# EFFECT OF AUXINS AND CYTOKININS ON THE *IN VITRO* PRODUCTION OF DATE PALM (*PHOENIX DACTYLIFERA* L.) BUD GENERATIVE TISSUES AND ON THE NUMBER OF DIFFERENTIATED BUDS.

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## ABSTRACT

The present study includes the effect of 18 different media developed from various combinations of two auxins (IAA and NAA) tested at the following concentrations: 0.1, 0.4, 0.8, 1.6, and 3.2 mg/l in addition to the control (no growth regulators), and also it presents the effect of 23 different media obtained from the combination of three different cytokinins (Kin, BAP, and 2iP) at different concentrations (0.1, 0.4, 0.8, 1.6, and 3.2 mg/l) on the date palm shoot bud generation from meristematic tissues of Khenezi cultivar.

The maximum percentage of bud generative tissue was induced by the addition of 1.6 mg/l IAA alone or 0.4 mg/l of both IAA and NAA to the initiation medium, and also by the addition of 3.2 mg/l 2iP or 1.6 mg/l BAP. The maximum number of differentiated buds per bud generative tissue resulted from the addition to the initiation medium of 0.8 mg/l IAA as well as to the addition of 3.2 mg/l 2iP.

Both auxins and cytokinins proved to be essential for the induction of bud generative tissues and for the differentiation of shoot buds from cultured explants.

The initiation medium contained Murashige and Skoog (1962) inorganic salts supplemented with 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 0.1 mg/l thiamine-HCl, 2 mg/l Glycine, 40 mg/l adenine sulfate, 2 g/l polyvinylepyrrolidone (PVP 40000), 3 mg/l activated charcoal, and 40 g/l sucrose.

**Additional Index words:** date palm, *Phoenix dactylifera* L., tissue culture, *In Vitro,* propagation.

# INTRODUCTION

Biotechnology has provided a promising alternative to the demand on date planting material. Plant tissue culture techniques have been employed to clone a wide range of economically important palms such as, coconuts, around the world oil palms, and date palms. These techniques cover a wide range of methodologies for the reproduction of whole plant organs such as shoots, roots and embryos under sterile conditions. They also include the culture of masses of unorganized callus or single cells, or even protoplasts. This occurs because each individual cell of a plant is totipotent (the capacity to form a whole organism when cultured under certain growth conditions). Tissue culture of date palms in the UAE gained an important momentum with the establishment of the Plant Tissue Culture Laboratory in the UAE University in February 1989.

Date palm tissue culture follows one of two methods: Asexual embryogenesis and organogenesis. The first method generates an embryogenic callus obtained from the cotyledonary sheath of date palm embryos, especially apical meristems and lateral buds. Organogenesis, on the other hand, is the method of generating a plant through culturing small plant parts (apical meristem, lateral buds or primary basis) on defined nutrient media. This results in obtaining a large number of plantlets without passing through the callus stage. Accordingly, the possibility of induced genetic variation is eliminated in the organogenesis method.

Research in the area of date palm cultivation using organogenesis technique is deficient. Little is known about the interaction between different cultivars of date palms, the time of the year during which the shoot tip is selected from the mother, and the effect of various factors on the development and growth of the tissue.

In the light of the above, the present research was conducted with the following main objectives:

1. To develop a culture medium that optimizes tissue development through the testing of various media, and

2. To quickly establish a reliable, reproducible and efficient shoot bud regeneration system for date palms using organogenesis technique.

## MATERIALS AND METHODS

The present studies were conducted through three successive seasons (1996-1998), at the Plant Tissue Culture Laboratory and its greenhouse facilities of the UAE University at Al-Ain. The studies aimed at the establishment of efficient multiplication systems from excised date palm tissues.

### Plant Materials

The conducted experiments used "Khenezi" date palm (*Phoenix dactylifera* L.) offshoots, a well-known cultivar throughout the UAE. The offshoots were collected from good renown farms in Al-Ain palm grove and transferred to the laboratory at Al-Oha area. The offshoots were 3-4 years old, collected from healthy, disease-free mother palms (Fig.1.a), and weighted approximately 7-10 kg per each bulb offshoot. The offshoots base was cleaned by running water and the outer large leaves and fibers were carefully and gradually removed by s sharp knife until the appearance of the shoot tip zone (Fig. 1.b). Special care was taken not to injure the meristematic region. Shoot tips were then carefully delimited to approximately 5-7 cm in length and 3-5 cm in width (Fig.1.c).

### Shoot tip disinfection

The excised shoot tips were cleaned by distilled water then subjected to disinfection procedure. The excised shoot tips were subjected to two consecutive disinfection steps. Firstly, the isolated shoot tips were sterilized by soaking them for 20 minutes in a fungicide solution, (Benlate at a concentration of 5 g/l). Secondly, the shoot tips were dipped in 33% commercial Clorox solution (5.2% sodium hypochlorite) for 20-25 minutes. The explants were then rinsed three times with autoclaved distilled water, each for 5 minutes under aseptic conditions provided by a laminar airflow hood, to remove any residual disinfectant before cultures are initiated.

### Treating explants with an antioxidant solution

The disinfected explants were then soaked in an antioxidant solution to minimize production of phenols (causing the browning), and to protect them from desiccation. The antioxidant solution consisted of 2 g/l polyvinylpyrolydon (PVP, Mw = 40,000), 100 mg/l sodium diethyldithiocarbonate AR (Mw=225.30), and 200 mg/l anhydrous caffeine (Mw=194.2). The shoot tips were kept in this solution until culture time.

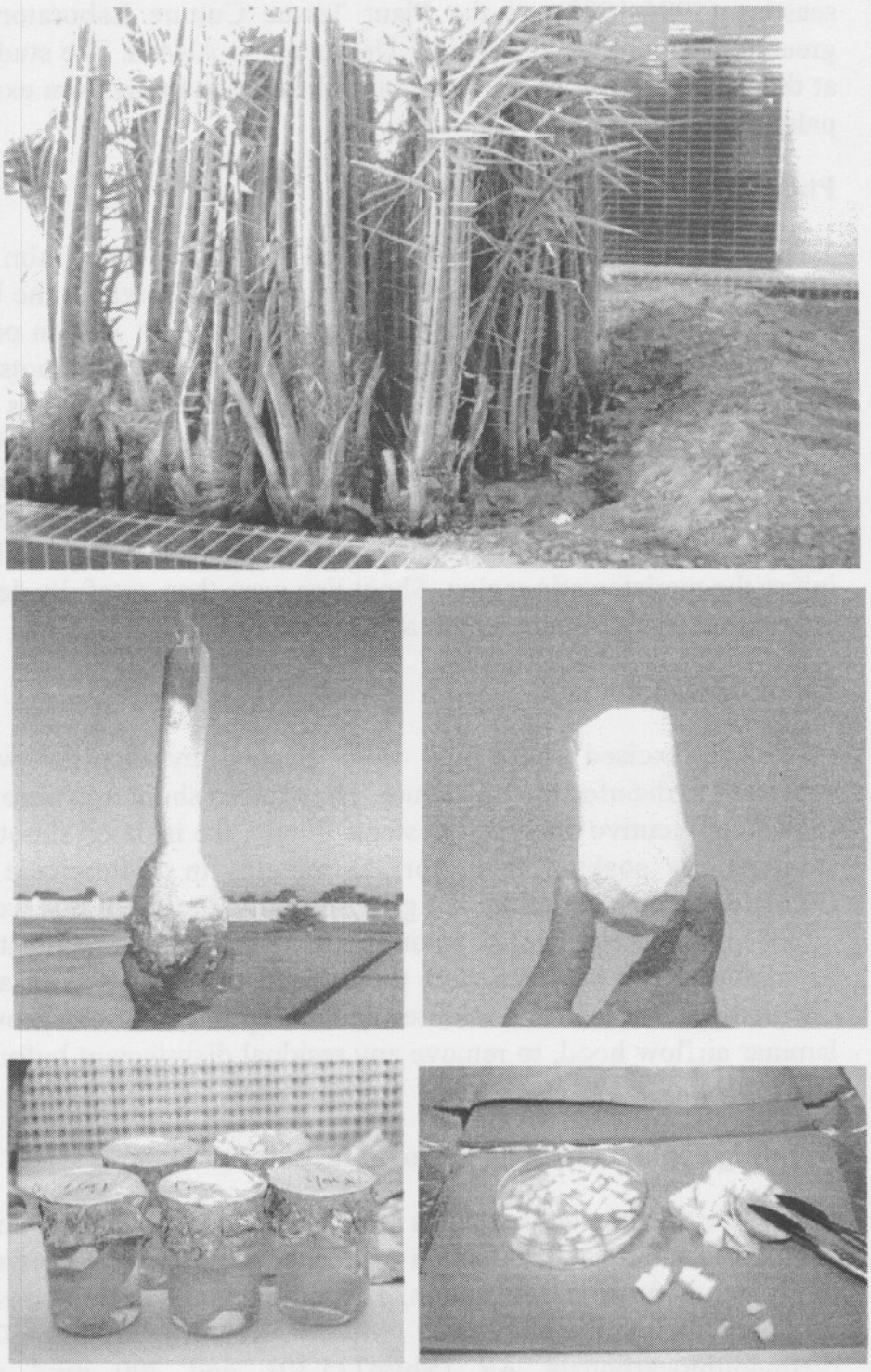


**Fig.1. Plant materials (Offshoots) used as a source of explants.**

**Culture procedure of shoot tips**

Isolated shoot tips were taken from the antioxidant solution and placed in a sterilized Petri dish containing some of the antioxidant solution. The primary xylem and bases of leaves were then cut off from the shoot tips. The rest of each explant was cut in half at right angles around the apical dome. The apical meristematic area was then divided into small pieces each of about 3-5 $mm^3$, and consideration was taken to leave some leaf primordia per explant. Each explant was then cultured on a 20 ml initiation medium in 24x200 mm test tubes.

**Initiation stage**

The initiation medium contained Murashige and Skoog (1962) inorganic salts and supplemented with 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 0.1 mg/l thiamine-HCl, 2 mg/l glycine, 40mg/l adenine sulfate, 2g/l polyvinylpyrolydon (PVP 40000), 3 g/l activated charcoal, 40 g/l sucrose, and solidified with 7 g/l agar agar. The pH was adjusted to 5.7 prior to the addition of agar agar and autoclaving was for 15 minutes at 121°C (Fig.2).

The initiation medium was supplemented with different growth regulators combinations as presented in experimental procedures. The initiation medium included activated charcoal for 2 subcultures, and then the explants were sub cultured on the same media, without charcoal until the end of the experiments. During the first four months of the initiation stage, cultures were incubated in darkness, at 28°C ± 1.

**Multiplication stage**

After four months on the initiation medium, cultures were transferred to a multiplication medium containing the same components as in initiation medium, but devoid of activated charcoal and supplemented with 30 g/l sucrose instead of 40 g/l as in the initiation medium (Fig.3). The growth regulators added to the multiplication medium were Indol acetic acid (IAA) at 0.4 mg/l, Naphthaline acetic acid (NAA) at 0.1mg/l, Kinetin (KIN) at 0.1 mg/l and $N^6$-(2-isopentyl) adenine (2iP) at 1.5 mg/l. All growth regulators were added to the medium before autoclaving, except IAA, which was added to the medium after autoclaving, at a temperature of about 55°C (sterilized through a 22µm Millipore sterilized filter). In this stage, cultures were maintained under light conditions of a 16/8-hr photoperiod at 30µMol $m^{-2}$ $sec^{-1}$. Cultures were then sub cultured every four weeks.

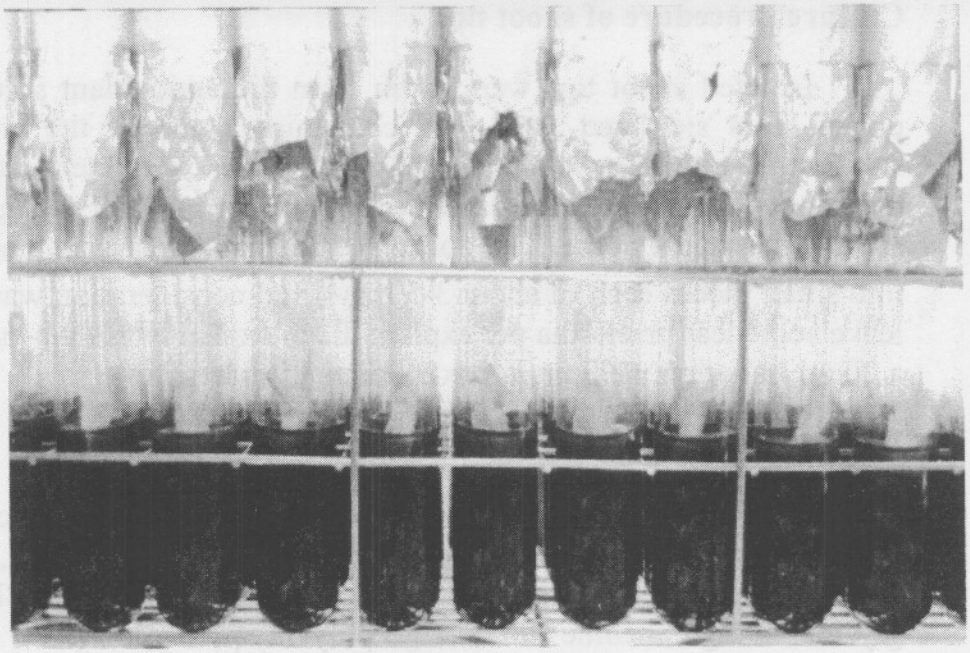


**Fig.2. The initiation stage.**

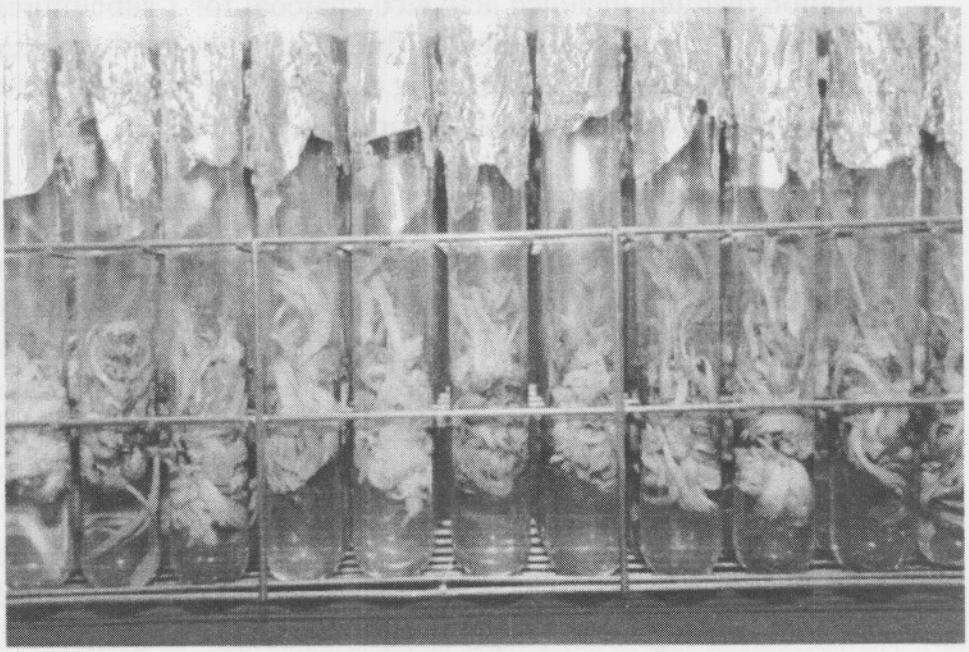


**Fig.3. The multiplication stage.**

**Elongation stage**

Multibuds formed on explants in the multiplication medium were isolated and individually separated, then cultured on an elongation medium. The elongation medium contained the same components as in initiation medium devoid of activated charcoal and growth regulators, but supplemented with 30 g/l sucrose. The cultures were kept for one month under a 16/8-hr photoperiod regime, at 30μmol $m^{-2}$ $sec^{-1}$ before being transferred to the rooting stage (Fig.4).

**Rooting stage**

Elongated shoots, 13-18 cm in length, were transferred to a rooting medium containing the same basic components as in the initiation medium, but without charcoal, and supplemented with 30g /l sucrose and 1 mg/l NAA. Cultures were kept under the same light regime as previously described in the multiplication and elongation stages, where they became ready to transfer to the greenhouse conditions (Fig.5, 6 a,b). However the plants acclimatized.

**Experimental Procedures**

**Part 1: Effect of different auxins at various concentrations on the production of bud generative tissues and number of differentiated buds per explant.**

The effect of 18 different media developed from various combinations of auxin types and concentrations, in addition to control (free hormones medium) at the initiation stage on bud regeneration from shoot-tips, was investigated. The initiation medium was supplemented with two different auxins namely indol acetic acid (IAA), and naphthalene acetic acid (NAA), each at seven different concentrations, 0.0, 0.1, 0.4, 0.8, 1.6, 3.2, and 6.4 mg/l. In addition, five media were developed from an equal combination of IAA + NAA at 0.1 mg/l each, 0.4 mg/l each, 0.8 mg/l each, 1.6 mg/l each, and 3.2 mg/l each. In addition to the tested auxins, naphthoxy acetic acid (NAO), 6-Benzylaminopurine (BAP), Kinetin (Kin) and $N^6$-(2-isopentyl) adenine (2iP) were added to all media at a fixed concentration of 4 mg/l, 0.4 mg/l, 0.4 mg/l, and 0.4 mg/l, respectively. The experiment had 16 replications (test tubes) per treatment and each tube had one explant. The experiment was set up in a randomized complete block design, and data were analyzed by analysis of variance using SAS program (SAS, 1989), with means separated by the least significant difference (LSD) test (Gomez

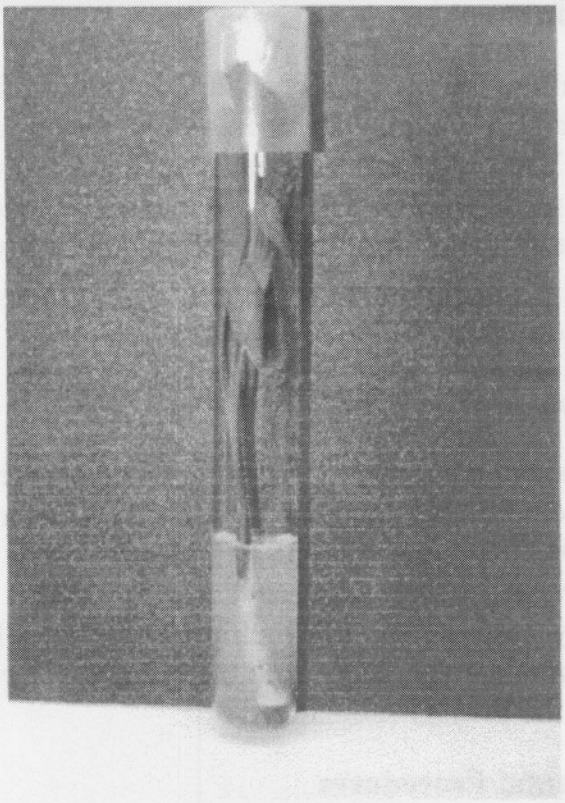


**Fig.4. The elongation stage.**

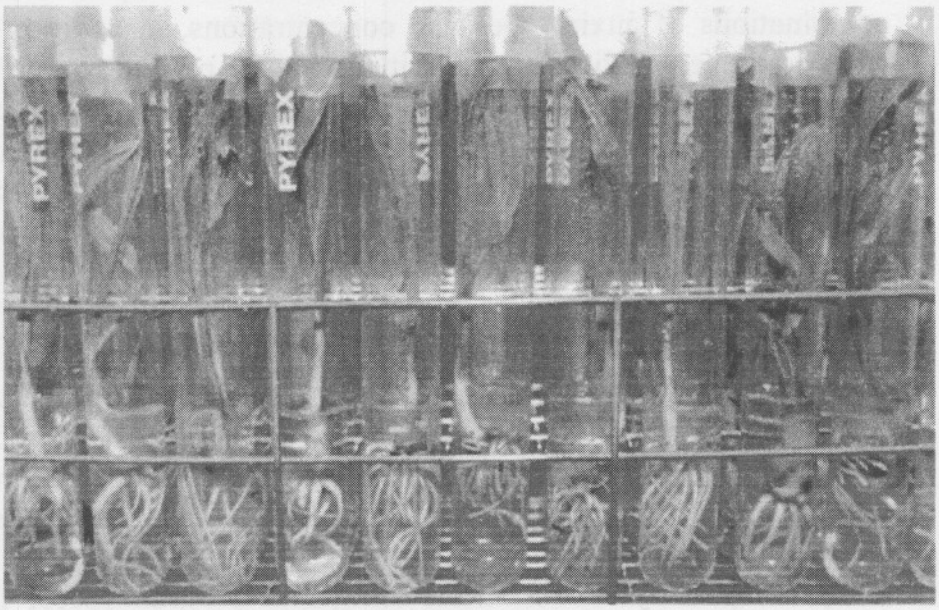
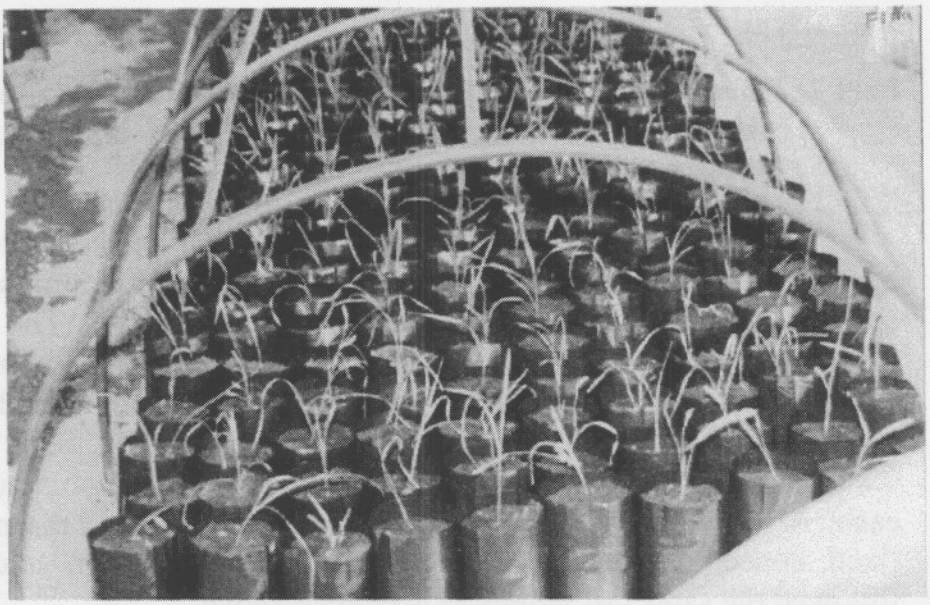



**Fig.5. The rooting stage.**



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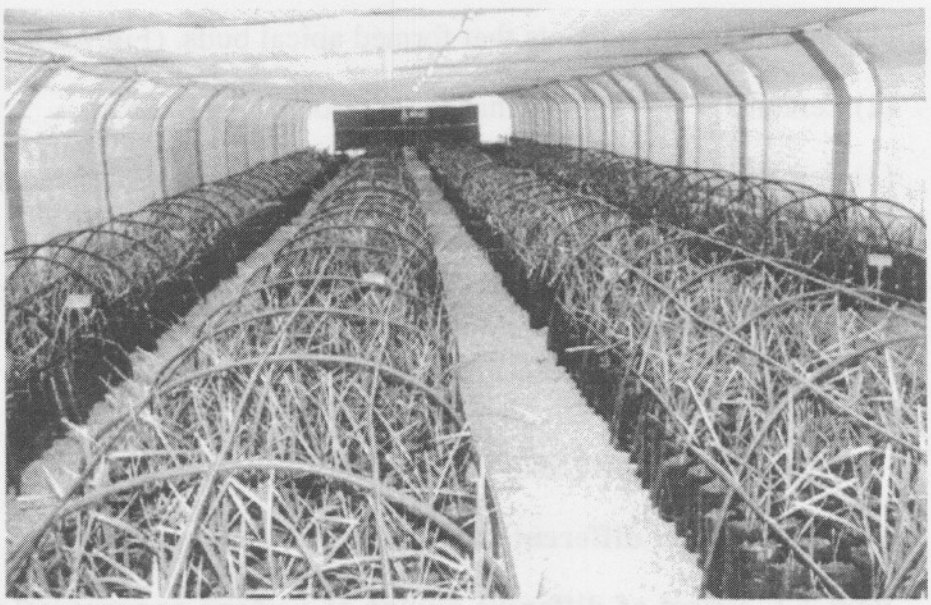


(b)

**Fig.6.a,b. Transfer of the date plantlets to the Green House.**

and Gomez, 1984). Contaminated cultures were not included in the analysis.

**Part 2: Effect of different cytokinins at various concentrations on the production of bud generative tissue and number of differentiated buds per explant.**

The effect of 23 different media developed from various combinations of cytokinin types and concentrations at the initiation stage on bud regeneration from shoot-tip, was investigated. The initiation medium was supplemented with three different cytokinins namely BAP, Kin and 2iP, each at 0.1, 0.4, 0.8, 1.6, 3.2, and 6.4 mg/l. In addition, five media were developed from an equal combination of Kin, BAP and 2iP at 0.1 mg/l each, 0.4 mg/l each, 0.8 mg/l each, 1.6 mg/l each, and 3.2 mg/l each. All auxins (IAA, NAA, and NOA) were added to the media at a fixed concentration of 0.4 mg/l. The statistical design and analysis followed the same procedures as explained above.

**Collected Data**

The following data were recorded in the experiments after 4 months in initiation culture:

(1) Percentage of explants that formed apical buds. (Fig.7.a,b,c,d).

(2) Percentage of explants that formed roots (Fig.8.a,b,c).

(3) Percentage of explants that formed bud generative tissues after 4, 5, 6, and 7 months. (Fig.9.a,b,c).

(4) Number of differentiated buds per explant was recorded after 5, 6, and 7 months from culture initiation. (Fig.10.a,b).

## RESULTS AND DISCUSSIONS

**Part 1: Effect of different auxins at various concentrations.**

**Part 1.1. Effect of different auxins at various concentrations on the percentage of explants that formed apical buds and roots from cultured shoot tips.**

The obtained results indicated that the presence of an auxin in the culture medium was not an essential requirement for the formation of apical buds from the shoot tips. However, it was essential for the rooting

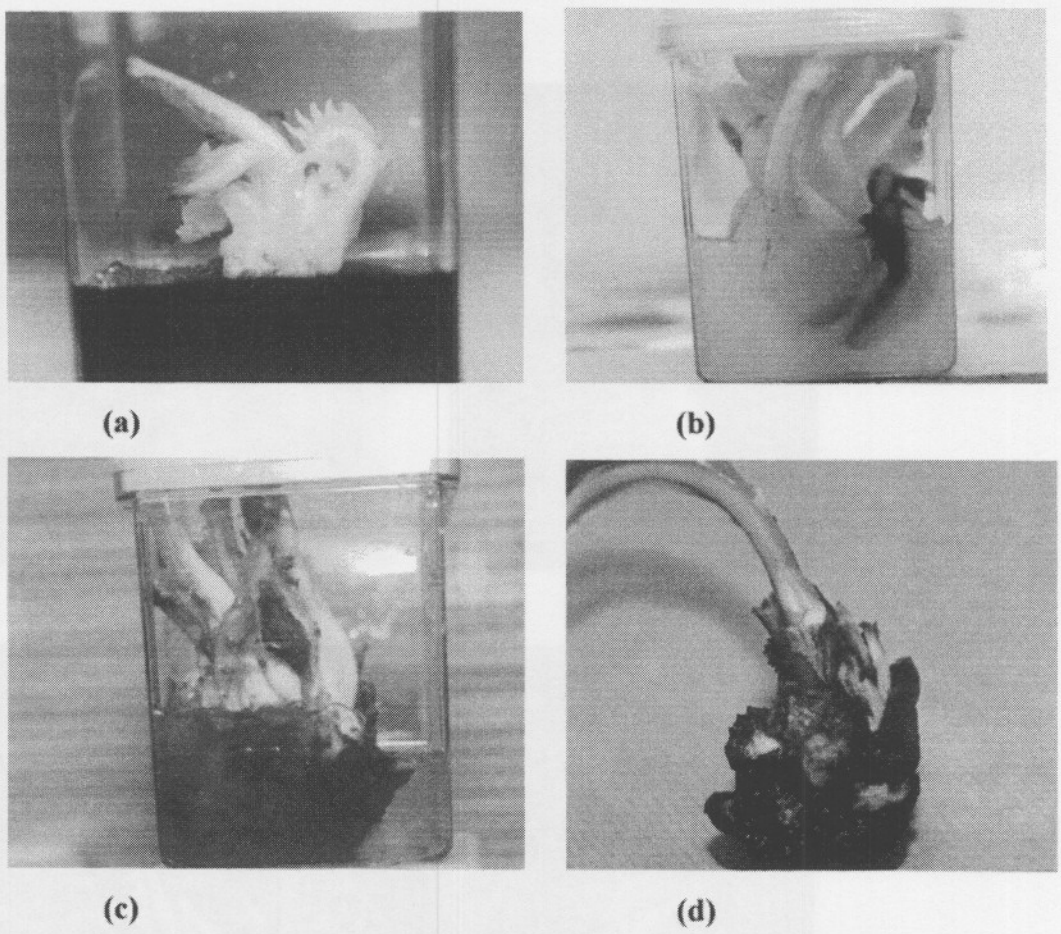
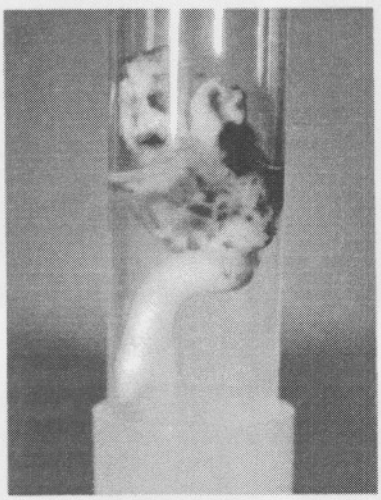



**Fig.7.a,b,c Apical bud formation at various stages of development.**



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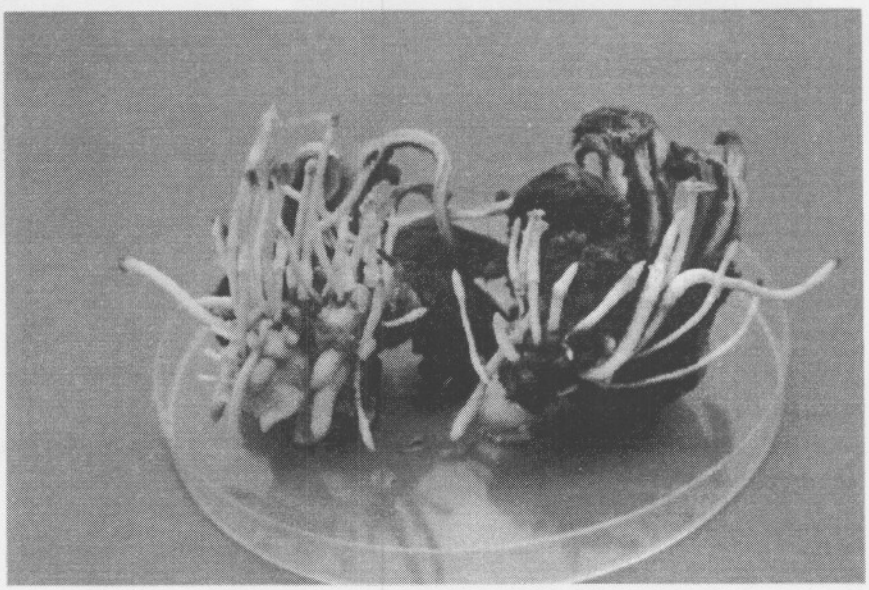

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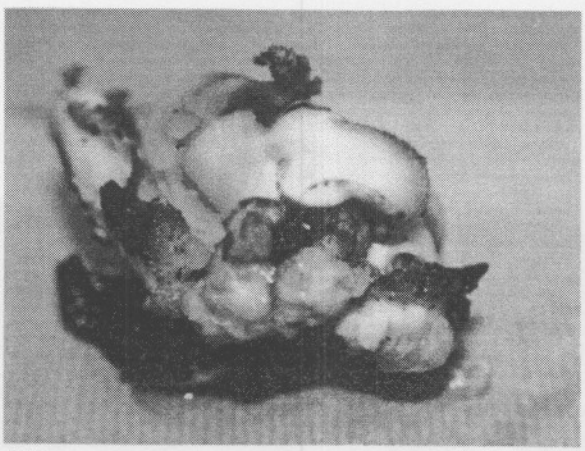
**Fig.8.a,b,c. Root formation on the explants at various stages of development.**

(a)



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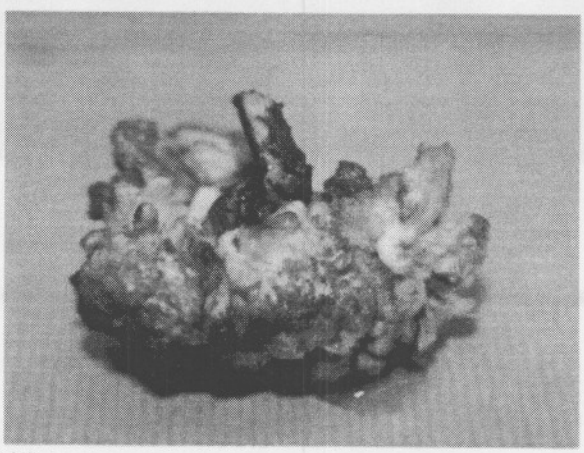

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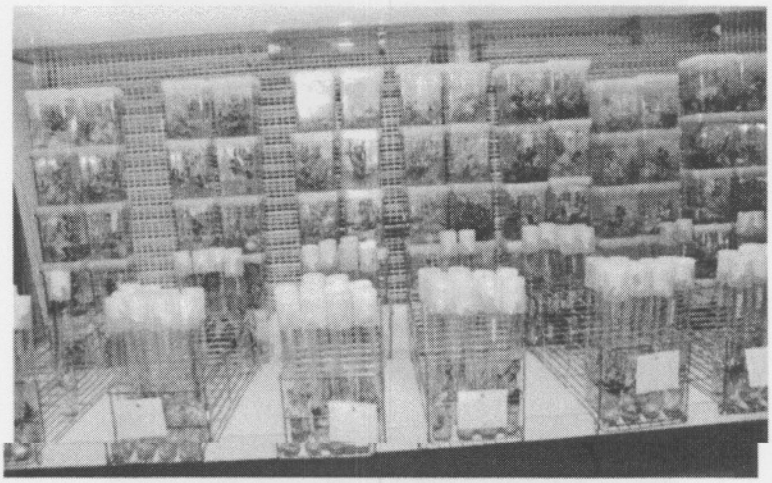
**Fig.9.a,b,c Differentiation of bud generative tissues.**

(a)



(b)

**Fig.10.a,b. Regeneration of shoots from differentiated buds.**

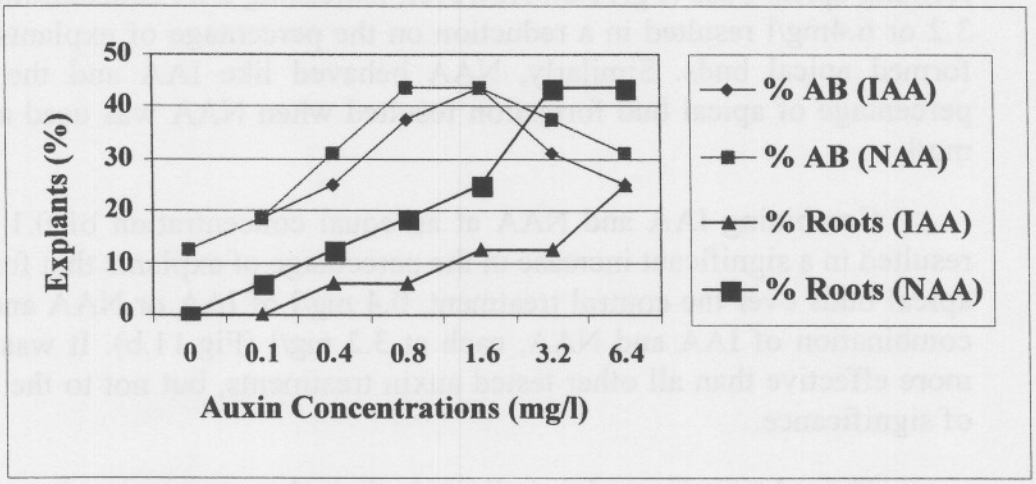
of explants (Fig.11). Increasing the level of either tested auxin IAA or NAA to 1.6 mg/l resulted in an increase in the number of explants forming apical buds (Fgi11.a). However, increasing IAA concentration to 3.2 or 6.4mg/l resulted in a reduction on the percentage of explants that formed apical buds. Similarly, NAA behaved like IAA and the best percentage of apical bud formation resulted when NAA was used at 1.6 mg/l.

Combining IAA and NAA at an equal concentration of 0.1 mg/l resulted in a significant increase in the percentage of explants that formed apical buds over the control treatment, 0.4 mg/l of IAA or NAA and the combination of IAA and NAA, each at 3.2 mg/l (Fig.11.b). It was also more effective than all other tested auxin treatments, but not to the level of significance.

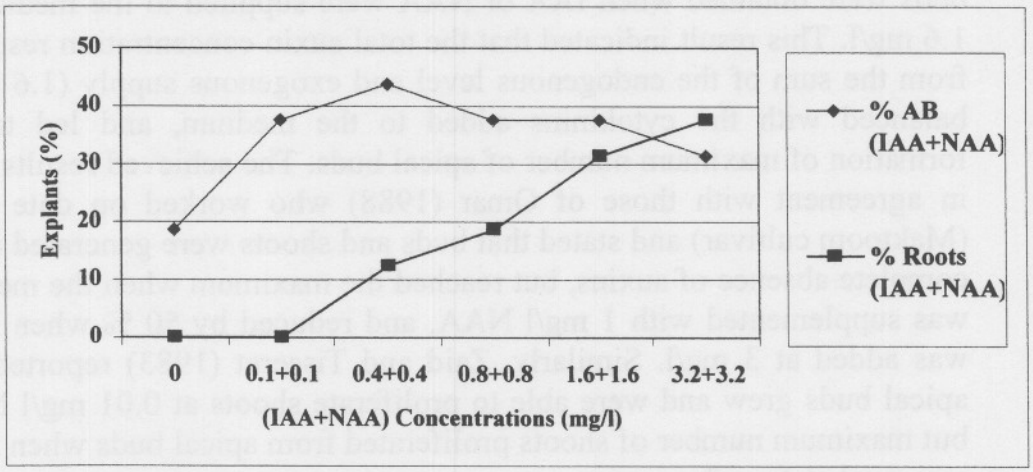
The obtained results indicated that the presence of enough endogenous auxins in the cultured explant tissues was enough to enhance the regeneration of apical buds, but it was not sufficient to induce the maximum apical bud regeneration capacity. Maximum numbers of apical buds were obtained when IAA or NAA were supplied to the medium at 1.6 mg/l. This result indicated that the total auxin concentration resulting from the sum of the endogenous level and exogenous supply (1.6 mg/l) balanced with the cytokinins added to the medium, and led to the formation of maximum number of apical buds. The achieved results were in agreement with those of Omar (1988) who worked on date palm (Maktoom cultivar) and stated that buds and shoots were generated at the complete absence of auxins, but reached the maximum when the medium was supplemented with 1 mg/l NAA, and reduced by 50 % when NAA was added at 3 mg/l. Similarly, Zaid and Tisserat (1983) reported that apical buds grew and were able to proliferate shoots at 0.01 mg/l NAA, but maximum number of shoots proliferated from apical buds when NAA was used at 1 mg/l.

Concerning the effect of different concentrations of auxin on percentage of explants formed roots, data are illustrated in (Fig.11.a,b). These results showed that increasing the level of auxins, regardless of the different of auxin, was associated with the increase in percentage of explants that formed roots.

Culturing explants on a medium containing NAA at 3.2 or 6.4 mg/l resulted in the production of the highest and most significant percentage of rooted explants. The obtained results were even higher than the percentage rooted explants treated with the highest IAA concentration




(a)




(b)

**Fig.11.a,b. Effect of different auxins at various concentrations on the percentage of explants that formed apical buds and roots from cultured shoot tips of Khenezi date cultivar. AB (Apical Bud).**

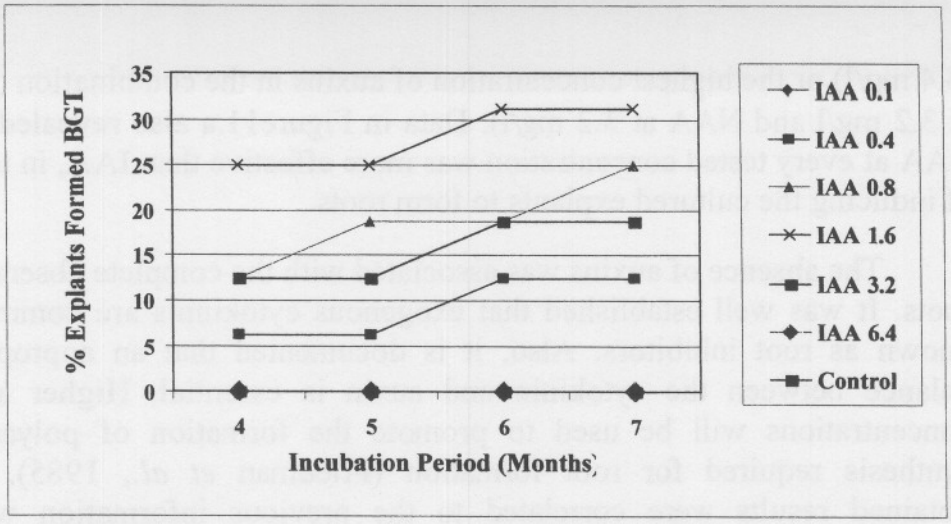
(6.4 mg/l) or the highest concentration of auxins in the combination (IAA at 3.2 mg/l and NAA at 3.2 mg/l). Data in Figure11.a also revealed that NAA at every tested concentration was more effective than IAA, in terms of inducing the cultured explants to form roots.

The absence of auxins was associated with the complete absence of roots. It was well established that exogenous cytokinins are commonly known as root inhibitors. Also, it is documented that an appropriate balance between the cytokinin and auxin is essential. Higher auxin concentrations will be used to promote the formation of polyamine synthesis required for root formation (Friedman *et al.*, 1985). The obtained results were correlated to the previous information where maximum rooting occurred at the highest tested auxin level, 6.4 mg/l IAA or 3.2 and 6.4 mg/l NAA. The achieved results are supported by the those of Omar (1988), Zaid and Tisserat (1983) and Vernamendi and Navarro (1996). They indicated that a relatively high auxin concentration is required to obtain roots.

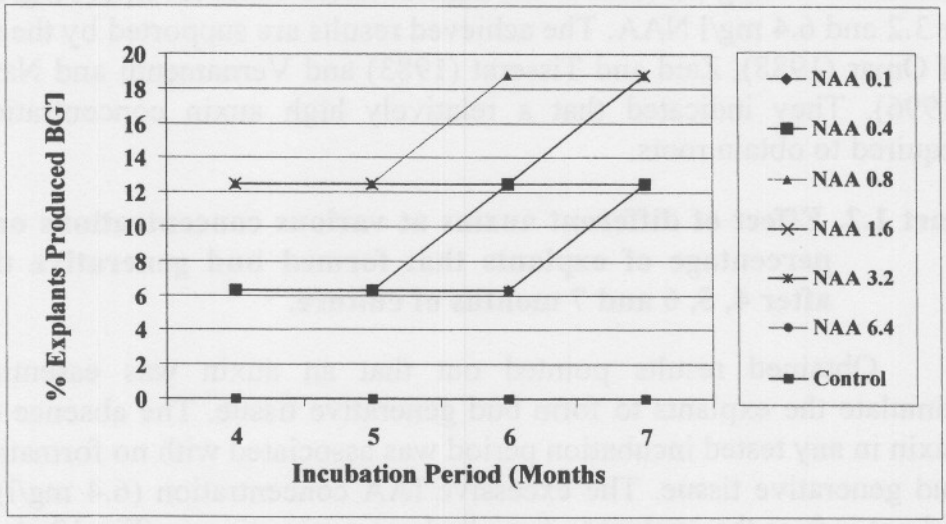
**Part 1.2. Effect of different auxins at various concentrations on the percentage of explants that formed bud generative tissue after 4, 5, 6 and 7 months of culture.**

Obtained results pointed out that an auxin was essential to stimulate the explants to form bud generative tissue. The absence of an auxin in any tested incubation period was associated with no formation of bud generative tissue. The excessive IAA concentration (6.4 mg/l) also did not induce the explant to form bud generative tissues (Fig.12.a).

There was a gradual increase in the percentage of explants that produced bud generative tissues with the increase of IAA concentration from 0.4 mg/l to 0.8 mg/l and then to 1.6 mg/l. Increasing the level of auxin in the medium to 3.2 mg/l or higher (6.4 mg/l) resulted in a reduction in the formation of bud generative tissues. However, the concentration of 3.2 mg/l proved to be better than 0.4 mg/l IAA. The obtained results were consistent through all tested incubation periods, i.e. 4, 5, 6, and 7 months. There was more produced bud generative tissue associated with increasing the incubation period, especially if the number of generative tissue after 4, and 5 months were compared to those after 6 and 7 months. On the other hand, the low concentration of NAA (0.1) was promotive for the production of bud generative tissue from explants after 4, 5, 6 or 7 months. The increase in NAA concentration from 0.1 mg/l to 1.6 mg/l resulted also in a gradual increase in the percentage of explants that formed bud generative tissue (Fig.12.b). The NAA concentration above 1.6 mg/l caused a reduction in the tested parameter




(a)

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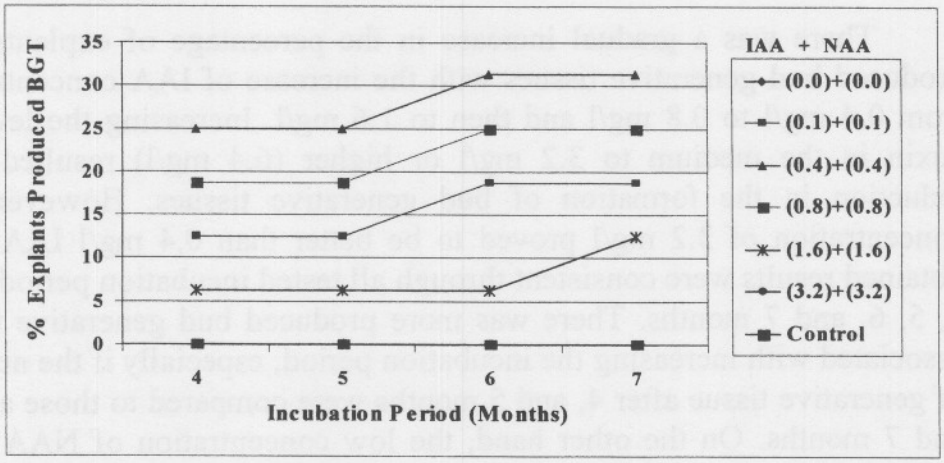



(c)

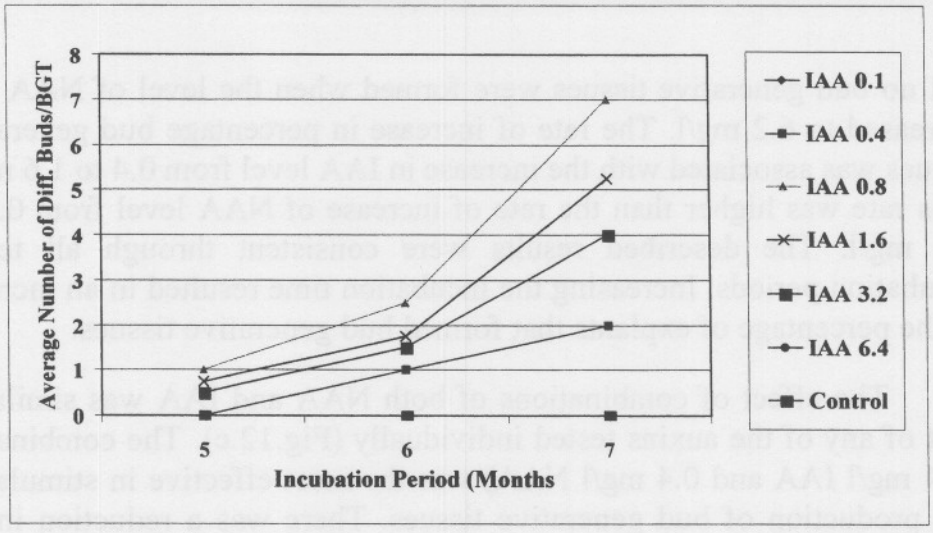
**Fig.12.a,b,c. Effect of different auxins at various concentrations on the percentage of explants that produced bud generative tissues after 4, 5, 6 and 7 months of incubation of cultured shoot tips of Khenezi date palm cultivar. BGT (Bud generative tissue).**

and no bud generative tissues were formed when the level of NAA was increased to 6.2 mg/l. The rate of increase in percentage bud generative tissues was associated with the increase in IAA level from 0.4 to 1.6 mg/l. This rate was higher than the rate of increase of NAA level from 0.4 to 1.6 mg/l. The described results were consistent through all tested incubation periods. Increasing the incubation time resulted in an increase in the percentage of explants that formed bud generative tissues.

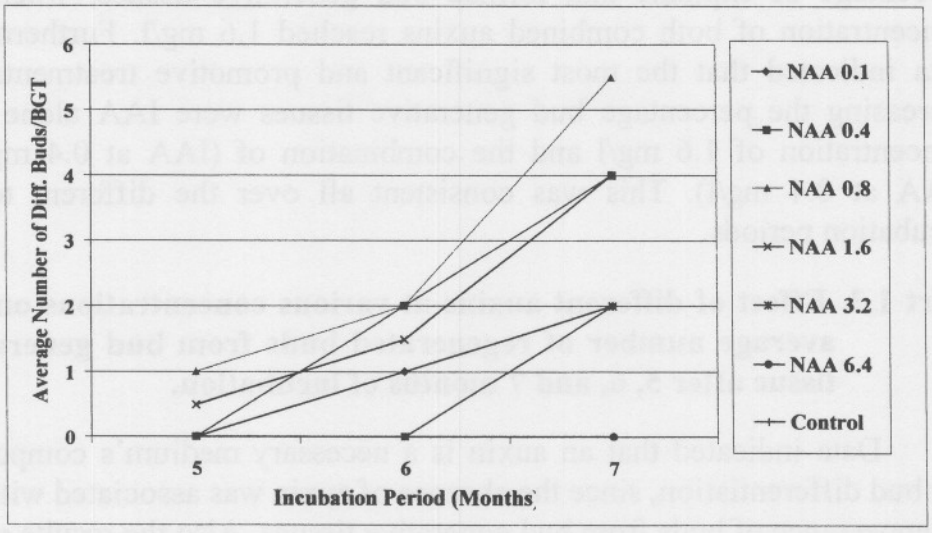
The effect of combinations of both NAA and IAA was similar to that of any of the auxins tested individually (Fig.12.c). The combination (0.4 mg/l IAA and 0.4 mg/l NAA) was the most effective in stimulating the production of bud generative tissues. There was a reduction in the percentage of explants that formed bud generative tissues, when the concentration of both combined auxins reached 1.6 mg/l. Furthermore, data indicated that the most significant and promotive treatments for increasing the percentage bud generative tissues were IAA alone at a concentration of 1.6 mg/l and the combination of (IAA at 0.4 mg/l + NAA at 0.4 mg/l). This was consistent all over the different tested incubation periods.

**Part 1.3. Effect of different auxins at various concentrations on the average number of regenerated buds from bud generative tissue after 5, 6, and 7 months of incubation.**

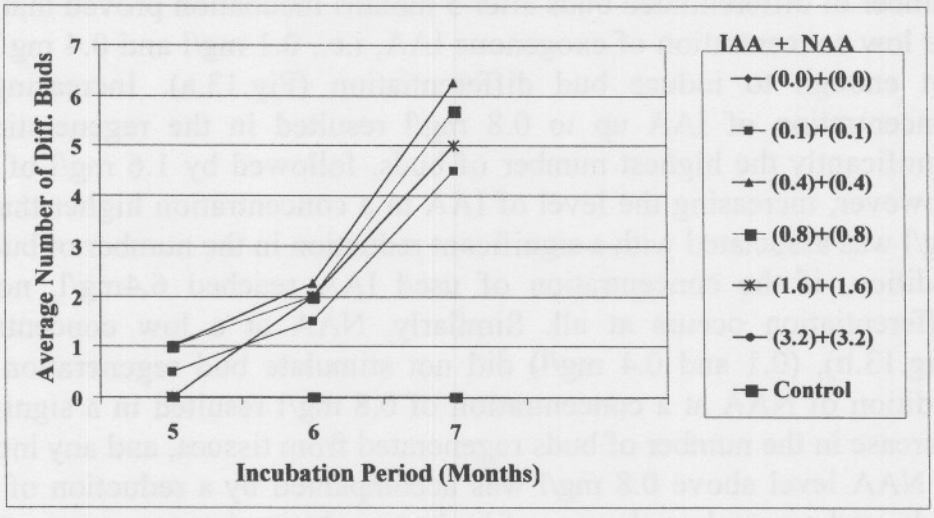
Data indicated that an auxin is a necessary medium's component for bud differentiation, since the absence of auxin was associated with the disappearance of buds from bud generative tissues. Also the results of the number of differentiated buds after 5 months incubation proved that even the low concentration of exogenous IAA, i.e., 0.1 mg/l and 0.4 mg/l was not enough to induce bud differentiation (Fig.13.a). Increasing the concentration of IAA up to 0.8 mg/l resulted in the regeneration of significantly the highest number of buds, followed by 1.6 mg/l of IAA. However, increasing the level of IAA to a concentration higher than 0.8 mg/l was associated with a significant reduction in the number of buds. In addition, if the concentration of used IAA reached 6.4mg/l, no bud differentiation occurs at all. Similarly, NAA at a low concentration (Fig.13.b), (0.1 and 0.4 mg/l) did not stimulate bud regeneration. The addition of NAA at a concentration of 0.8 mg/l resulted in a significant increase in the number of buds regenerated from tissues, and any increase in NAA level above 0.8 mg/l was accompanied by a reduction of these buds, and a complete absence of buds was obtained at a concentration of 3.2mg/l or 6.4mg/l. The most significant combinations of auxins were those containing IAA and NAA at 0.4 mg/l or 0.8 mg/l each (Fig.13,c).




(a)




(b)




(c)

**Fig.13.a,b,c. Effect of different auxins at various concentrations on the average number of differentiated buds per BGT after 5, 6 and 7 months of incubation of cultured shoot tips of Khenezi date palm cultivar.**

Any other tested combination of IAA and NAA resulted in a significant reduction in the number of differentiated buds. After 6 and 7 months of incubation, it was quite clear that IAA at 0.8 mg/l was the most effective and significant treatment in increasing the number of differentiated buds from bud generative tissues. The low concentrations of IAA, i.e. 0.1 and 0.4 mg/l showed a positive increase of bud regenerated from tissues after 6 or 7 months of incubation but they were at the least in terms of significance. Increasing the level of IAA to above 1.6 mg/l caused a significant reduction in the number of differentiated buds after 6 and 7 months of incubation. Similarly, data in (Fig.13.b) showed that the best and most effective concentration of NAA was 0.8 mg/l. After 6 months of incubation, 0.4 mg/l NAA was equal in its effect to 0.8 mg/l, but was less effective compared to 0.8 mg/l after 7 months of incubation. Increasing the level of NAA to a concentration higher than 0.8 mg/l significantly reduced the number of regenerated buds per bud generative tissues. IAA and NAA, both at 0.4 mg/l, were the most effective and significant combination that improved number of differentiated buds from bud generative tissues after 6 or 7 months of incubation, followed by the combinations of (0.8mg/l IAA, 0.8mg/l NAA) and (1.6mg/l IAA + 1.6mg/l NAA). One general observation can be concluded from the data illustrated in (Fig.13), was that the number of differentiated buds from bud generated tissue increased with the increase of incubation period.

The number of differentiated buds behaved similarly to the percentage of formed bud generative tissues, where both required low auxins and high cytokinins. The increase in auxin concentration to above 0.8 mg/l was associated with a significant reduction in the number of differentiated buds, regardless of auxin type. The formation of shoot buds, whether directly from explanted tissues or indirectly from callus, is regulated by the interaction between auxins and cytokinins, with the cytokinin higher in balance. High concentrations of auxin will promote either undifferentiated callus or root formation.

These results correspond to those obtained by (Omar, 1988; Gabr and Tisserat, 1985; and Zaid and Tisserat, 1983). Also there was a significant increase in the average number of differentiated buds with progress in time, indicating a successful differentiation of meristemoids into buds.

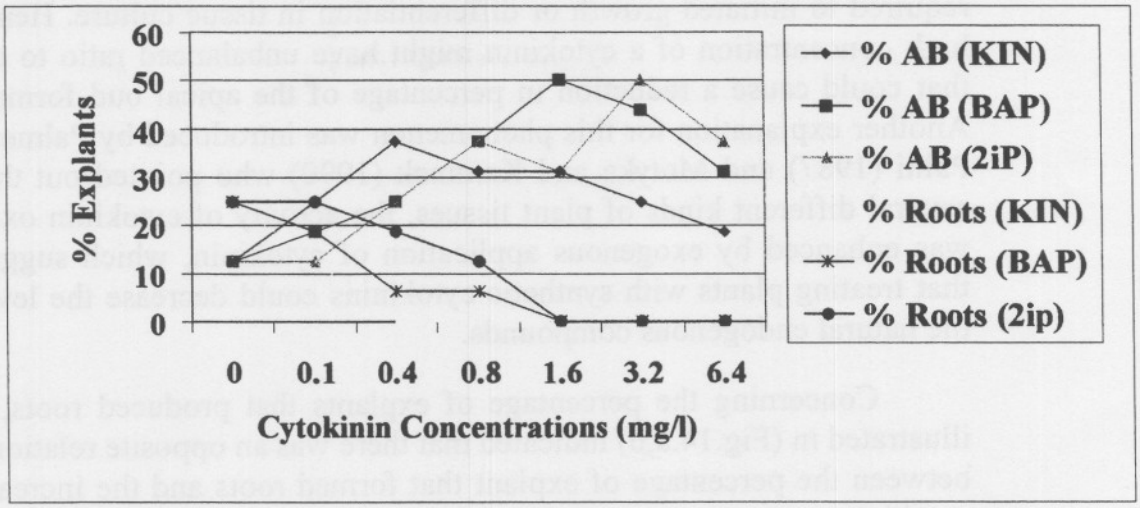
**Part 2: Effect of different cytokinins at various concentrations on the production of bud generative tissue and number of differentiated buds per explant.**

**Part 2.1. Effect of different cytokinins at various concentrations on the percentage of explants that formed apical buds and roots from cultured shoot tips of Khenezi date palm cultivar.**

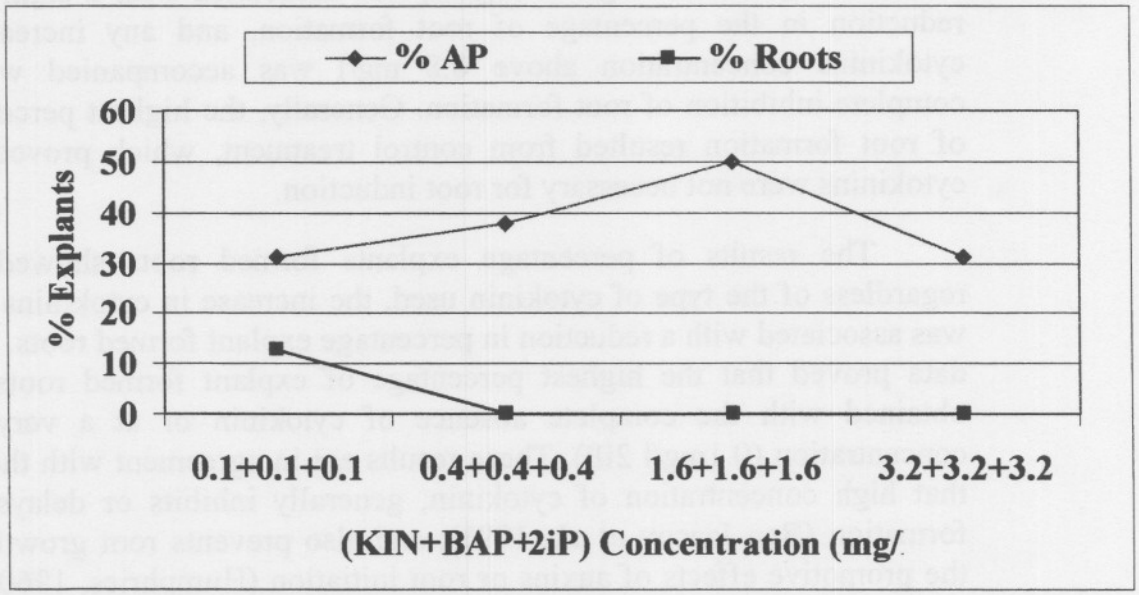
The results showed that a cytokinin was not an essential requirement for the production of apical buds where 12.5% of the control explants succeeded to form apical buds (Fig.14.a). However, the addition of a cytokinin improved the percentage of explants that formed apical buds, and this improvement was significant in most cases. Data also pointed out that Kinetine alone was not effective in increasing apical buds formation as compared to BAP and 2iP as well as the combination of all three cytokinins. The highest percentage explants that formed apical buds in the case of Kin treatment was (37.5%), which was achieved at a concentration of 0.4 mg/l, compared to 50% apical bud formation that resulted from BAP at 1.6 mg/l., 2iP at 3.2 mg/l, and the combination of (Kin + BAP + 2iP) each at 1.6 mg/l (Fig.14.b) It was also clear that the low concentration of a cytokinin (0.1 mg/l), as well as the high concentration (6.4 mg/l) reduced the percentage of explants formed apical buds, even in the case of a combination of the three cytokinins. The most effective concentration varied depending on the cytokinin type, it was 0.4 mg/l in the case of Kin, 1.6 mg/l in the case of BAP, 3.2 mg/l with regard to 2iP and the combination of Kin + BAP + 2iP at 1.6 mg/l each.

The results which indicated the ability of control explants to form apical buds may be attributed to the presence of sufficient endogenous level of a cytokinin in their tissues, which was enough to induce bud formation at a low percentage. These results are correlated to those obtained by Sunderland and Wells (1968), who stated that in the tissues of *Oxalis dispar*, cell division proceeds with the addition of a cytokinin to the culture medium. Similarly, Skoog *et al.* (1973) succeeded to isolate three natural cytokinins from a cytokinin-independent strain of tobacco callus.

Since different cytokinin types differ in their effectiveness, different concentrations of tested cytokinins, i.e. Kin, BAP, 2iP and their combination resulted in a maximum apical bud formation. However, the results showed that using the highest concentration of a cytokinin led to reduction in percentage of the apical bud formation. The reason for this phenomenon could be that many aspects of cellular differentiation and organogenesis in tissue and organ culture have been found to be controlled by an interaction between cytokinin and auxin concentrations. The balance between the two sorts of growth regulators is usually




(a)




(b)

**Fig.14.a,b. Effect of different cytokinins at various concentrations on the percentage of explants that formed apical buds and roots from cultured shoot tips of Khenezi date palm cultivar.**

required to initiated growth or differentiation in tissue culture. Hence, a high concentration of a cytokinin might have unbalanced ratio to auxin that could cause a reduction in percentage of the apical bud formation. Another explanation for this phenomenon was introduced by Palmer and Palni (1987) and Motyka and Kaminek (1990) who pointed out that in several different kinds of plant tissues, the activity of cytokinin oxidase was enhanced by exogenous application of cytokinin, which suggested that treating plants with synthetic cytokinins could decrease the level of the natural endogenous compounds.

Concerning the percentage of explants that produced roots, data illustrated in (Fig.14.a,b) indicated that there was an opposite relationship between the percentage of explant that formed roots and the increase in cytokinin concentrations. Increasing the level of cytokinins above 0.1 mg/l in the case of kin (Fig.14.a) or the combination of the three cytokinin (Fig.14.b) at 0.1 mg/l resulted in an inhibition of root formation. Also, in the case of BAP and 2iP, increasing the concentration from 0.1 mg/l to 0.8 mg/l or higher, was associated with a significant reduction in the percentage of root formation, and any increase in cytokinins concentration above 0.8 mg/l was accompanied with a complete inhibition of root formation. Generally, the highest percentage of root formation resulted from control treatment, which proved that cytokinins were not necessary for root induction.

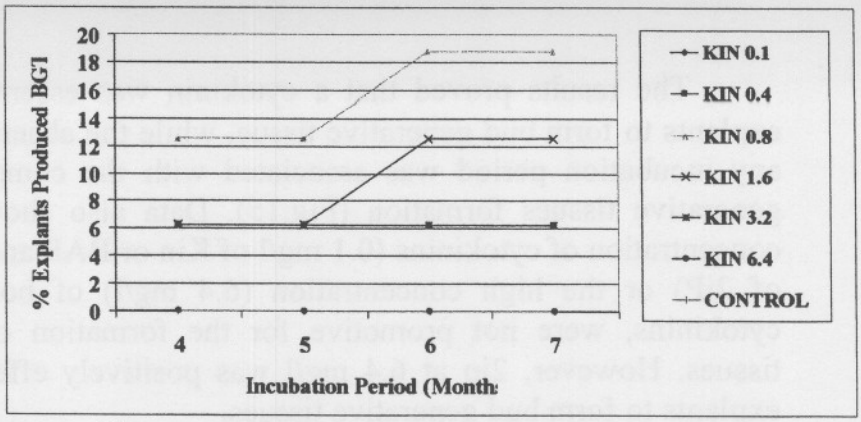
The results of percentage explants formed roots showed that regardless of the type of cytokinin used, the increase in cytokinins level was associated with a reduction in percentage explant formed roots. Also, data proved that the highest percentage of explant formed roots was obtained with the complete absence of cytokinin or at a very low concentration (0.1mg/l 2iP). These results are in agreement with the fact that high concentration of cytokinin, generally inhibits or delays root formation (Ben-Jaacov *et al.*, 1991), and also prevents root growth and the promotive effects of auxins or root initiation (Humphries, 1960). On the other hand, Fries, (1960) demonstrated that low concentration of cytokinins can sometimes induce or promote root growth. Also, Boxus and Terzi (1988) advocated the addition of 0.5 mg/l Kin and an auxin to the rooting medium for strawberries and several woody plants, finding that the cytokinin had a bacteriostatic effect and rooting was not impaired

**Part 2.2 Effect of different cytokinins at various concentrations on the percentage of explants that formed bud generative tissues after 4, 5, 6 and 7 months of incubation.**

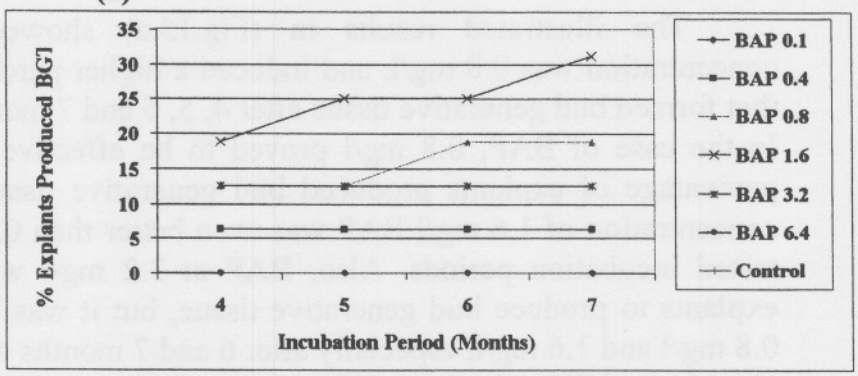
The results proved that a cytokinin was essential to induce the explants to form bud generative tissue, while the absence of cytokinin at any incubation period was associated with the complete lack of bud generative tissues formation (Fig.15). Data also showed that the low concentration of cytokinins (0.1 mg/l of Kin or BAP and 0.1 and 0.4 mg/l of 2iP) or the high concentration (6.4 mg/l) of both Kin and BAP cytokinins, were not promotive for the formation of bud generative tissues. However, 2ip at 6.4 mg/l was positively effective in inducing explants to form bud generative tissues.

The illustrated results in (Fig.15.a) showed that best Kin concentration was 0.8 mg/l, and induced a higher percentage of explants that formed bud generative tissue after 4, 5, 6 and 7 months of incubation. In the case of BAP, 0.8 mg/l proved to be effective in improving the percentage of explants produced bud generative tissues. However, the concentration of 1.6 mg/l BAP was even better than 0.8 mg/l BAP in all tested incubation periods. Also, BAP at 3.2 mg/l was able to induce explants to produce bud generative tissue, but it was less effective than 0.8 mg/l and 1.6 mg/l, especially after 6 and 7 months of incubation. Data also showed (Fig.15.c) that 2iP at 3.2mg/l was more effective than other tested 2iP concentration in increasing the percentage of explants produced bud generative tissues, followed by 1.6 mg/l 2iP. Increasing the concentration of 2iP to 6.4 mg/l was associated with a reduction in percentage explants formed bud generative tissues and was similar in its effect to that of 2iP at 0.8 mg/l. The tested cytokinin combination was less effective than individual cytokinins, e.g. BAP and 2ip at 0.4 mg/l was the most positive. The combination of cytokinins increased the percentage of explant that formed bud generative tissues. In general, the best and significantly effective cytokinin treatments were BAP at 1.6mg/l and 2iP at 3.2mg/l. These two treatments also showed a clear increase in percentage explants formed bud generative tissues, which was associated with the increase in length of incubation periods, especially after 7 months.

The achieved results of complete absence of adventitious buds at zero cytokinin may be attributed to the effect of cytokinin in encouraging the growth and formation of adventitious buds, whether directly, from explanted tissues or indirectly from callus, the point that is regulated by an interaction between auxins and cytokinins. Also, increasing the level of cytokinin, regardless of the used type, to 6.4mg/l caused a reduction in the case of 2iP or complete absence in the case of Kin and BAP of bud generative tissue formation (Fig.20). These results may be due to the negative effect of the inhibition of endogenous cytokinin level as a result




(a)

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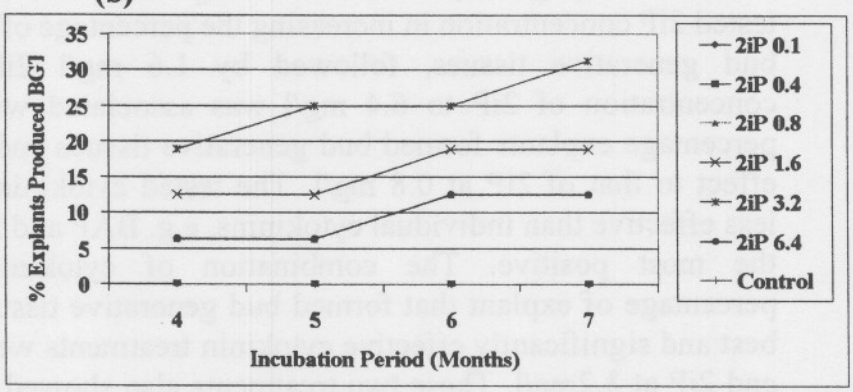


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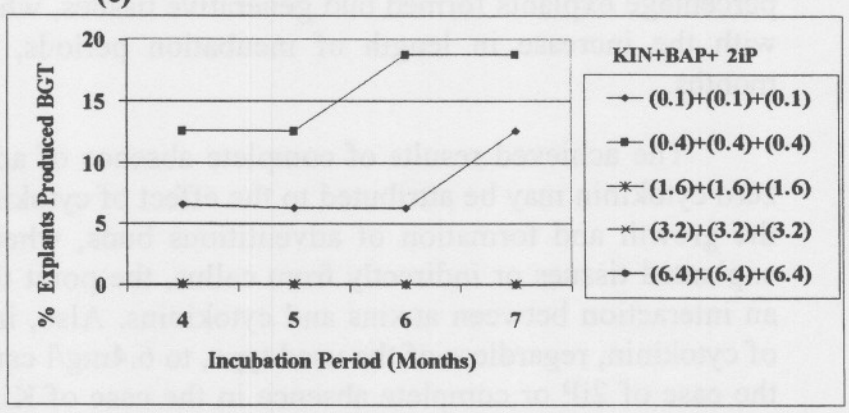


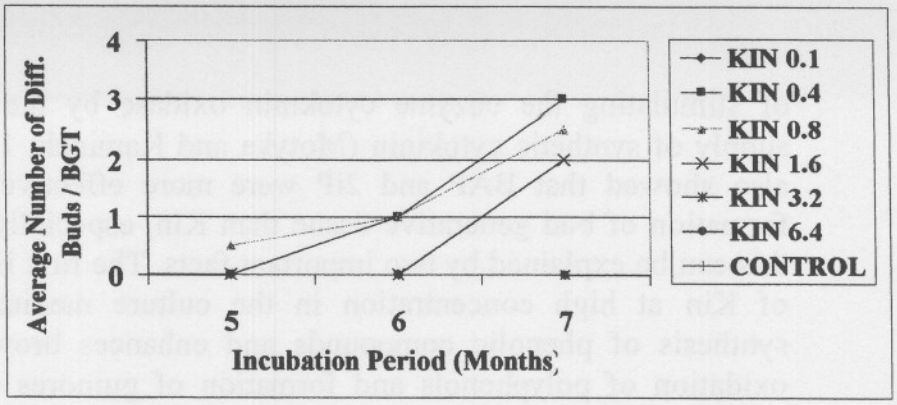

(d)

**Fig.15.a,b,c,d. Effect of different cytokinins at various concentrations on the percentage of explants that produced bud generative tissues after 4, 5, 6, and 7 months of incubation time, from cultured shoot tips of Khenezi date palm cultivar.**

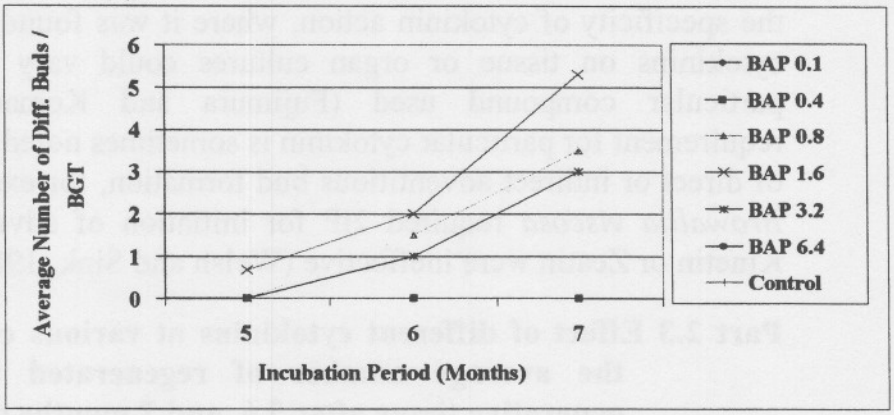
of stimulating the enzyme cytokinin oxidase by the high exogenous supply of synthetic cytokinin (Motyka and Kaminek, 1990). The results also showed that BAP and 2iP were more effective in inducing the formation of bud generative tissue than Kin, especially at 1.6 mg/l, and this can be explained by two important facts. The first is that the addition of Kin at high concentration in the culture medium stimulates the synthesis of phenolic compounds and enhances browning due to the oxidation of polyphenols and formation of guinores which are highly reactive and toxic to the tissues (Zaid, 1984). The second fact is due to the specificity of cytokinin action, where it was found that the effect of cytokinins on tissue or organ cultures could vary according to the particular compound used (Fujimura and Komamine, 1975). A requirement for particular cytokinin is sometimes noted for the promotion of direct or indirect adventitious bud formation, for example, cultures of *Browallia viscosa* required 2iP for initiation of adventitious bud, but Kinetin or Zeatin were ineffective (Welsh and Sink, 1981).

**Part 2.3 Effect of different cytokinins at various concentrations on the average number of regenerated buds from bud generative tissue after 5,6, and 7 months of incubation.**

Effect of cytokinin type and concentration on average number of buds differentiated from each bud generative tissue was studied. The results showed that cytokinins are absolutely necessary for bud differentiation, where the complete absence of cytokinin or even using it at low concentration (0.1 and 0.4 mg/l) did not stimulate bud differentiation at all in the case of BAP and 2iP. However, the concentration of 0.4mg/l Kin induced bud generation only after 6 and 7 months incubation, (Fig.16.a). Increasing the level of cytokinin to 1.6 mg/l in the case of BAP or 2iP increased bud differentiation frequency after 5 months of incubation (Fig.16.a,b). The maximum number of differentiated buds after 5 months was achieved when the medium was supplemented with 3.2 mg/l 2iP. Increasing the level of Kin or BAP to higher concentrations than 1.6 mg/l resulted in the absence of bud differentiation and the increase of phenolic components production. However, increasing the level of 2iP to 3.2 mg/l or 6.4 mg/l did not enhance oxidation of phenolic components and, therefore, stimulated bud regeneration (Fig.16.c). After 6 months of incubation, or after the accumulation of up taken cytokinins, the concentration of 0.8 mg/l of any tested cytokinins started showing positive effect on bud differentiation. The 2iP was the most effective cytokinin at 0.8 mg/l. Increasing the level of Kin to more than 0.8 mg/l inhibited the regeneration of buds, but increased bud regeneration in the case of BAP and 2iP, especially at 1.6




(a)

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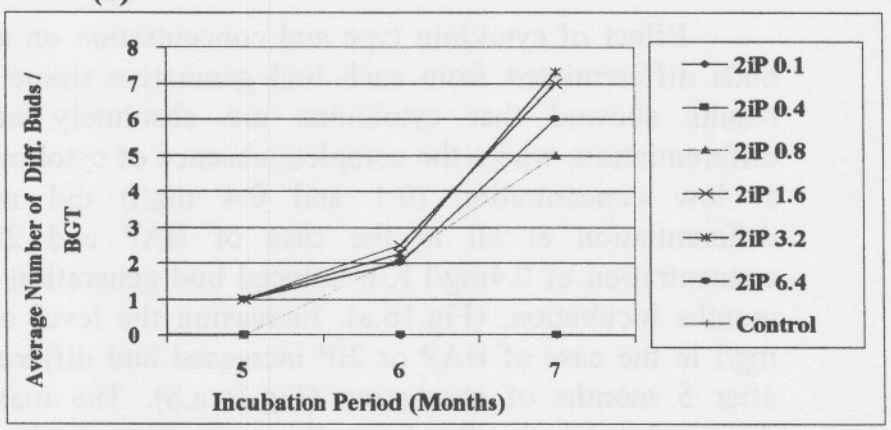


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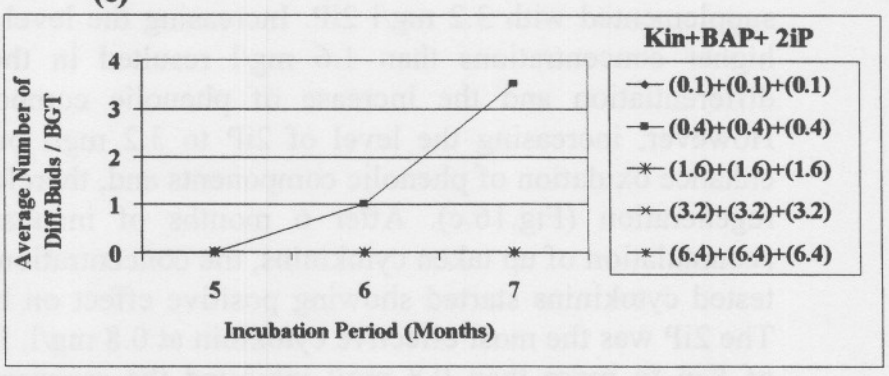


(d)

**Fig.16.a,b,c,d. Effect of different cytokinins at various concentrations on the average number of differentiated buds per bud generative tissues of cultured shoot tips of Khenezi date palm cultivar.**

mg/l. Again, increasing the level of 2iP to 3.2 or 6.4 mg/l caused a non significant reduction in the number of differentiated buds, compared to the level of 1.6mg/l. After seven months of incubations, there was a clear increase in number of regenerated buds from bud generative tissues. The low concentration of Kin (0.4 mg/l) was associated with less browning and highest number of bud differentiation that resulted from any kin concentration. Increasing the level of Kin above 1.6 mg/l inhibited bud differentiation. Increasing BAP level to above 3.2mg/l seemed to be inhibitory for bud differentiation. Also the data indicated that best BAP concentration was 1.6mg/l, where it resulted in 5.3 buds / bud generative tissue. Concerning the effect of 2iP, it was clear that using 2iP at 0.8 mg/l or higher was of a promotive effect for induction of bud differentiation. Number of regenerated buds resulted from the application of 2iP at 1.6mg/l or higher was more than any tested level of Kin or BAP. The most significantly effective concentration of 2iP was at 3.2 mg/l, followed by 1.6mg/l when they produced 7.3 and 7.0 regenerated buds / bud generative tissue respectively. In fact, these two treatments of 2iP (3.2 and 1.6mg/l) were significantly better than all other tested hormonal concentrations, regardless of the cytokinin type.

The combinations of cytokinins were significantly less effective in inducing bud regeneration compared to separate cytokinin treatments (Fig.16.d). Only the combination of Kin, BAP and 2iP at 0.4mg/l each, resulted in bud differentiation, but at a significantly lower level compared to the other discussed cytokinin treatments.

The results reflected the strength of the fact that cytokinins are required and are very effective in promoting bud or shoot differentiation. A balance between cytokinin and auxin normally gives the most effective organogenesis (George 1993). Also the highest number of differentiated bud / bud generative tissues was achieved when the cytokinin was 2iP at 1.6 or 3.2 mg/l, followed by BAP at 1.6 mg/l. Decreasing the level of cytokinin to less than 1.6 mg/l was associated with a reduction in number of regenerated buds due to the change of cytokinin: auxin ratio required for initiation of bud formation (George 1993). On the other hand, increasing the cytokinin concentration to 6.4 mg/l resulted in a reduction in number of regenerated buds / bud generative tissues, and this phenomenon may be due to the activity of cytokinin oxidase which is enhanced by the high exogenous application of cytokinins (Palmer

and Palni, 1987; Motyka and Kaminek, 1990). The data also proved that 2iP is more effective type of cytokinin than Kinetin or BAP. This may be attributed to the specificity of cytokinin type and action,

where a requirement for a particular cytokinin is sometimes noted for the induction of adventitious shoot. For example, cultures of *Browallia viscosa* required 2iP for the initiation of adventitious shoot buds, and Kin, BAP or Zeatin were ineffective (Welsh and Sink 1981).

## CONCLUSIONS

The main goal of the research is to study the effect of hormonal combinations on the *In Vitro* organogenesis of date palm (*Phoenix dactylifera* L., cv. Khenezi) *In vitro.*

The presence of an auxin in a culture medium was not an essential requirement for the formation of apical buds from shoot tips but it is essential for developing apical buds. Increasing the level of either tested auxin (IAA or NAA) to 1.6 mg/l resulted in an increase in the number of explants that formed apical buds. Increasing IAA concentration to 3.2 or 6.4 mg/l resulted in a reduction on the percentage of explants that formed apical buds. Similarly, the auxin NAA behaved like IAA. Combining IAA and NAA together at an equal concentration of 0.1 mg/l resulted in a significant increase in the percentage of explants that formed apical buds over the control treatment.

The presence of endogenous auxins in the cultured explant tissues was sufficient to enhance the regeneration of apical buds, but it was not enough to induce the maximum apical bud regeneration capacity.

NAA at every tested level was more effective than IAA at any tested concentration, in terms of inducing the cultured explants to form roots. The absence of auxins was associated with the complete absence of roots, and it was well established that exogenous cytokinins are commonly known as root inhibitors.

Data pointed out that auxin was essential to stimulate the explants to form bud generative tissue, while the absence of auxin after any tested incubation period was associated with no bud generative tissue. Furthermore, there was a gradual increase in the percentage of explants that produced bud generative tissues with the increase of IAA concentration from 0.4 to 0.8 and then to 1.6 mg/l.

The combination of both NAA and IAA behaved in the same manner as any of the auxins alone, with the combination (0.4mg/l each) being the most effective in stimulating the production of bud generative tissues.

Increasing the used auxin level to a higher concentration reduced significantly the percentage bud generative tissue formation.

The results indicated that auxin is a necessary medium component for bud differentiation, since the absence of auxin was associated with the disappearance of buds from bud generative tissues. Also the results of the number of differentiated buds after 5 months incubation period proved that even the low concentrations of exogenous IAA, i.e., 0.1 and 0.4 mg/l, were not enough to induce bud differentiation. Increasing the concentration of IAA up to 0.8 mg/l resulted in the regeneration of significantly highest number of buds from bud generative tissues, followed by 1.6 mg/l of IAA.

The data of the number of differentiated buds behaved similarly to percentage explants formed bud generative tissue, where both required low auxin and high cytokinin concentrations. The increase in auxin concentration to above 0.8 mg/l was associated with a significant reduction in number of differentiated buds, regardless of auxin type. The formation of shoot buds whether directly from explanted tissues, or indirectly from callus, is regulated by the interaction between auxins and cytokinins, with the cytokinins generally should be higher in balance, where high concentration of auxins will promote either undifferentiated callus or root formation.

The results showed that cytokinin was not an essential requirement for the production of apical buds where 12.5% of the control explants succeeded to form apical buds. However, the addition of cytokinin improved the percentage of explants that formed apical buds, and this improvement was significant in most cases.

The most effective concentration varied depending on the type of cytokinin, it was 0.4 mg/l in the case of Kin, 1.6 mg/l in the case of BAP, 3.2 mg/l with regard to 2iP and the combination of Kin + BAP + 2iP at 1.6 mg/l each.

In addition, the ability of control explants to form apical buds may be attributed to the presence of enough endogenous level of cytokinins in their tissues, which was enough to induce bud formation at a low percentage.

Regarding the percentage explants formed roots results showed that there was an opposite relationship between percentages explant formed roots and the increase in cytokinin concentrations. The obtained results showed that regardless of the type of cytokinin used, the increase in

cytokinin level was associated with a reduction in percentage explant formed roots. Also, data proved that the highest percentage explant formed roots was obtained with the complete absence of cytokinin or at a very low level (0.1mg/l 2iP).

The results proved that cytokinin was essential to induce the explants to form bud generative tissue, where the absence of cytokinin at any incubation period was associated with the complete absence of bud generative tissues formation.

The tested cytokinin combination was less effective than individual cytokinin, e.g. BAP and 2ip at 0.4mg/l was the most positive. The combination of cytokinins increased the percentage of explant that formed bud generative tissues. In general, the best and significantly effective cytokinin treatments were BAP at 1.6 mg/l and 2iP at 3.2 mg/l. These two treatments also showed a clear increase in percentage explants formed bud generative tissues, which was associated with the increase in length of incubation periods, especially after 7 months.

The results showed that cytokinins are absolutely necessary for bud differentiation where the complete absence of cytokinins or even their use at a low concentration (0.1 and 0.4 mg/l) did not stimulate bud differentiation at all.

The most significantly effective concentration of 2iP was 3.2 mg/l, followed by 1.6 mg/l when they produced 7.3 and 7.0 regenerated buds / bud generative tissue respectively. The combinations of cytokinins were significantly less effective in inducing bud regeneration compared to separate cytokinins treatments.

## APPENDIX

Table 1. Effect of different auxins at various concentrations on the percentage of explants that formed apical buds and roots, of cultured shoot tips of Khenezi date palm cultivar.

| Auxin (mg/l) | % Explants that formed apical buds | % Explants that formed roots. |
|---|---|---|
| Control | 12.5 | 0.00 |
| IAA (0.1) | 18.75 | 0.00 |
| IAA (0.4) | 25.0 | 6.25 |
| IAA (0.8) | 37.5 | 6.25 |
| IAA (1.6) | 43.75 | 12.5 |
| IAA (3.2) | 31.25 | 12.5 |
| IAA (6.4) | 25.0 | 25.0 |
| NAA (0.1) | 18.75 | 6.25 |
| NAA (0.4) | 31.25 | 12.5 |
| NAA (0.8) | 43.75 | 18.75 |
| NAA (1.6) | 43.75 | 25.0 |
| NAA (3.2) | 37.5 | 43.75 |
| NAA (6.4) | 31.25 | 43.75 |
| IAA (0.0), NAA (0.0) | 18.75 | 0.00 |
| IAA (0.1), NAA (0.1) | 37.5 | 0.00 |
| IAA (0.4), NAA (0.4) | 43.75 | 12.5 |
| IAA (0.8), NAA (0.8) | 37.5 | 18.75 |
| IAA (1.6), NAA (1.6) | 37.5 | 31.25 |
| IAA (3.2), NAA (3.2) | 31.25 | 37.5 |
| LSD (5%) | 16.02 | 21.34 |

Control: medium free from any growth regulators.

Table 2. Effect of different auxins at various concentrations on the percentage of explants that produced bud generative tissues after 4,5,6 and 7 months of incubation of cultured shoot tips of Khenezi date cultivar.

| Auxin (mg/l) | % Explants produced BGT after 4 months. | % Explants produced BGT after 5 months. | % Explants produced BGT after 6 months. | % Explants produced BGT after 7 months. |
|---|---|---|---|---|
| Control | 0.00 | 0.00 | 0.00 | 0.00 |
| IAA (0.1) | 0.00 | 0.00 | 0.00 | 0.00 |
| IAA (0.4) | 6.25 | 6.25 | 12.5 | 12.5 |
| IAA (0.8) | 12.5 | 18.75 | 18.75 | 25.0 |
| IAA (1.6) | 25.0 | 25.0 | 31.25 | 31.25 |
| IAA (3.2) | 12.5 | 12.5 | 18.75 | 18.75 |
| IAA (6.4) | 0.0 | 0.0 | 0.0 | 0.0 |
| NAA (0.1) | 6.25 | 6.25 | 6.25 | 12.5 |
| NAA (0.4) | 6.25 | 6.25 | 12.5 | 12.5 |
| NAA (0.8) | 12.5 | 12.5 | 18.75 | 18.75 |
| NAA (1.6) | 12.5 | 12.5 | 12.5 | 18.75 |
| NAA (3.2) | 6.25 | 6.25 | 6.25 | 12.5 |
| NAA (6.4) | 0.0 | 0.0 | 0.0 | 0.0 |
| IAA (0.0), NAA | 0.0 | 0.0 | 0.0 | 0.0 |
| IAA (0.1), NAA | 12.5 | 12.5 | 18.75 | 18.75 |
| IAA (0.4), NAA | 25.0 | 25.0 | 31.25 | 31.25 |
| IAA (0.8), NAA | 18.75 | 18.75 | 25.0 | 25.0 |
| IAA (1.6), NAA | 6.25 | 6.25 | 6.25 | 12.5 |
| IAA (3.2), NAA | 0.0 | 0.0 | 0.0 | 0.0 |
| LSD (5%) | 14.09 | 15.09 | 19.32 | 21.45 |

BGT: Bud generative tissue.

Table 3. Effect of different auxins at various concentrations on the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissue, of cultured shoot tips of Khenezi date palm cultivar.

| Auxin (mg/l) | No. of Differentiated buds / BGT After 5 months. | No. of Differentiated buds / BGT After 6 months. | No. of Differentiated buds / BGT After 7 months. |
|---|---|---|---|
| Control | 0.0 | 0.0 | 0.0 |
| IAA (0.1) | 0.0 | 0.0 | 0.0 |
| IAA (0.4) | 0.0 | 1.0 | 2.0 |
| IAA (0.8) | 1.0 | 2.5 | 7.0 |
| IAA (1.6) | 0.75 | 1.75 | 5.25 |
| IAA (3.2) | 0.50 | 1.5 | 4.0 |
| IAA (6.4) | 0.0 | 0.0 | 0.0 |
| NAA (0.1) | 0.0 | 1.0 | 2.0 |
| NAA (0.4) | 0.0 | 2.0 | 4.0 |
| NAA (0.8) | 1.0 | 2.0 | 5.5 |
| NAA (1.6) | 0.5 | 1.5 | 4.0 |
| NAA (3.2) | 0.0 | 0.0 | 2.0 |
| NAA (6.4) | 0.0 | 0.0 | 0.0 |
| IAA (0.0), NAA | 0.0 | 0.0 | 0.0 |
| IAA (0.1), NAA | 0.50 | 1.5 | 4.5 |
| IAA (0.4), NAA | 1.0 | 2.25 | 6.25 |
| IAA (0.8), NAA | 1.0 | 2.0 | 5.70 |
| IAA (1.6), NAA | 0.0 | 2.0 | 5.0 |
| IAA (3.2), NAA | 0.0 | 0.0 | 0.0 |
| LSD (5%) | 0.2242 | 0.5776 | 1.328 |

Table 4. Effect of different cytokinins at various concentrations on the percentage of explants that formed apical buds and roots of cultured shoot tip of Khenezi date palm cultivar.

| Cytokinin (mg/l) | % Explants formed apical buds | % Explants formed roots. |
|---|---|---|
| Control (0.0) | 12.5 | 25.0 |
| KIN (0.1) | 25.0 | 12.5 |
| KIN (0.4) | 37.5 | 0.0 |
| KIN (0.8) | 31.25 | 0.0 |
| KIN (1.6) | 31.25 | 0.0 |
| KIN (3.2) | 25.0 | 0.0 |
| KIN (6.4) | 18.75 | 0.0 |
| BAP (0.1) | 18.75 | 18.75 |
| BAP (0.4) | 25.0 | 6.25 |
| BAP (0.8) | 37.5 | 6.25 |
| BAP (1.6) | 50.0 | 0.0 |
| BAP (3.2) | 43.75 | 0.0 |
| BAP (6.4) | 31.25 | 0.0 |
| 2iP (0.1) | 12.5 | 25.0 |
| 2iP (0.4) | 25.0 | 18.75 |
| 2iP (0.8) | 37.5 | 12.5 |
| 2iP (1.6) | 31.25 | 0.0 |
| 2iP (3.2) | 50.0 | 0.0 |
| 2iP (6.4) | 37.5 | 0.0 |
| KIN (0.1), BAP (0.1), 2iP | 31.25 | 12.5 |
| KIN (0.4), BAP (0.4), 2iP | 37.5 | 0.0 |
| KIN (1.6), BAP (1.6), 2iP | 50.0 | 0.0 |
| KIN (3.2), BAP (3.2), 2iP | 31.25 | 0.0 |
| LSD (5%) | 16.01 | 14.75 |

Table 5. Effect of different cytokinins at various concentrations on the percentage of explants that produced bud generative tissue after 4, 5, 6 and 7 months of incubation of cultured shoot tips of Khenezi date palm cultivar.

| Cytokinin (mg/l) | % Explants produced BGT after 4 months | % Explants produced BGT after 5 months | % Explants produced BGT after 6 months | % Explants produced BGT after 7 months |
|---|---|---|---|---|
| Control (0.0) | 0.00 | 0.00 | 0.00 | 0.00 |
| KIN (0.1) | 0.00 | 0.00 | 0.00 | 0.00 |
| KIN (0.4) | 6.25 | 6.25 | 6.25 | 6.25 |
| KIN (0.8) | 12.5 | 12.5 | 18.75 | 18.75 |
| KIN (1.6) | 6.25 | 6.25 | 12.5 | 12.5 |
| KIN (3.2) | 6.25 | 6.25 | 6.25 | 6.25 |
| KIN (6.4) | 0.00 | 0.00 | 0.00 | 0.00 |
| BAP (0.1) | 0.00 | 0.00 | 0.00 | 0.00 |
| BAP (0.4) | 6.25 | 6.25 | 6.25 | 6.25 |
| BAP (0.8) | 12.5 | 12.5 | 18.75 | 18.75 |
| BAP (1.6) | 18.75 | 25.0 | 25.0 | 31.25 |
| BAP (3.2) | 12.5 | 0.1250 | 12.5 | 12.5 |
| BAP (6.4) | 0.00 | 0.00 | 0.00 | 0.00 |
| 2iP (0.1) | 0.00 | 0.00 | 0.00 | 0.00 |
| 2iP (0.4) | 0.00 | 0.00 | 0.00 | 0.00 |
| 2iP (0.8) | 6.25 | 6.25 | 12.5 | 12.5 |
| 2iP (1.6) | 12.5 | 12.5 | 18.75 | 18.75 |
| 2iP (3.2) | 18.75 | 25.0 | 25.0 | 31.25 |
| 2iP (6.4) | 6.25 | 6.25 | 12.5 | 12.5 |
| KIN (0.1), BAP (0.1), 2iP | 6.25 | 6.25 | 6.25 | 12.5 |
| KIN (0.4), BAP (0.4), 2iP | 12.5 | 12.5 | 18.75 | 18.75 |
| KIN (1.6), BAP (1.6), 2iP | 0.00 | 0.00 | 0.00 | 0.00 |
| KIN (3.2), BAP (3.2), 2iP | 0.00 | 0.00 | 0.00 | 0.00 |
| LSD Value (5%) | 13.2 | 14.75 | 18.66 | 21.55 |

Table 6. Effect of different cytokinins at various concentrations on the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissue, resulted of shoot tips of Khenezi date palm cultivar.

| Cytokinin (mg/l) | No. of Differentiated buds / BGT After 5 months | No. of Differentiated buds / BGT After 6 months | No. of Differentiated buds / BGT After 7 months |
|---|---|---|---|
| Control (0.0) | 0.0 | 0.0 | 0.0 |
| KIN (0.1) | 0.0 | 0.0 | 0.0 |
| KIN (0.4) | 0.0 | 1.0 | 3.0 |
| KIN (0.8) | 0.5 | 1.0 | 2.5 |
| KIN (1.6) | 0.0 | 0.0 | 2.0 |
| KIN (3.2) | 0.0 | 0.0 | 0.0 |
| KIN (6.4) | 0.0 | 0.0 | 0.0 |
| BAP (0.1) | 0.0 | 0.0 | 0.0 |
| BAP (0.4) | 0.0 | 0.0 | 0.0 |
| BAP (0.8) | 0.0 | 1.5 | 3.5 |
| BAP (1.6) | 0.67 | 2.0 | 5.3 |
| BAP (3.2) | 0.0 | 1.0 | 3.0 |
| BAP (6.4) | 0.0 | 0.0 | 0.0 |
| 2iP (0.1) | 0.0 | 0.0 | 0.0 |
| 2iP (0.4) | 0.0 | 0.0 | 0.0 |
| 2iP (0.8) | 0.0 | 2.125 | 5.0 |
| 2iP (1.6) | 1.0 | 2.5 | 7.0 |
| 2iP (3.2) | 1.0 | 2.25 | 7.3 |
| 2iP (6.4) | 1.0 | 2.0 | 6.0 |
| KIN (0.1), BAP (0.1), 2iP | 0.0 | 0.0 | 0.0 |
| KIN (0.4), BAP (0.4), 2iP | 0.0 | 1.0 | 3.5 |
| KIN (1.6), BAP (1.6), 2iP | 0.0 | 0.0 | 0.0 |
| KIN (3.2), BAP (3.2), 2iP | 0.0 | 0.0 | 0.0 |
| LSD Value (5%) | 0.3125 | 0.5665 | 0.8340 |

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