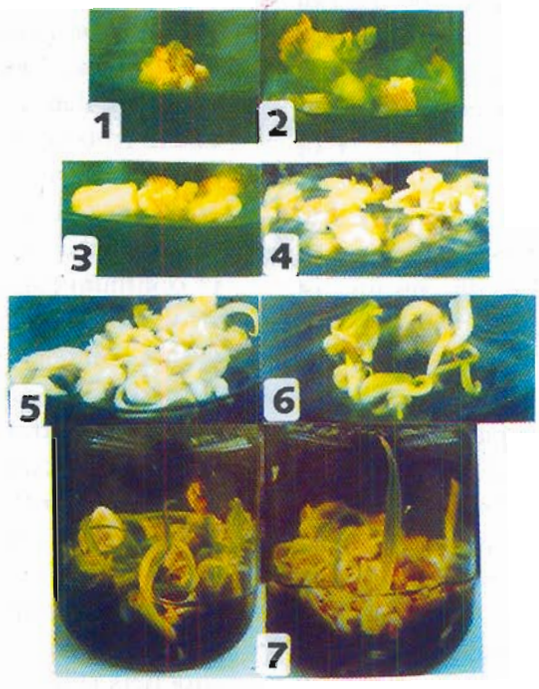


*Fig. (1): Emergence of shoot tip of date palm (Samany) cultured on Msm medium supplemented with 100 mg/l 2,4-D.*

*Fig. (2 and 3): Induction of creamy white callus on the end of the middle rib explants with or without leaflets on MSm medium supplemented with 100 mg/l 2,4-D.*

*Fig. (4): Enhancing the growth of embryogenic callus and embryo-like structures on MSm medium amended with 100 mg/l 2,4-D and 3 mg/l of each of 2ip and Kin .*

*Fig. (5): Enhancing of shootlets on MSm medium free of plant hormones.*

*Fig. (6): Development of the shootlets on MSm medium supplemented with 100 mg/l 2,4-D + combinations of 1 or 2 mg/l of each of 2ip and Kin.*

*Fig. (7): Induced and developed date palm Samany plantlets on MSm medium amended with 3 mg/l of each of 2ip, Kin and NAA.*

***Table (4): Induction of shoots with or without roots after 3 months of culturing 1 gram of embryongenic calli with embryo-like structures on MSm medium with or without plant growth regulators.***

| MSm with plant growth regulators (mg/l) | | | Induction of shoots with and without roots | | |
|---|---|---|---|---|---|
| Kin | 2ip | NAA | No. tested cultures | No. un-rooted shoots | No. rooted shoots |
| 0 | 0 | 0 | 40 | 70 | 0 |
| 1 | 1 | 1 | 50 | 10 | 0 |
| 2 | 2 | 2 | 50 | 15 | 0 |
| 3 | 3 | 3 | 50 | 0 | 23 |
| 4 | 4 | 4 | 50 | 0 | 0 |

Found that increasing the concentration of each plant growth regulator to 4 mg/l was not effective for shoot initition, but it was stimulative for callus mass growth. Such results agreed with those of Ibrahim and Hegazy (1999), who could obtain *in vitro* date palm shootlets on medium amended with 2ip, Kin and NAA. They also reported that *in vitro* rooting of date palm shoolets proved to be difficult on medium free of plant growth regulators. It was observed that the high concentration of plant growth regulators (4 mg/l) inhibited the organogenesis and incouraged the callus mass growth. This result is in agreement with Tisserat (1979), Abo El-Nil (1986) and Mater (1986). However, Zaid and Tisserat (1983) and Tisserat (1984) stated that growth regulators were not necessary to stimulate the shootlet proliferation or enhance the shoot differentiation, while addition of NAA may help root initiation from *in vitro* shootlets. Abo El-Nil (1986) used IBA for root initiation of date palm shootlets.

All the previous reported works on other cultivars of date palm dealt with embryogenic calli formed from the shoot tips or primordial leaf explants, whereas, the presented data indicated the initiation of embryogenic calli followed by plant regeneration from the middle rib explants, meaning that the genotype, type of explant and plant growth regulator play important roles in shootlet proliferation and root induction in date palm tissue culture.

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## الملخص العربي

### طريقة للإكثار الدقيق المعملي لنخيل البلح المصري صنف السماني

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تم في هذا البحث استخدام مجموعة من البادئات النباتية ( القمم النامية – البراعم الجانبية – الأوراق الأولية وكذلك العرق الوسطي للأوراق) من فسائل امية لنخيل البلح السماني مع رشها بالكحول 70% اثناء عمليات القطع لها. وقد تم نقعها في محلول من حمض الأسكوربك وحمص الستريك السابق تعقيمهما بالمرشحات البيولوجية كمانعات للأكسدة للمواد المفرزة من البادئات النباتية المستخدمة .وقد عقمت هذه البادئات بمعاملات تعقيم مختلفة لتطهيرها من أي ملوثات ميكروبية تكون عالقة بها. و كانت أفضل طريقه للتعقيم السطحي هى استخدام كحول الإيثانول بتركيز70% لمدة 90 ثانية ثم 100% كلوراكس لمدة 30 دقيقة ، يلى ذلك التعقيم بـــ 1.5 جرام/ لتر كلوريد الزئبق لمدة 5 دقائق وقد وجد أن إضافة 80 100 مج/لتر من منظم النمو الـــ 2,4-D أعطت أفضل نتائج لتحفيز تكوين الكالوس على البادئات النباتية من العرق الوسطي للأوراق فقط دون البادئات النباتية الأخرى ، ووجد أن إضافة 100 مج/لتر من الـــ 2,4-D مع 3 مج/لتر من كل من منظمى النمو الـــ Kin والـــ 2ip الى بيئة النمو أدى الى إنتاج كالوس غني بالتراكيب الجنينية، ووجد أن أفضل بيئة لإنتاج المستولدات النباتية هي بيئة الـــ MSm والمحتوية علي 3 مج/ لتر من كل منظم النمو الـــ Kin والـــ 2ip والـــ NAA . واتضح من الدراسة أن التعقيم السطحي الجيد وكذلك منظمات النمو ونوع البادئ النباتي كل منها يلعب دوراً هاما في إكثار النخيل معملياً. ويعتبر البادئ النباتي من العرق الوسطى للأوراق Middle rib أول محاولة ناجحة للإكثار النسيجي لنخيل البلح من خلاله.