EFFECT OF EXPLANTS INTRODUCTION TIME ON THE *IN VITRO* DATE PALM (*PHOENIX DACTYLIFERA* L.) PRODUCTION OF BUD GENERATIVE TISSUES AND ON THE NUMBER OF DIFFERENTIATED BUDS.

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ABSTRACT

Meristematic tissues of date palm Khenezi cultivar were monthly cultured from September 1996, and for 12 successive months, on two different media with the following growth regulator:

Medium 1 (M1): 0.4 mg/l IAA, 0.4 mg/l NAA, 4 mg/l NOA, 0.4 mg/l Kin, 0.4 mg/l BAP and 0.4 mg/l 2iP.

Medium 2 (M2): 0.4 mg/l IAA, 0.4 mg/l NAA, 0.4 mg/l NOA, and 3.2 mg/l 2iP.

The two media contained Murashige and Skoog 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, 2g/l polyvinylepyrrolidone (PVP 40000), 3 mg/l activated charcoal, and 40 g/l sucrose.

The maximum percentage of bud generative tissues was obtained during spring season, especially in March. Similarly, the maximum number of buds was produced during the same season, and in particular at the month of April, regardless of the medium type. The hot environment in summer inhibited the introduced-offshoots to produce bud generative tissues, as well as the differentiation of shoot buds per bud generative tissues.

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

INTRODUCTION

Date Palm (*Phoenix dactylifera L.*) *in vitro* mass propagation has been attempted by a few laboratories around the world and a various success was achieved depending, among others, on the used multiplication technique and the variety factor. The present study used organogenesis as the main *in vitro* multiplication technique. Organogenesis, based on meristematic tissues potentiality, avoids callus formation and does not use 2,4-D. Growth substances included in the media are used as low as possible. Organogenesis technique ensures the true to typeness of the produced date palm material. Indeed, Organogenesis technique used in this study is totally different from the asexual embryogenesis used elsewhere. Asexual (called also somatic) embryogenesis, is based on the callus production and multiplication, followed by the germination and elongation of somatic embryos. However, both techniques have their advantages and disadvantages and in regard to the Organogenesis, several problems are still waiting to be solved. These various problems are met at the initiation phase, the multiplication, the rooting and elongation, and at the acclimatization phase. Furthermore, various problems met at these levels have their origin at the initiation phase and could be summarized as follows: i) Physiological stage of the offshoot, weight, age, Lignification's degree, period of introduction; ii) Initiation (too long); iii) Bacterial and fungal contamination; iv) Browning phenomenon; v) Varietal response to the technique/ Lack of reactions of some clones and varieties; vi) Yield of the technique/ offshoot; vii) Precocious root development; and the viii) Lack of results repetition. Indeed, the period of introduction of the offshoot to the *in vitro* conditions plays an important role in the success of the multiplication process. The appropriate introduction period is essential in the initiation phase and efforts are to be focused in this issue for each date palm selected variety.

MATERIALS AND METHODS

The present studies were conducted through three successive seasons (1996-1998), at the Plant Tissue Culture Laboratory and its respective greenhouse facilities of the UAE University at Al-Ain. The studies aimed at the assessment of the effect of seasonal variations on date palm regeneration capacity.

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 renowned 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. 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 disinfections

The excised shoot tips were cleaned by distilled water then subjected to disinfections procedure. The excised shoot tips were subjected to two consecutive disinfections 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 (Fig.4) 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.

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

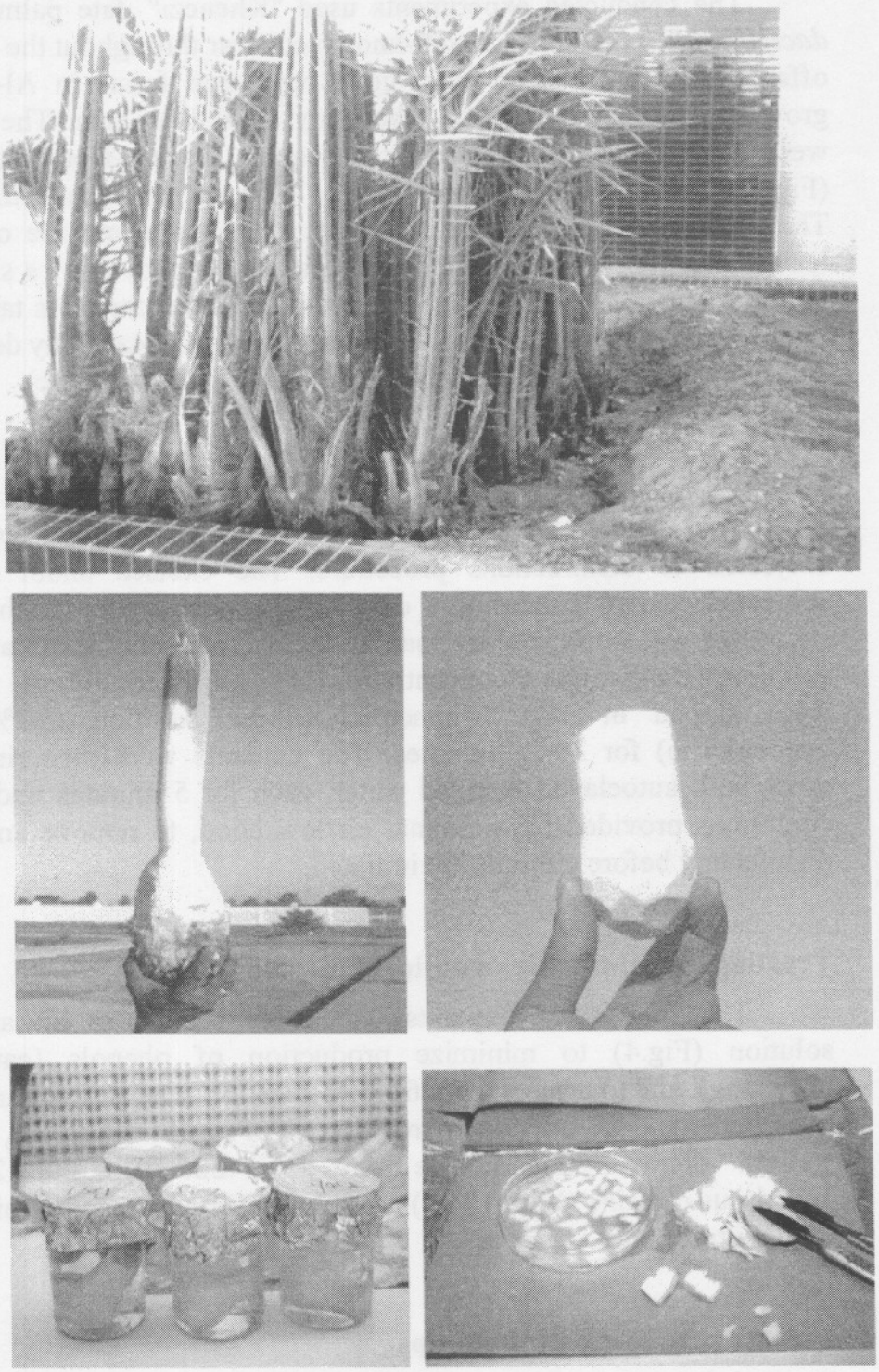


Fig.1. Plant materials (Offshoots) used as a source of explants.

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 should be 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 hormonal 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, but without charcoal until the end of the experiments. During the four first months of the initiation stage, cultures were incubated in darkness, at a temperature of 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, naphthalene 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 using 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.

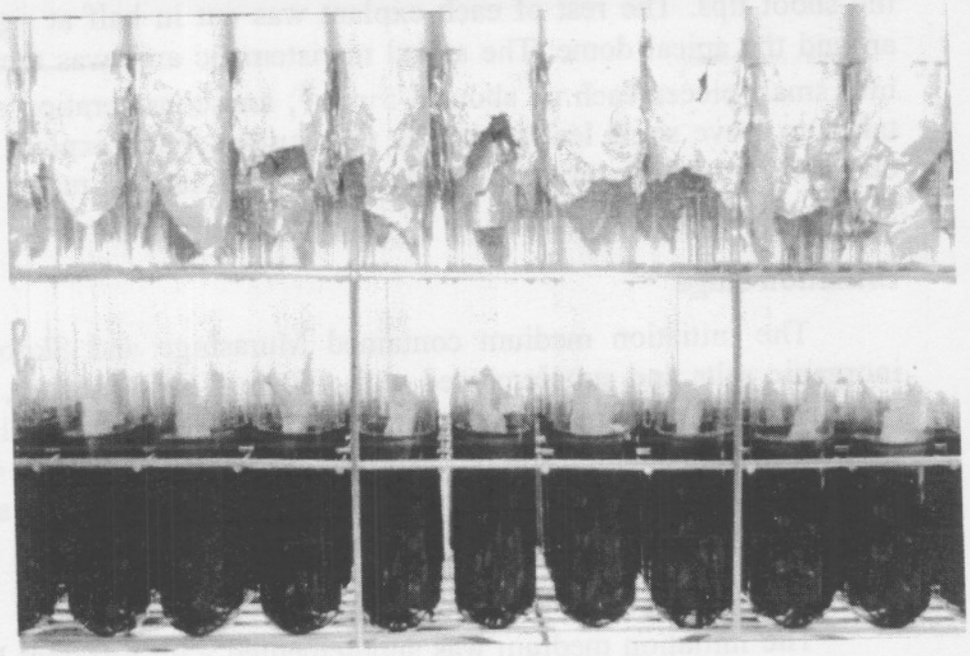


Fig.2. The initiation stage.

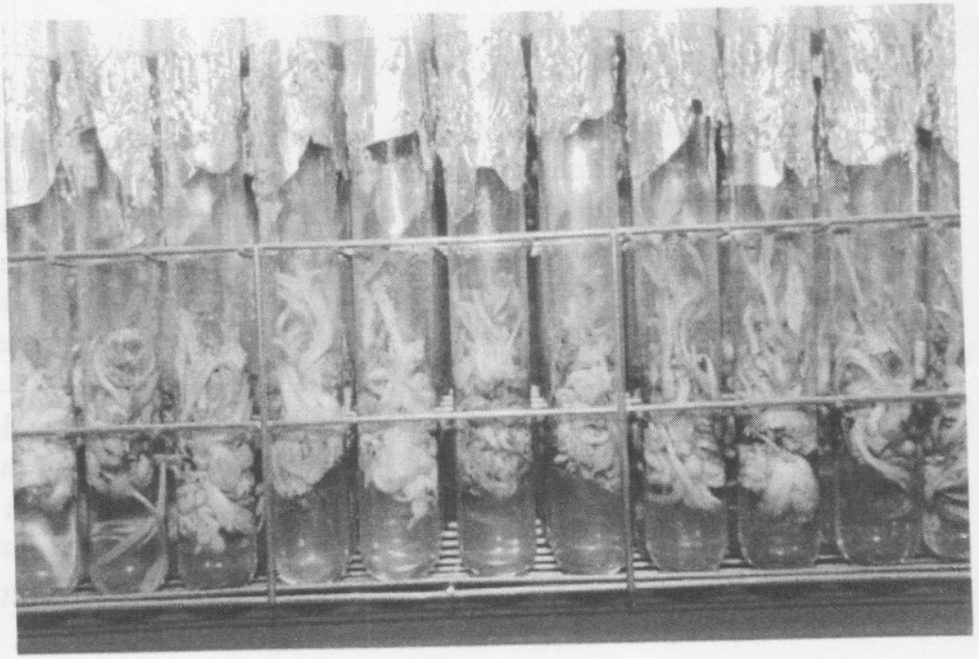


Fig.3. The multiplication stage.

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).

Experimental Procedures

Effect of culturing time on percentage explants produced bud generative tissues and number of differentiated buds per explant.

Effect of culturing time on shoot bud generative tissues and shoot bud regeneration from shoot tips of Khenezi date palm cultivar was investigated. In this experiment, the two best hormonal combinations were selected based on the results of previous experiments. These combinations were as follows:

Medium 1 (M1): 0.4 mg/l IAA, 0.4 mg/l NAA, 4 mg/l NOA, 0.4 mg/l Kin, 0.4 mg/l BAP and 0.4 mg/l 2iP.

Medium 2 (M2): 0.4 mg/l IAA, 0.4 mg/l NAA, 0.4 mg/l NOA, and 3.2 mg/l 2iP.

Culture media in this experiment were the same as in the initiation medium in both previous experiment, but supplemented with one of the above hormonal combinations.

Shoot tips were isolated from offshoots and cultured on an initiation medium containing one of the 2 hormonal combinations. Culturing started under *in vitro* conditions from September 5, 1996 and continued for 12 months, with one-month intervals. The last culture was conducted in August 5, 1997. The experiment had 32 replications (test

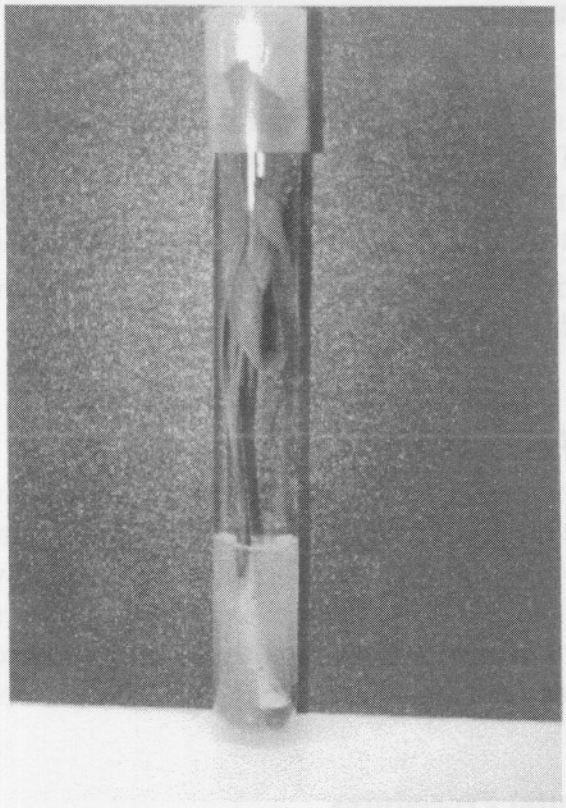


Fig.4. The elongation stage.

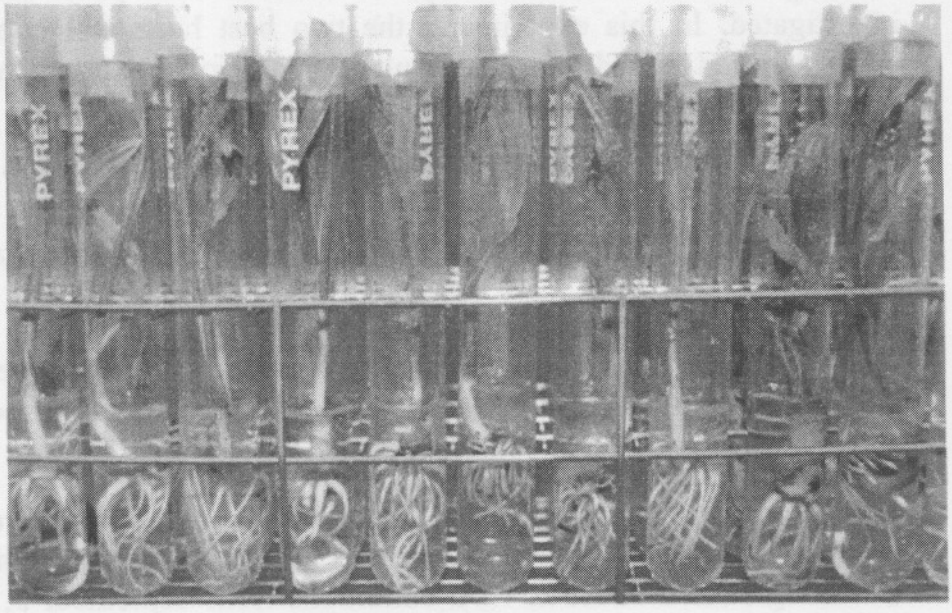
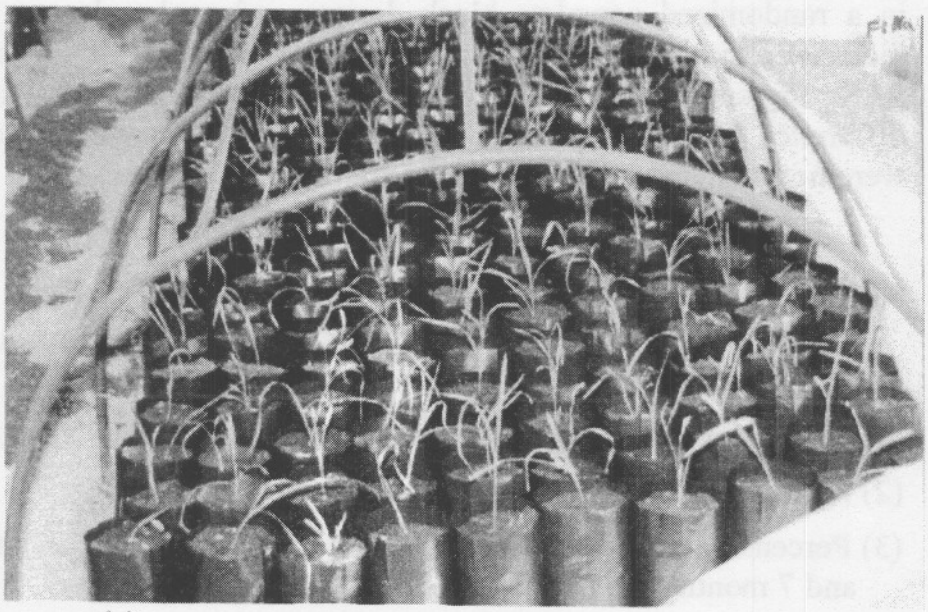
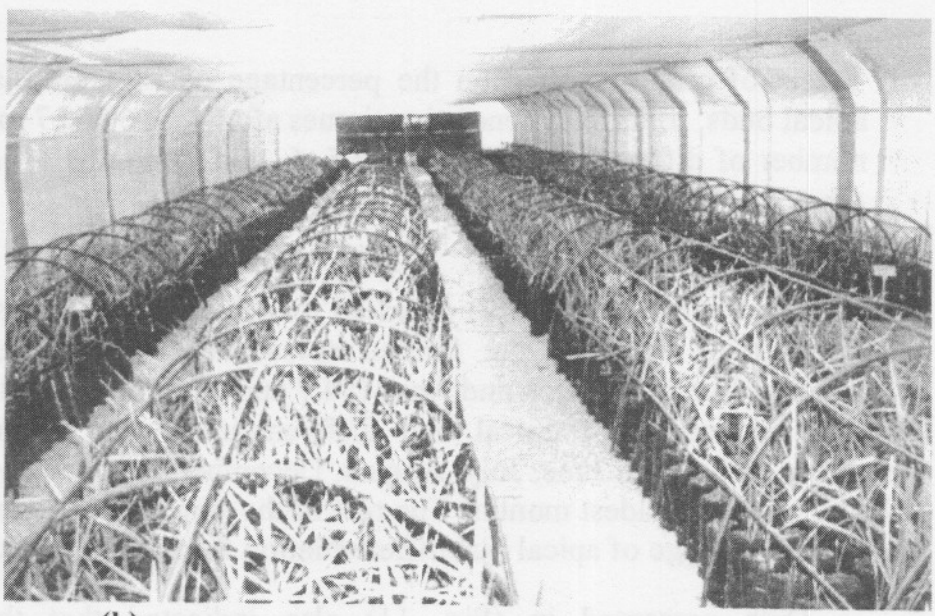


Fig.5. The rooting stage.



(a)



(b)

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

tubes) per treatment and each had one explant. The experiment was set up in a randomized complete block design, and analyzed as a 2 factors factorial experiment. Data were analyzed by analysis of variance using SAS program (SAS, 1989), with means separated by the least significant difference (LSD) test (Gomez and Gomez, 1984). Contaminated cultures were not included in the analysis.

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 were recorded after 5, 6, and 7 months from culture initiation. (Fig.10.a,b).

RESULTS AND DISCUSSION

1. Effect of culturing time on the percentage of explants that formed apical buds, roots, bud generative tissues after 4, 5, 6 and 7 months and number of differentiated buds after 5, 6, and 7 months of incubation time.

The results in (Fig. 11) revealed that the highest, and significant percentage of explants that formed apical buds was achieved from explants cultured in March and April (54.7 and 51.6 %, respectively). The lowest percentage of apical bud differentiation was obtained when explants cultured in June, July, August, December and January or during the hottest and coldest months. The rest of the year had a moderate effect on the percentage of apical bud differentiation from cultured explants.

Data presented in (Fig. 11) also indicated that the highest significant percentage of explants that formed root was obtained when explants were cultured during June, followed by explants cultured during May (17.2 and 12.5 %, respectively). There were no explants that formed roots during August and December. The ratio of explants that formed roots was significantly low during July, September, November and

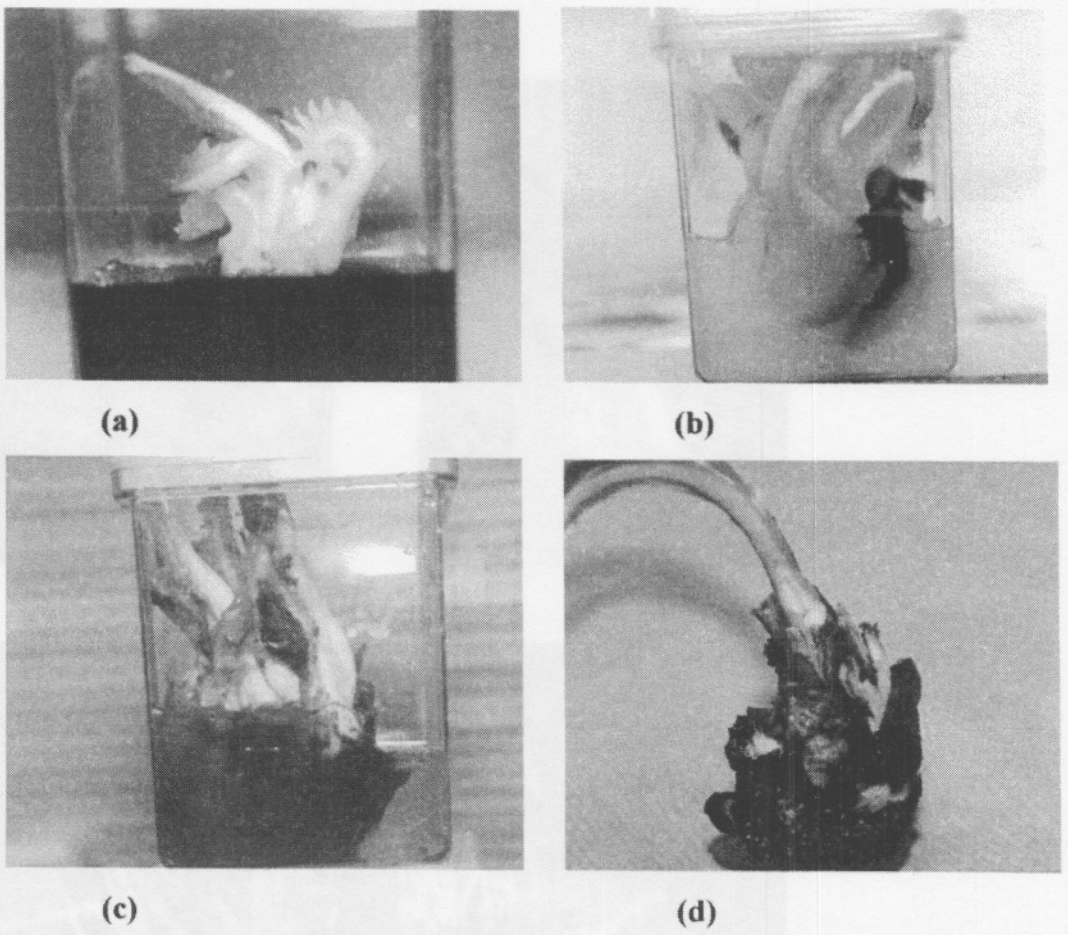
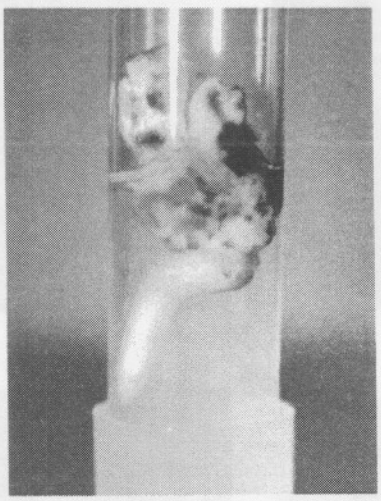
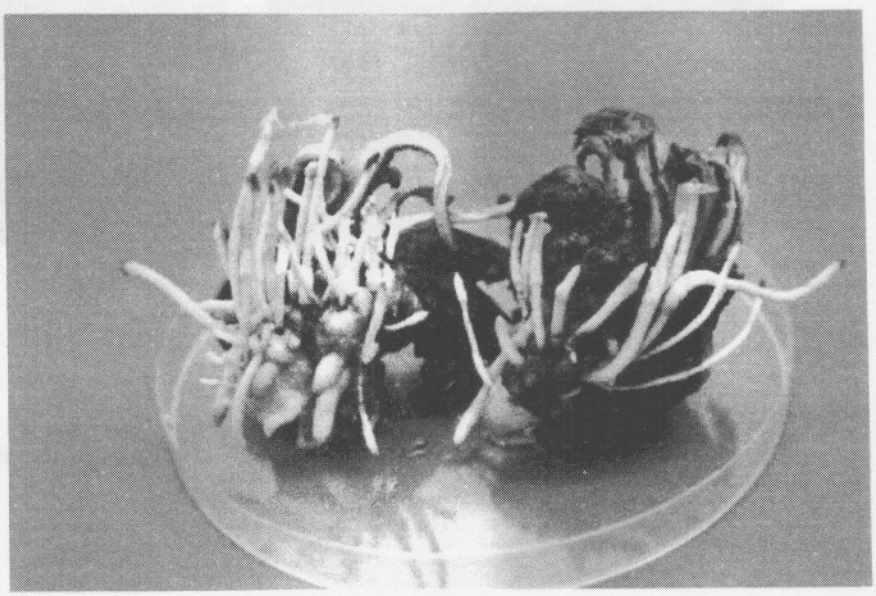


Fig.7.a,b,c Apical bud formation at various stages of development.



(a)

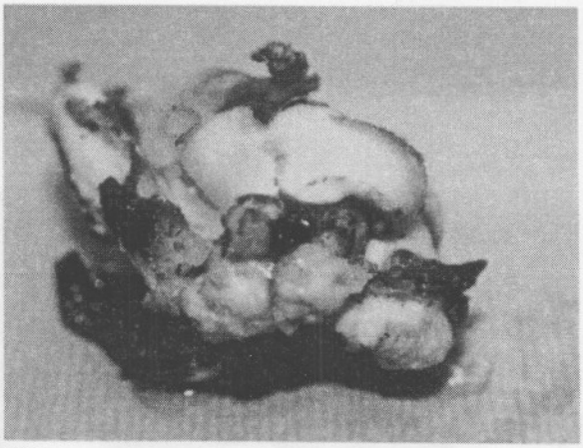
(b)



(c)

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

(a)



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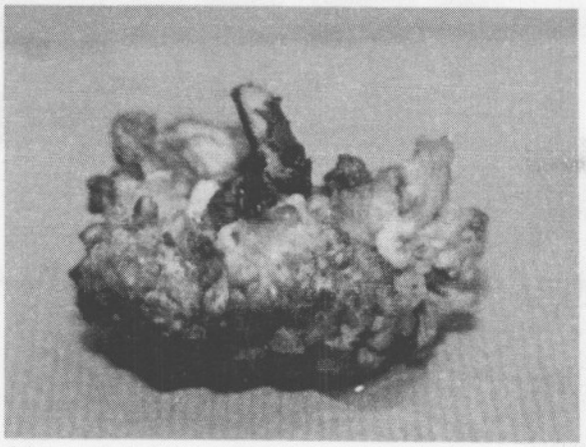

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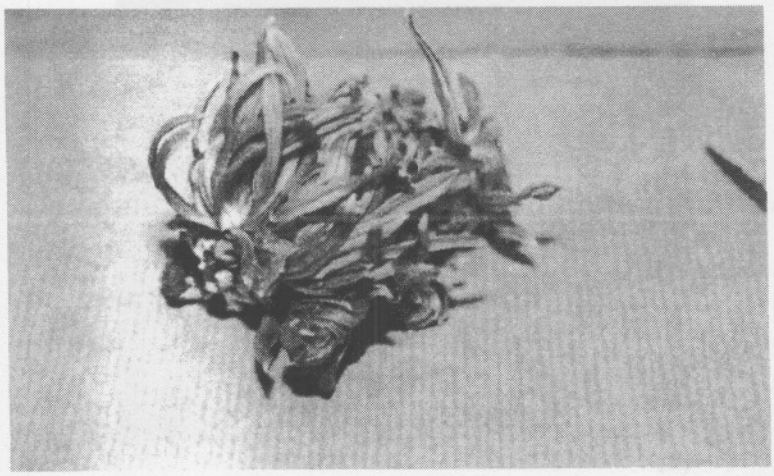
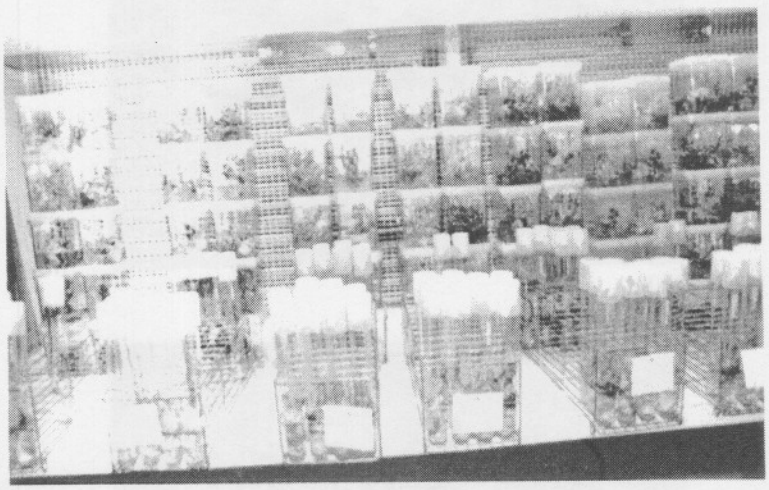
Fig.9.a,b,c Differentiation of bud generative tissues.



(a)



(b)

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

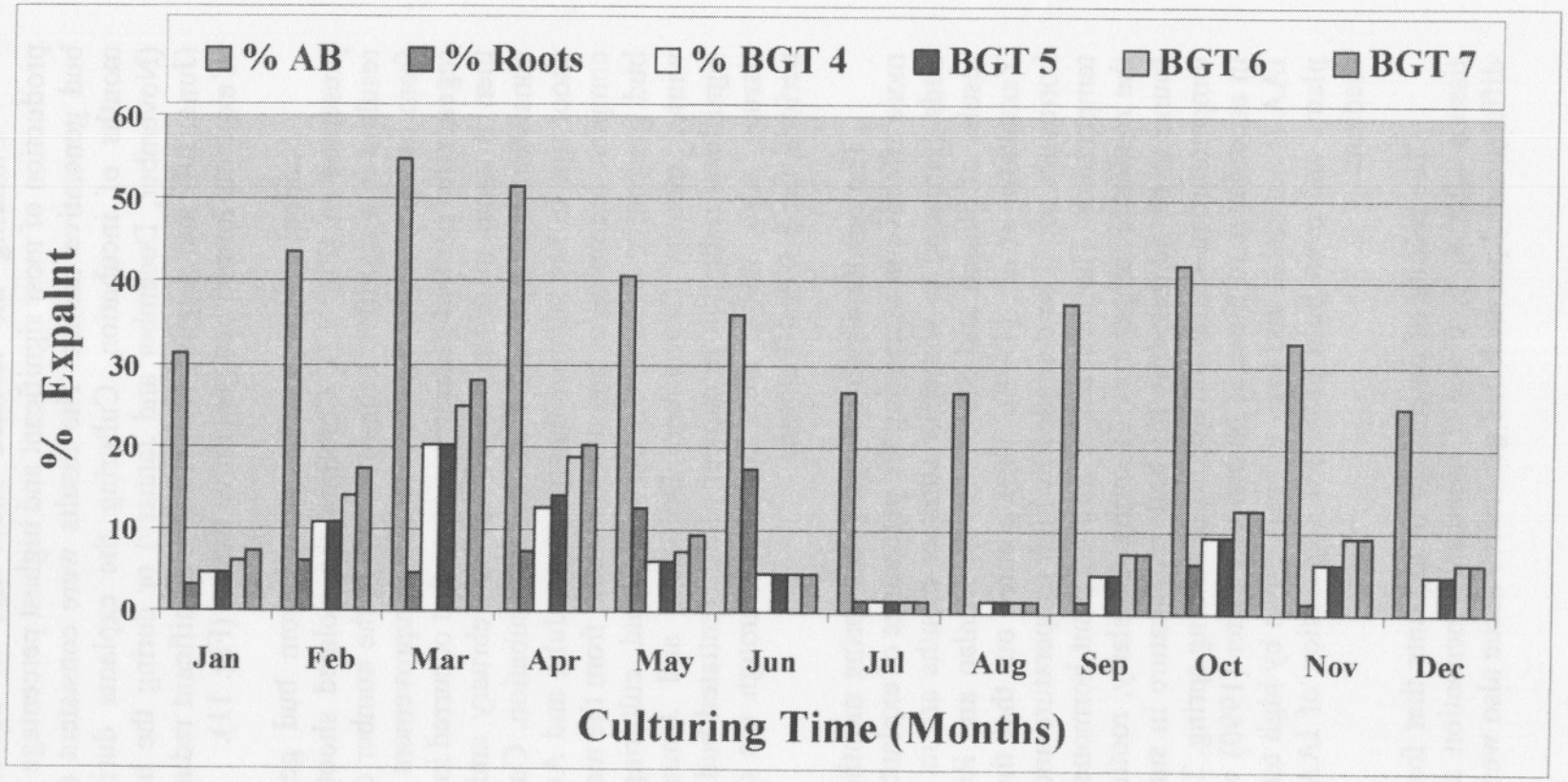


Fig.11. Effect of culturing time on the percentage of explants that formed apical buds, roots, and bud generative tissues after 4, 5, 6, and 7 months of incubation, of cultured shoot-tips of Khenezi cultivar. AB (Apical Bud), BGT (Bud generative tissues).

January. The rest of culturing months had a similar moderate effect on the percentage explant that formed roots ranging from 4.9 to 7.8 %.

Culturing the isolated shoot tips in March resulted in the production of most significant and highest percentage of explants formed bud generative tissues. The results were consistent after 4, 5, 6 and 7 months of incubation. Culturing the explants during winter months (November, December and January) or during the hot summer months (June, July and August) resulted in a significant reduction in percentage of explants formed bud generative tissues (Fig. 11).

Number of buds differentiated from bud generative tissues is presented in (Fig. 12). Culturing the isolated shoot tips during April resulted in a significant improvement in the number of buds regenerated from bud generative tissues. The same improvement in number of buds regenerated per bud generative tissues that occurred in October and April, then it came in order the months of February and March. This was consistent after 5, 6 and 7 months of incubation. Culturing the isolated shoot tips in hot summer months (June, July, and August) resulted in a complete inhibition of bud differentiation from the meristemoid arisen on bud generative tissues. On the other hand, culturing shoot tips during winter months (November, December and January) resulted in a significant reduction in number of differentiated bud per bud generative tissues. Also, results were consistent through the different incubation periods, i.e. 5, 6 and 7 months.

The results which indicated that spring months were significantly more effective in increasing the percentage of explants that formed apical buds compared to winter or summer months are in agreement with the results of Nissen and Sutter (1990); Dunlap and Robacker (1988) and Yamakawa *et al.*, (1979). They pointed out that the endogenous plant hormones could be degraded by high temperature and inactivated by low temperature. The most active form of plant hormones is associated with the moderate temperature of spring. Similarly, rooting of explants were found to be inhibited by the high temperature in summer or by the low temperature in winter and maximized during spring. These results are also in accordance to those of Nissen and Sutter (1990) who pointed out that IAA is not stable and easy to break down by high temperature in mother plants and even light promoted degradation of IAA and IBA in liquid medium.

The results of percentage of explants that formed bud generative tissues after 4, 5, 6 and 7 months of incubation and number of buds differentiated per each bud generative tissue also were consistent and

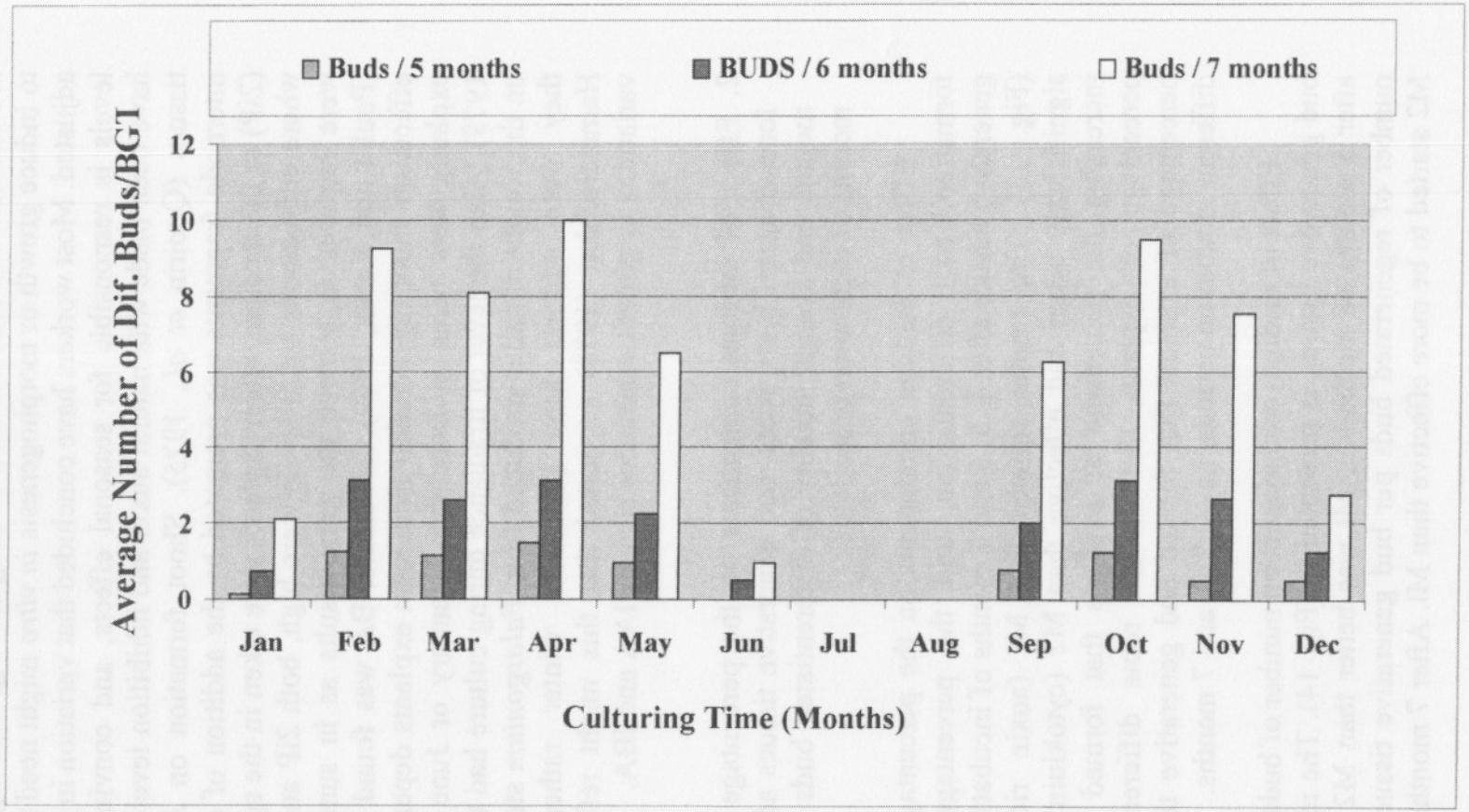


Fig.12. Effect of culturing time on the average number of differentiated buds regenerated after 5, 6, 7 months per bud generative tissues, of cultured shoot-tips of Khenezi cultivar.

related to seasonal variation in temperature. In case of mother plants, they are not kept in growth chamber under constant conditions, their tissues are likely to experience seasonally induced changes in natural growth substances levels, and /or the system which control them. This could indicate that the concentration of exogenous growth regulators necessary to induce growth or morphogenesis *in vitro* might need to be periodically adjusted. Most workers have concluded that variation in growth substance levels is responsible for seasonal effects, and convincing correlations have been made with natural auxin and inhibition level within explanted tissues (Quoirin *et al.*, 1975). Shoot formation on *Nicotiana glauca* internode fragments was promoted by the addition of only a cytokinine (2iP) to the medium, when explants were taken in the spring, in a period, where endogenous auxin levels were high, both 2iP and an auxin (IAA) were required to achieve the same results as in summer and autumn (Poulet and Ketata, 1969). In tomato, BA was found to have different effects on shoot regeneration from stem explants depending on whether explants were taken in December / January or June / July (Cassells, 1979). Also the level of irradiance during culture had the greatest effect on the weight of callus produced from *Palargonium* stem explants when they were excised from plants in winter rather than summer. Hammerschlog (1978) suggested that this might reflect the seasonal variations in growth substances such as IAA and IBA.

2. Effect of medium components on the percentage of explants that formed apical buds, roots, bud generative tissues after 4, 5, 6 and 7 months and average number of differentiated buds after 5, 6, and 7 months of incubation time.

Effect of medium components on the percentage of apical buds formation, root differentiation, and the percentage formation bud generative tissues after 4, 5, 6 and 7 months of incubation is presented in (Fig. 13). The results showed that Ml (auxin rich medium) was significantly better and effective than M2 (cytokinin rich medium) in increasing the percentage of explants that formed apical buds, the percentage of explants that formed roots differentiation, and the percentage of explants that formed bud generative tissues after the 4 different incubation periods, i.e. 4, 5, 6, and 7 months.

Effect of medium components on number of buds regenerated from bud generative tissues, is presented in (Fig. 14). The results showed that after 5 months of incubation, Ml was better than M2 in increasing the number of regenerated buds per bud generative tissues. But with time, M2 started to be more effective than Ml. After 7 months of incubation, it

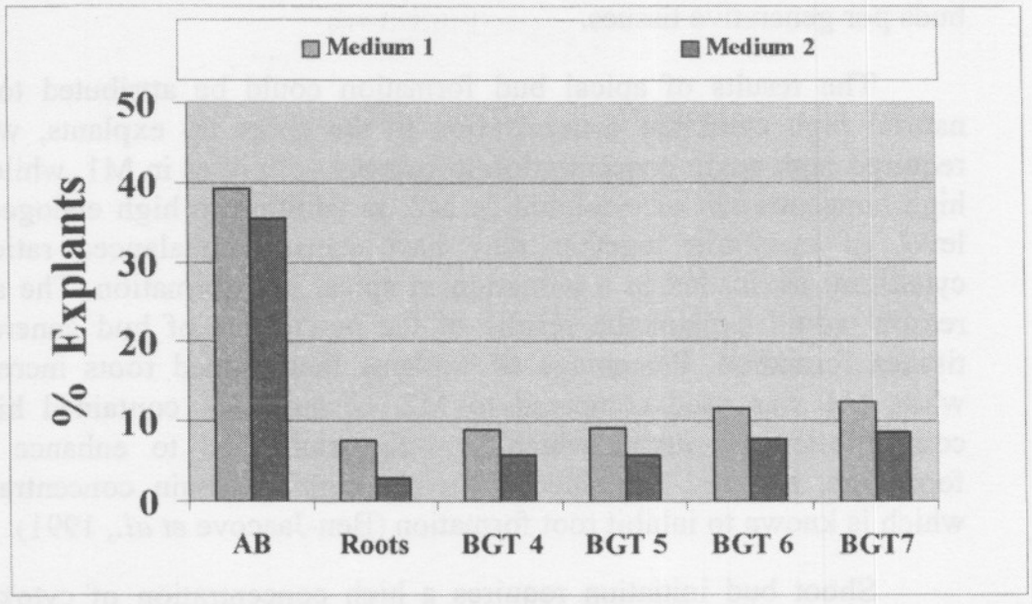


Fig.13. Effect of media components on the percentage of explants that formed apical buds, roots, and bud generative tissues after 4, 5, 6, and 7 months, of cultured shoot-tips of Khenezi cultivar. AB (Apical bud), BGT (Bud generative tissues).

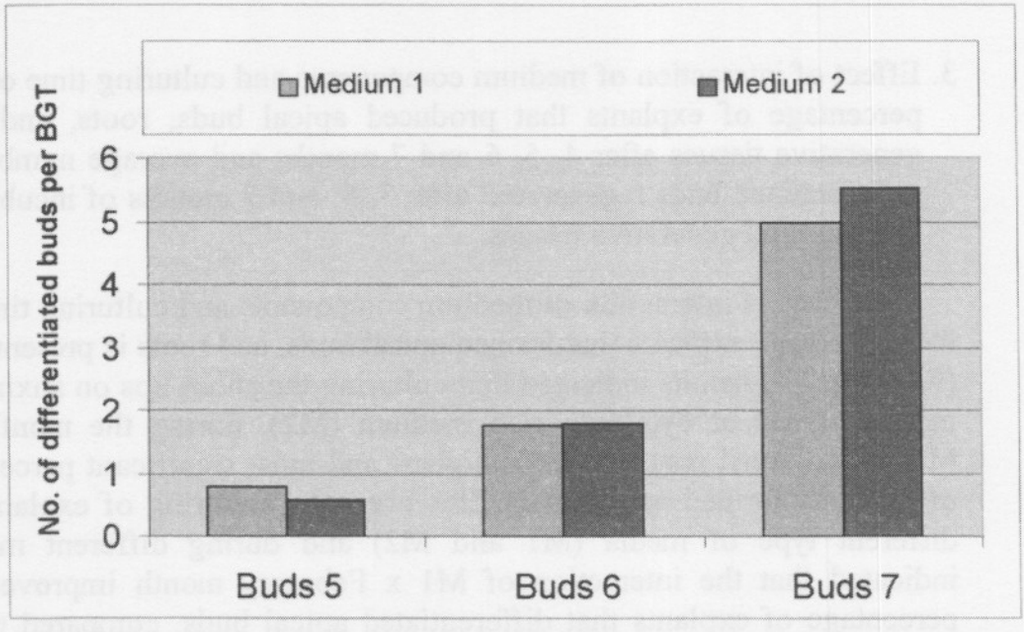


Fig.14. Effect of media components on the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissues, of cultured shoot-tips of Khenezi cultivar.

was quite clear that M2 significantly enhanced the regeneration of more buds per generative tissues.

The results of apical bud formation could be attributed to the natural high cytokinin concentration in the shoot tip explants, which required high auxin concentration to balance with it, as in M1, while the high concentration of cytokinin in M2, in addition to high endogenous level of cytokinin together may have caused imbalanced ratio of cytokinin: auxin, led to a reduction in apical bud formation. The same reason would explain the results of the percentage of bud generative tissues formation. Percentage of explants that formed roots increased when M1 was used compared to M2, because M1 contained higher concentration of auxin, which is well established to enhance root formation, and M2 contained relatively high cytokinin concentration, which is known to inhibit root formation (Ben-Jaacove *et al.*, 1991).

Shoot bud initiation requires a high concentration of cytokinin. This requirement was covered by the application of M2, which contained higher cytokinin concentration compared to M1. There were no significant differences between the effect of both media after 5 months, then after 6 and 7 months, or after the accumulation of more cytokinin in the tissues. The medium M2 proved to be better in stimulating shoot formation, especially after 7 months of incubation.

3. Effect of interaction of medium components and culturing time on the percentage of explants that produced apical buds, roots, and bud generative tissues after 4, 5, 6 and 7 months and average number of differentiated buds regenerated after 5, 6, and 7 months of incubation time per bud generative tissues.

Effect of interaction of medium components and culturing time on the percentage explants that formed apical buds, and roots is presented in (Fig. 15). The results indicated that culturing the shoot tips on auxin rich medium (M1) or cytokinin rich medium (M2), during the months of March and April resulted in the highest and most significant percentage of explants formed apical buds. However, the culturing of explants on different type of media (M1 and M2) and during different months indicated that the interaction of M1 x February month improved the percentage of explants that differentiated apical buds, compared to the interaction of M2 x February month, but not to a significant level. The results also proved that, except when explants were cultured in March and April, the interaction of M1 with any culturing time resulted in improving the percentage of explants that formed apical buds, regardless of its

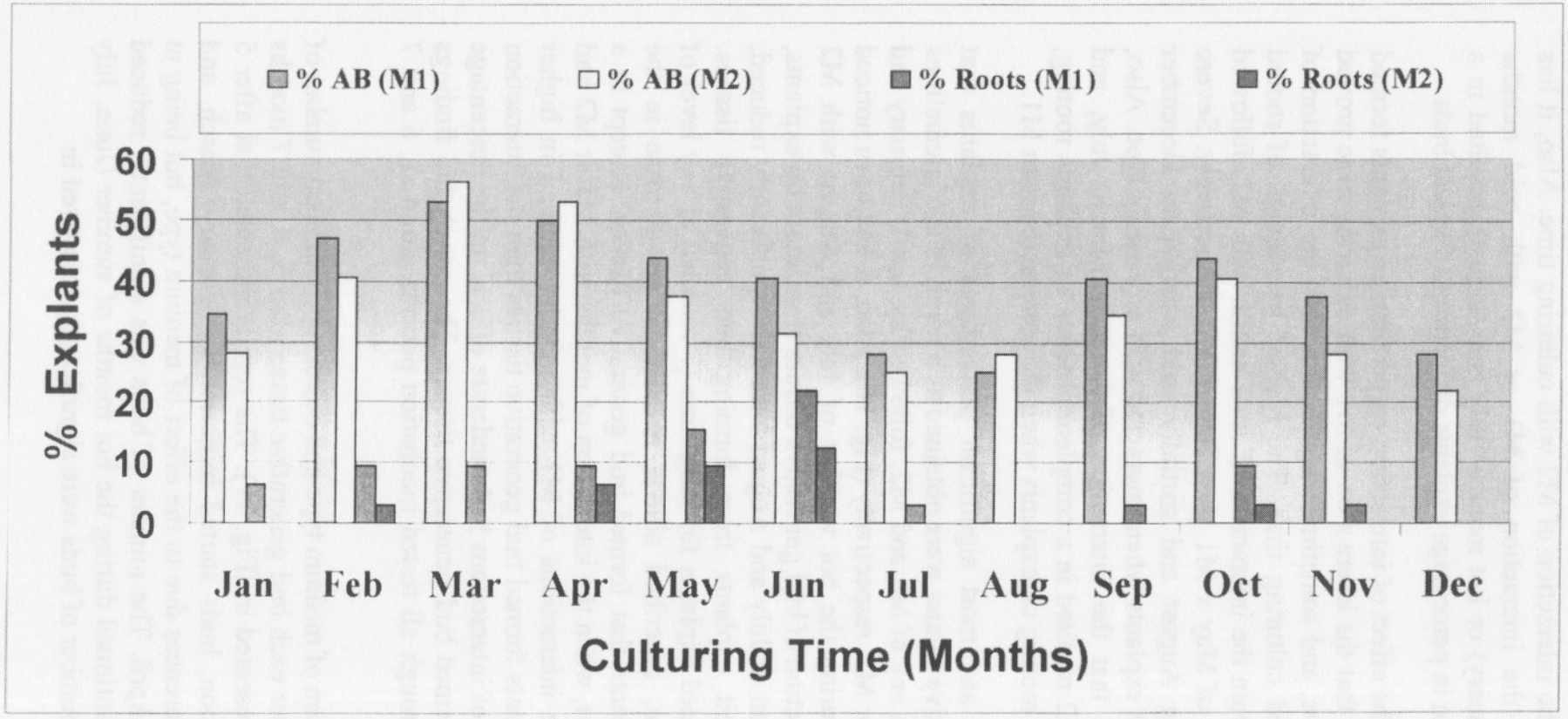


Fig.15. Effect of the interaction of medium components and culturing time on the percentage of explants that formed apical buds and roots after 4, 5, 6 and 7 months, of cultured shoot-tips of Khenezi cultivar.

significance, than the interaction of M2 with culturing time. Also, it has been noticed that the interaction of M1 or M2 with cold months (December and January) or hot months (July and August), resulted in a significant reduction in percentage explants differentiated apical buds.

Concerning the effect of interaction on percentage explants formed roots, data showed that the interaction of M1 with culturing time proved to be more effective, and sometimes significant, than the interaction of M2 with any tested culturing time. The highest percentage of rooted explants resulted from the interaction of June month with M1, followed by the interactions of May x M1, then June x M2, respectively. Severe hot weather during August and partially cold weather in December inhibited rooting of explants when interacted with any media type. Also, the data indicated that the interaction of January, March, July, and November, with M2 resulted in a complete absence of explants rooting, but it did stimulate rooting on explants when they interacted with M1.

The highest and most significant percentages of explants that formed bud generative tissues were obtained as a result of the interactions of March date with any of M1 and M2, followed by April, February and October with M1 or M2, respectively (Fig. 16). Also, it had been noticed that interactions during the hot weather of July and August with M2 inhibited the production of bud generative tissues from shoot tip explants, while the interaction of July and August with M1 significantly reduced, but did not inhibit explants from forming bud generative tissues. Incubating the treated explants for long time (7 months) at any level of positive interaction, described above, resulted in an increase in the percentage of explants that formed bud generative tissues. Except in a few countable cases, when the interaction of months with M1 or M2 had similar effects, the interactions of M1 with months resulted in higher percentage of explants formed bud generative tissues than the interaction with M2. The rest of interactions had moderate effects on the percentage of explants that formed bud generative tissues. The concluded findings were consistent through all tested incubation periods, i.e. 4, 5, 6 and 7 months.

The interaction of medium type and culturing months on number of buds regenerated per each bud generative tissue after 5, 6, and 7 months of incubation is presented in (Fig. 17). The results indicated that after 5 months of incubation, buds started initiation in February, March, and April, with no differences due to the effect of medium type, but being at maximum rate in April. The number of buds was significantly reduced started May and continued during the hot months of summer (June, July and August). The number of buds were significantly improved in

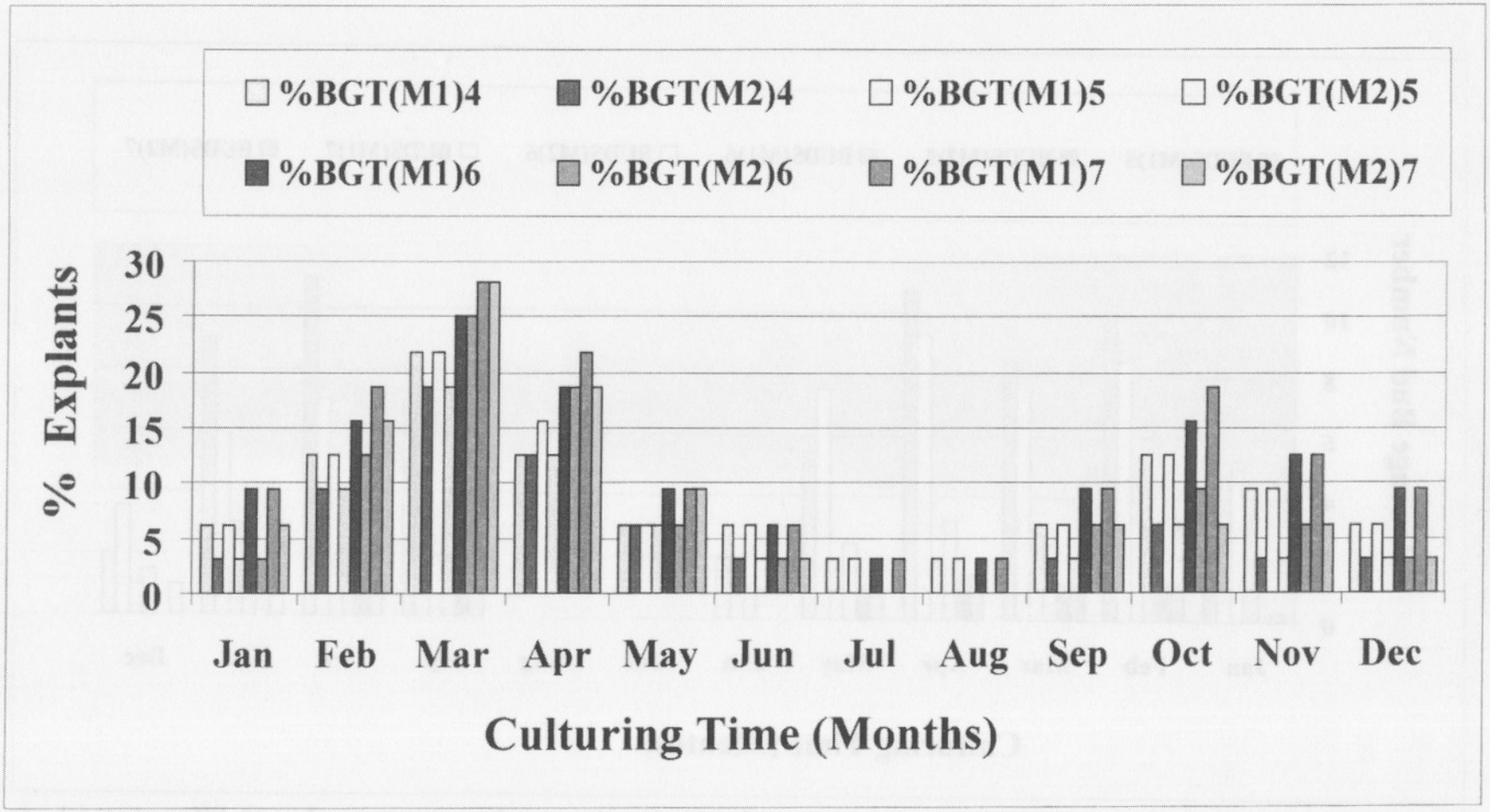


Fig.16. Effect of the interaction of medium components and culturing time on bud generative tissues after 4, 5, 6 and 7 months of cultured shoot-tips of Khenezi cultivar.

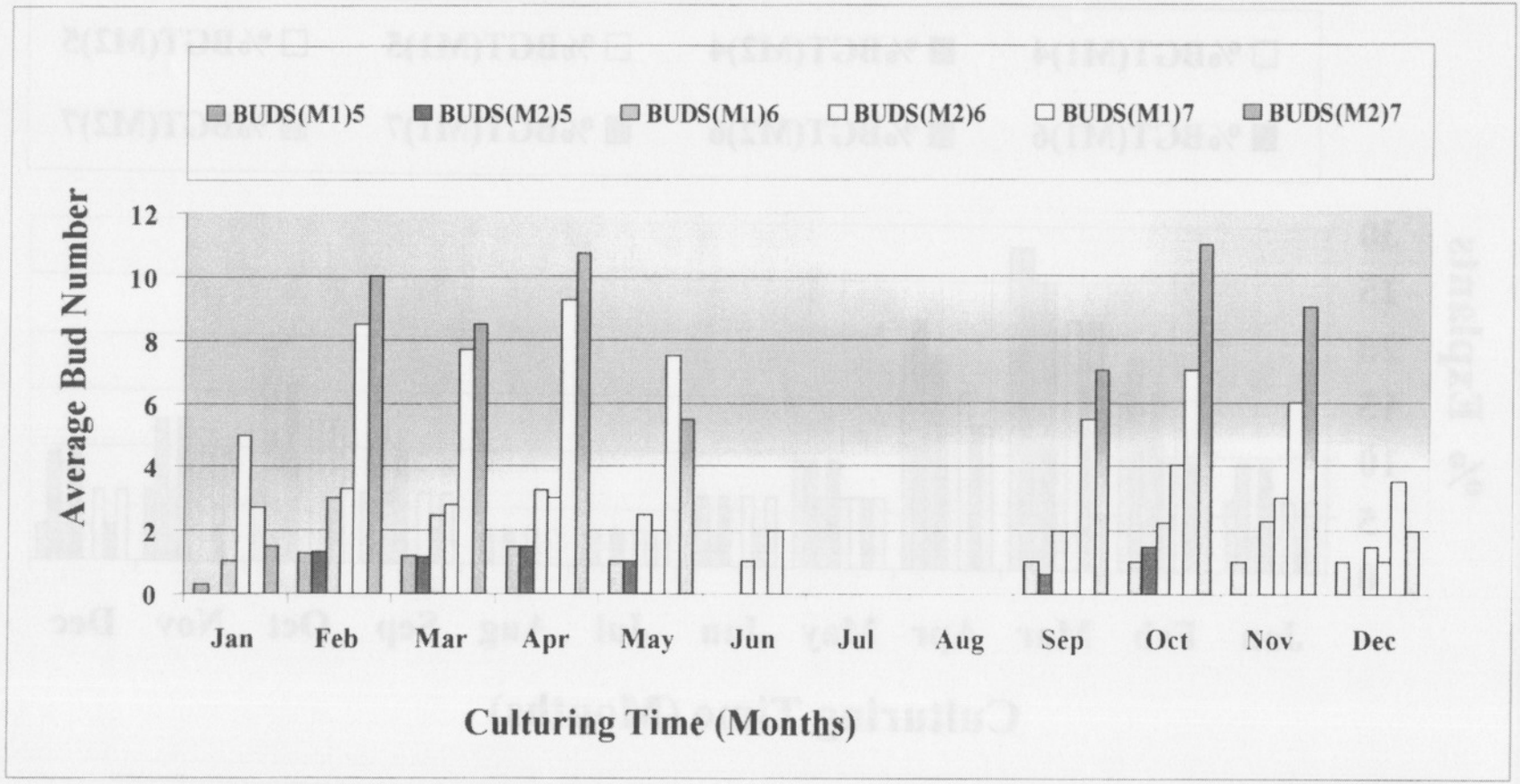


Fig.17. Effect of the interaction of medium components and culturing time on the average number of differentiated buds regenerated after 5, 6, 7 months per bud generative tissues of cultured shoot-tips of Khenezi cultivar.

October, especially when M2 was used, but it was reduced again during the winter months (November, December and January). Maximum number of buds was obtained during April, with similar effects for medium type, and during October, when the explants cultured on M2 medium. Similar trend was observed on buds generated after 6 months of incubation, when buds increased in number during February, March and April, then they were totally absent during the hot months of summer and started to develop again during October. The maximum number of buds was obtained when explants were cultured during October on M2 medium. The same trend was observed again after 7 months of incubation, but M2 was significantly better than M1 during both the spring and fall seasons.

Because of the break down of endogenous auxin by high temperature or the inactivation of plant hormones during winter months (Nissen and Sutter, 1990; Dunlap and Robacker, 1988; and Yamakawa *et al.*, 1979), culturing the explants on auxin rich medium during hot summer months or cold winter months would result in an improvement of percentage explants forming bud generative tissues, compared to the interaction with cytokinin rich medium. However, when the explants were collected in March and April, both media, M1 and M2 were similar in their effect when there was enough endogenous auxin level to induce the development of apical bud and bud generative tissues. Since rooting requires a relatively high auxin concentration, rooting was almost inhibited during winter and summer months because of the absence or unavailability of auxin. The use of an auxin rich medium was even not enough to induce rooting. The interaction of March or April with M1 or M2 resulted in the best rooting percentage due to the presence of enough endogenous hormones level available in mother tissues during spring. Number of regenerated buds/bud generative tissues reached its maximum when M2 (cytokinin rich medium) interacted with the months of April or October after 7 months of incubation. These results are in agreement with the fact that shoot bud differentiation required a relatively high concentration of cytokinin (George 1993) so the naturally endogenous balanced auxin and cytokinin were supported with the exogenous supplement in M2, and resulted in a significant increase in bud differentiation. In contrast, the low temperature in winter months reduced, but did not inhibit, bud regeneration. This observation indicated that cytokinin are not completely inactivated by low temperature as in the case of auxin.

CONCLUSIONS

The main goals of the this research is to study the effect of culturing time and hormonal combinations on the *In Vitro* organogenesis of date palm (*Phoenix dactylifera* L., cv. Khenezi).

Data showed that the highest, and significant percentage of explants that formed apical buds was achieved from explants cultured in March and April (54.7 and 51.6 %, respectively). The lowest percentage of apical bud differentiation was obtained when explants cultured in June, July, August, December and January or during the hottest and coldest months. The rest of the year had a moderate effect on the percentage of apical bud differentiation from cultured explants.

Culturing the isolated shoot tips in March resulted in the production of most significant and highest percentage of explants that formed bud generative tissues. Culturing the explants during winter months (November, December and January) or during the hot summer months (June, July and August) resulted in a significant reduction in percentage of explants formed bud generative tissues. The most active form of plant hormones was associated with the moderate temperature of spring. Similarly, rooting of explants were found to be inhibited by the high temperature in summer or by the low temperature in winter and maximized during spring.

The results showed that Ml (auxin rich medium) was significantly better and effective than M2 (cytokinin rich medium) in increasing the percentage of explants that formed apical buds, the percentage root differentiation, and the percentage bud generative tissues after the 4 different incubation periods, i.e. 4, 5, 6, and 7 months.

Regarding the effect of media components on the number of buds regenerated from bud generative tissues, the results showed that after 5 months of incubation, Ml was better than M2. But with the advancing of time, M2 started to be more effective than M1. After 7 months of incubation, it was quite clear that M2 significantly enhanced the regeneration of more buds generative tissues compared to M1.

Shoot bud initiation required a high concentration of cytokinin. This requirement was covered by the application of M2. There were no differences between the effect of both media after 5 months, then after 6 and 7 months, or after the accumulation of more cytokinin in the tissues. The M2 medium proved to be better in stimulating shoot formation, especially after 7 months of incubation.

The results indicated that after 4 months of incubation, buds started initiation in February, March, and April, with no differences due to the effect of medium type, but being at maximum rate in April. The number of buds was reduced significantly started from May and continued during the hot months of summer (June, July and August). The number of buds was significantly improved in October, especially when M2 was applied, but it was reduced again during the winter months (November, December and January). Maximum number of buds was produced during the month of April, with similar effects for medium type, and during October, when the explants were cultured on M2 medium. Similar trend was observed on buds generated after 6 months of incubation, when buds increased in number during February, March and April, then they were totally absent during the hot months of summer and started to develop again during October. The maximum number of buds was achieved when explants were cultured during October on M2 medium. The same trend was repeated again after 7 months of incubation, but with M2 significantly better than M1 during the spring and fall seasons. Because of the break down of endogenous auxin by high temperature or the inactivation of plant hormones during winter months, culturing the explants on an auxin-rich medium during hot summer months or cold winter months would result in an improvement of the percentage of explants forming bud generative tissues, compared to the interaction with a cytokinin-rich medium. Rooting was almost inhibited during winter and summer months because of the absence or unavailability of auxin. The use of an auxin-rich medium was even not enough to induce rooting. The results are in agreement with the fact that shoot bud differentiation requires a relatively high concentration of cytokinin so the naturally endogenous balanced auxin and cytokinin were supported with the exogenous supplement, and resulted in a significant increase in bud differentiation. In contrast the low temperature in winter months reduced, bud did not inhibit, bud regeneration. This finding indicated that cytokinins are not completely inactivated by low temperature as in the case of auxin.

Table 1. Effect of culturing time on the percentage of explants that formed apical buds, roots and bud generative tissues after 4, 5, 6, and 7 months and the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissues, of cultured shoot tips of Khenezi cultivar.

Month	% AB	% Roots	% BGT4	% BGT5	% BGT6	% BGT7	# Buds5	# Buds6	# Buds7
January	31.25	3.12	4.68	4.68	6.25	7.81	0.15	0.75	2.1
February	43.75	6.25	10.94	10.94	14.06	17.19	1.27	3.15	9.25
March	54.69	4.68	20.31	20.31	25.00	28.13	1.17	2.62	8.1
April	51.56	7.81	12.50	14.06	18.75	20.31	1.50	3.12	10.0
May	40.63	12.50	6.25	6.25	7.81	9.37	1.0	2.25	6.5
June	35.94	17.19	4.68	4.68	4.68	4.68	0.0	0.5	1.0
July	26.56	1.56	1.56	1.56	1.56	1.56	0.0	0.0	0.0
August	26.56	0.0	1.56	1.56	1.56	1.56	0.0	0.0	0.0
September	37.50	1.56	4.68	4.68	7.81	7.81	0.82	2.0	6.25
October	42.19	6.25	9.37	9.37	12.50	12.50	1.25	3.12	9.5
November	32.81	1.56	6.250	6.25	9.37	9.37	0.5	2.65	7.5
December	25.00	0.0	4.68	4.68	6.25	6.25	0.5	1.25	2.75
LSD	7.68	7.11	5.80	5.91	7.28	8.28	0.19	0.36	0.25

Table 2. Effect of media components on the percentage of explants that formed apical buds, roots and bud generative tissues after 4, 5, 6, and 7 months and the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissues, of cultured shoot tips of Khenezi cultivar.

Media	% AB	% Root	% BGT	% BGT	% BGT	% BGT	# Buds	# Buds	# Buds
M1	39.3	7.6	8.9	9.1	11.5	12.5	0.771	1.771	4.971
M 2	35.4	2.9	5.7	5.7	7.8	8.6	0.591	1.8	5.521
LSD	1.13	1.05	0.85	0.87	1.07	1.21	0.028	0.053	0.036

M1: medium 1 (Auxin rich medium)

M2: medium 2 (Cytokinin rich medium).

Table 3. Effect of the interaction of medium components and culturing time on the percentage of explants that formed apical buds, roots, and bud generative tissues after 4, 5, 6, and 7 months, of cultured shoot tips of Khenezi cultivar.

	% Apical Buds		% Roots		% BGT after 4 months		% BGT after 5 months		% BGT after 6 months		%BGT after 7 months	
Time (month)	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
January	34.	28.	6.2	0.0	6.2	3.1	6.2	3.1	9.3	3.1	9.3	6.2
February	46.	40.	9.3	3.1	12.	9.3	12.	9.3	15.	12.	18.	15.
March	53.	56.	9.3	0.0	21.	18.	21.	18.	25.	25.	28.	28.
April	50.	53.	9.3	6.2	12.	12.	15.	12.	18.	18.	21.	18.
May	43.	37.	15.	9.3	6.2	6.2	6.2	6.2	9.3	6.2	9.3	9.3
June	40.	31.	21.	12.	6.2	3.1	6.2	3.1	6.2	3.1	6.2	3.1
July	28.	25.	3.1	0.0	3.1	0.0	3.1	0.0	3.1	0.0	3.1	0.0
August	25.	28.	0.0	0.0	3.1	0.0	3.1	0.0	3.1	0.0	3.1	0.0
Septemb	40.	34.	3.1	0.0	6.2	3.1	6.2	3.1	9.3	6.2	9.3	6.2
October	43.	40.	9.3	3.1	12.	6.2	12.	6.2	15.	9.3	18.	6.2
Novemb	37.	28.	3.1	0.0	9.3	3.1	9.3	3.1	12.	6.2	12.	6.2
Decembe	28.	21.	0.0	0.0	6.2	3.1	6.2	3.1	9.3	3.1	9.3	3.1
LSD	10.86		10.6		8.21		8.35		10.3		11.72	

Table 4. Effect of the interaction of medium components and culturing time on the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissues, of cultured shoot tips of Khenezi cultivar

	# Bud5/BGT		# Bud6/BGT		# Bud7/BGT	
Time (month)	M1	M2	M1	M2	M1	M2
January	0.3	0.0	1.0	0.5	2.7	1.5
February	1.25	1.3	3.0	3.3	8.5	10.0
March	1.2	1.14	2.45	2.8	7.7	8.5
April	1.5	1.5	3.25	3.0	9.25	10.7
May	1.0	1.0	2.5	2.0	7.5	5.5
June	0.0	0.0	1.0	0.0	2.0	0.0
July	0.0	0.0	0.0	0.0	0.0	0.0
August	0.0	0.0	0.0	0.0	0.0	0.0
September	1.0	0.65	2.0	2.0	5.5	7.0
October	1.0	1.5	2.25	4.0	7.0	11.0
November	1.0	0.0	2.3	3.0	6.0	9.0
December	1.0	0.0	1.5	1.0	3.5	2.0
LSD	0.2713		0.5183		0.3541	

Table 5. Monthly rate of temperature average during the year of 1997 in Al-Ain City.

Months of the year	January	February	March	April	May	June	July	August	September	October	November	December
Temp. (°C)	17.0	20.3	21.6	25.3	30.4	35.1	36.0	36.1	34.2	29.8	24.0	19.7

Source: Ministry of Agriculture and Fisheries. UAE.

REFERENCES

Ben-Jaacove J., A. Ackerman, E. Tal and G. Jacops. (1991): Vegetative propagation of Alberta magna by tissue culture and grafting. Hortscience. 26, 74.

Cassells A.C. (1979): The effect of 2,3,5-tri- iodobenzoic acid on caulogenesis in callus cultures of tomato and Pelargonium. Physiol. Plant. 46, 159-164.

Dunlap, J. and K. Robacker. (1988): Light and nutrient salts interact to destroy IAA in tissue culture media. Plant Physiol. 86:120.

George Edwin. F. (1993): Plant propagation by tissue culture. Part1. Exegetics ltd., Endigton, Wilts. BA134QG, England.

Gomez, K. A. and A. A. Gomez. (1984): Statistical procedures for agricultural research. John Wiley and Sons, N. Y., USA.

Hammerschlog F. A. (1978): Influence of light intensity and date of explanation on growth of geranium callus. Hortscience 13, 153-154.

Nissen, S. J. and E G. Sutter. (1990): Stability of IAA and IBA in nutrient medium to several tissue culture procedures. Hortscience 25(7):800-802.

Poulet P. and S. Ketata. (1969): La regulation de I' organogenesis sur des cultures in vitro de fragments d' entre-noeuds de tiges de Nicotiana glauca Grah. Compt. Rend. Acad. Sci. Paris 268D, 1604-1606.

Quoirin M., Gaspart T. and Ph. Boxus. (1975): Substances de croissance endogenes liees a la reprise de meristemes de Prunus accolade en culture in vitro. Compt. Rend. Acad. Sci. Paris 281D, 1309-1312.

SAS. 1989. SAS/STAT Guifr of personal computer, 6th.ed. SAS Institute Inc., NC., USA.

Yamakawa, T., O. Kurashi, K. Ishida, K. Shigeaki, T. Kodama, and Y. Minoda. (1979): Stability of indole-3-acitic acid to autoclaving, aeration, and light illumination. Agr. Biol. Chem. 43: 879-880.

TO LARGE SCALE PROPAGATION OF SOME ELITE DATE PALM CULTIVARS THROUGH EMBRYOGENIC SUSPENSION CULTURES

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Somatic embryogenesis is believed by most research workers to be the *in vitro* system of choice for mass propagation of many species. However, with date palm, solid media did not permit a good exploitation of the embryogenic calli. This research reveals the advantages of the regeneration system based on the pro-embryogenic masses (PEMs) proliferation in suspension cultures. Our study proves that many elite date palm varieties, originated from the Maghreb and the Middle East, can produce a high number of pro-embryos using appropriate liquid media. Embryos at the globular stage complete their developmental program in the same fresh medium, and thousands of mature somatic embryos can be generated from a few grams of PEMs. Furthermore, problems with the germination of the somatic embryos have been overcome by desiccation treatment and plantlets are easily acclimatized. About genetic fidelity, flow cytometry analysis of the DNA showed that our *in vitro* conditions did not affect the ploidy level of somatic embryo-derived plantlets.