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Effect of Culturing Time and Hormonal Combinations on Organogenesis of Date Palm (Phoenix dactylifera L., CV. Khnazi) In Vitro

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EFFECT OF CULTURING TIME AND HORMONAL COMBINATIONS ON ORGANOGENESIS OF DATE PALM (*PHOENIX DACTYLIFERA* L., CV. KHNAZI) *IN VITRO*

Thesis

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ABSTRACT

Date palm (*Phoenix dactylifer*
one of the most important fruit crop trees in the Arabian Gulf region in general and in the UAE in particular.

Date palm is propagated sexually by seeds or vegetatively by offshoots. Seed propagation is not appropriate for commercial production because of the high genetic heterozygosity, which resulted in not true-to-type male and female seedlings. The vegetative propagation utilizing offshoots is slow and inefficient for rapidly growing demands of the date industry. Therefore, it seems essential to use plant tissue culture techniques for propagating and producing date palms.

The present study included three experiments that were conducted through three successive seasons (1996-1998). The first experiment included the effect of 18 different media developed from various combinations of different auxin and concentrations, in addition to the control (no hormones at all), on shoot bud generation from shoot tip of Khnazi cultivar. Maximum percentage of explants formed bud generative tissue were induced by the addition of 1.6mg/l IAA or 0.4mg/l of both IAA and NAA to the initiation medium. Maximum number of differentiated buds per bud generative tissue resulted from the addition of 0.8 mg/l IAA to initiation medium. The initiation medium contained Murashige and Skoog inorganic salts and supplemented with 100mg/l myo-inositol, 0.5mg/l nicotinic acid, 0.5mg/l pyridoxine, 0.1mg/l thiamine-HCl, 2mg/l Glycine, 40mg/l adenine sulfate, 2g/l polyvinile pyrolidon (PVP 40000), 3mg/l activated charcoal, and 40mg/l sucrose.

In the second experiment, 23 different media were developed from the combinations of different cytokinin and concentrations. Maximum percentage of explants formed bud generative tissue was induced by the addition of 3.2 mg/l 2iP or 1.6 mg/l BAP. Maximum number of generated buds per explants was induced by the addition of 3.2 mg/l 2iP to initiation medium. Both auxins and cytokinins proved to be essential for the induction of bud generative tissue and for differentiation of shoot buds from cultured explants.

In the third experiment, shoot tips of the tested cultivar were cultured monthly beginning from September 5, and continued for successive 12 months, on two different types of medium. Maximum percentage of explants formed bud generative tissue was attained during spring season, especially in March. Similarly, maximum number of buds was produced during the spring, and in particular at the month of April regardless of medium types. The hot environment in summer inhibited the formation bud generative tissues, and the differentiation of shoot buds per generative tissue.

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INTRODUCTION

CHAPTER 1

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is among the oldest plantation around the world in general and the Arabian Gulf region in particular. The tree is known to have been cultivated in this part of the world as early as 4500 B.C. and has since been an essential component of every household. Moreover, the tree has a sacred cherish in the cultures of the people of the region and is a national symbol. This is due to the fact that the tree has been the major source of stable food and fiber for the earlier societies and its groves were the locus of attraction around which towns were built and prospered. That was further supported with the mentioning of the tree in 29 different passages in the Holy Quran and many others in the Hadith.

The date palm is a tall unbranched evergreen tree that is common to almost all tropical and subtropical areas around the world. It well resists the stressful growth conditions predominant in the environments of arid-lands, especially excessive heat, salt, and water deficiency both qualitatively and quantitatively. It tolerates temperatures up to 50°C and irrigation water

salinity of over 10,000 ppm. It is also sustainable in high soil salinity and lives well in alkali soils.

The production of date palm fruit in 1996 was 4492 million metric ton per year (FAO 1996), and the main producers were the Arabian countries including (Egypt, Iraq, Saudi Arabia, UAE, Oman, Morocco and Tunisia). Dates are becoming a major export of a number of those countries and its industry is emerging as a major source of income in the agribusiness. Several highly advanced projects have been established in those countries to utilize the dates and the by-products of the tree in many profitable applications.

Commitment of the UAE government to the development of the date palm industry has sharply augmented the total number of trees from less than 2 million trees in 1971 to 25 million trees in 1997. This augmentation has been the direct result of the attention, interest and follow up of H.H. Sheikh Zayed Bin Sultan Al Nahyan, the president of the United Arab Emirates who has long directed for the; 1- subsidy to farmers growing date trees and provision of technical support and production incentives to them, 2 - establishment of new date farms and the expansion of production projects, and 3 - intensification of production operations through the utilization of advanced and modern production projects that apply

progressive technologies for sustainable date production. The increase in the total number of trees has also been complemented with improved date returns both qualitatively and quantitatively resulting in setting the UAE among the leading, if not the leader, producers in the world. The total land cultivated with dates makes up over 25 percent of the total agricultural land in UAE and over 80 percent of its fruit farms. A number of modern date processing plants have been constructed in the UAE for post-harvest processing and packaging and plans are underway for the foundation of a number of projects for the utilization of date tree by-products. Also, a number of projects have been established for the production of seedlings since the expansion in the date palm cultivation has resulted in increased demand on seedlings, especially high quality ones. Approximately more than 120 cultivars are grown in the country of which 25 are commercially utilized. Examples of those cultivars are Khlas, Bomaan, Burhi, Jabri, Khnazi, Khasab, Fardh, and. Lulu.

The date palm is a dioecious tree composed of separate male and female trees. It is an arborescent monocotyledon plant that matures into a single shoot. Flowers are born in bunches at the top of the tree and artificial pollination is a must for a marketable fruit. Conventionally, seeds from

those fruit are utilized for seedlings production but there are a number of limitations to this operation including:

1. Extended period of time required to attain the fruiting stage (3-5 years) from a seed,
2. Vast genetic variation among the seedling and the parent trees,
3. High possibility of infections of seedlings, and
4. High frequency (50%) of getting a male tree.

A faster conventional propagation process is the asexual method through the utilization of the offshoots (suckers - also known as the clone trees) taken from the base of a mature tree. This method is slow and inefficient for the rapidly growing demands of the date industry. This is due to the fact that offshoots start growing on mother tree as of the fifth year of its age and does not exceed 6-12 per tree throughout its lifetime.

Biotechnology has provided a promising asexual alternative to the demand on date seedlings. Plant tissue culture techniques have been employed to clone a wide range of economically important palms, coconuts, oil palms, and date palms around the world. Those 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 cells devoid

of cell walls as protoplasts. This occurs because each individual cell of a plant is totipotent and has 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 foundation of the Plant Tissue Culture Laboratory in the UAE University in 1989.

Date palm tissue culture follows one of two methods: 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 seedling 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 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 techniques 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 various synthesis of growth media, and their effect on the development and growth of the tissue.

Accordingly, the present research was conducted with the following main objectives:

1. To determine the best month of the year for date palm offshoot selection for *in vitro* culturing using Khnazi cultivar;
2. To develop a growth medium that optimizes tissue development through the testing of various media and the selection of the most adaptive one, and
3. To establish quick, reliable, reproducible and efficient shoot bud regeneration system for date palms using organogenesis technique.

CHAPTER II

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

2.1 Plant Regeneration

An important factor for the application of *in vitro* techniques to woody plant improvement is the development of efficient protocols for regeneration of plants from protoplasts, cells, tissues and organs. These techniques are not as well developed, especially in monocotyledon woody plants, as in herbaceous plants.

Plantlets can be regenerated from cells or tissues through organogenesis or embryogenesis, depending on plant developmental stage and culture medium and conditions. Organogenesis involves the differentiation of organs, e.g., root or shoot primordia from cells or tissues. Embryogenesis involves the development of an intact plant from somatic bipolar embryos. Embryogenesis proceeds a long typical developmental sequence including the globular heart and torpedo stage. This section will discuss organogenesis, embryogenesis and some of the factors that are involved in controlling plant regeneration in general.

2.2 Organogenesis

Many reports of *in vitro* organogenesis have been published since the early studies of plant tissue culture. A relatively high auxin: cytokinin ratio induced root formation in tobacco callus, while a high cytokinin: auxin ratio favored shoot production (Skoog and Miller, 1957).

The joint action of auxins and cytokinins seems to regulate cell division, where each of which appears to influence different phases of the cell cycle. Auxin exert an effect on DNA replication, while cytokinin seems to exert some control over the events leading to mitosis (Jouanneau, 1971). Normal cell division requires synchrony between the S phase and cell division, suggesting that auxin and cytokinin levels in culture need to be carefully matched. Late replication of DNA in cell cultures has been advanced as one cause of chromosome rearrangement (Lee and Phillips, 1988). Cells are not though to enter mitosis unless cytokinin is present whereas callus on suspension cultures is initiated on media, which only contain an auxin. Reliance is presumably being placed on endogenous cytokinins for completion of the cell cycle (Jouanneau, 1971). In some cases, the emission of auxin from the medium was sufficient to induce shoot formation (Street, 1977).

Similarly, Hassan *et al.* (1993) reported that cytokinins were necessary for organogenesis, but auxins were not.

Another factor controlling organogenesis is developmental regulation. Certain isolated tissue layers have a remarkable potential for organogenesis. For example, explants consisting of three to six layers of epidermal and subjacent collenchyma tissues removed from the region of leaf midvein of *Begoria rex*, readily produce shoots or roots from the epidermal cells (Chlyah and Tran Thanh, 1975). The types of organogenesis depend on the hormonal balance in culture medium. Again shoots formed in the presence of zeatin or benzylaminopurine (BAP) without auxin, while root initiation occurred in the presence of NAA and zeatin (Chlyah and Tran Thanh, 1975).

Many observations of organ formation in cultured tissues support the hypothesis that localized meristematic activity proceeds the organized development of shoots and roots (Torrey, 1966). The division of some cells may stimulate the division of adjacent cells or the premeristematic region acts as a sink and withdraws essential metabolites from surrounding cells, thus localizing the meristematic zone (Street, 1977).

In general buds can be initiated from callus or from cut edges of explants in the presence of high cytokinin: auxin ratio (Gresshoff, 1978). In

some species, the addition of cytokinins to the medium fails to induce shoots, suggesting that the accumulation of endogenous auxin or other hormones may result in an inhibitory effect on organogenesis which can not be reversed by exogenously applied hormones (Street, 1977). In these cases, additional hormones may be required for the cultural conditions, including nutritional and physical factors, which may block the onset of the process.

The conditions for the induction of roots vary considerably, and the appropriate conditions for root formation in some species or varieties may not be suitable in a related species or variety (Street, 1977). Usually a high concentration of auxin is favored to enhance root production, but in some cases exogenous auxin is inhibitory (Thomas and Street, 1970). Root initiation potential from *Brachycome* callus was lost after several subcultures perhaps due to changes in ploidy (Gould, 1978). Applied auxins seem to be capable of the genetically programmed physiology of whole plant tissues, which had previously determined their differentiated state. Cells which respond to auxin, revert to dedifferentiated state and begin to divide. This process had been explained by Terzi and Lo Schiava (1990) who found that auxins cause DNA to become more methylated than usual and suggested that this might be necessary for the re-programming of differentiation of cells. Tissue-specific programs specifically associated

with differentiation would become eradicated by hypermethylation, with perhaps a small fraction of the cells reaching an ultimate state of dedifferentiation in which they become capable of morphogenesis, or embryogenesis (Terzi and Lo Schiava, 1990).

In the last few decades, research has had limited success inducing organogenesis in woody plants, especially fruit crops. However, there have been several recent reports of successful organogenesis in black berry (Graham *et al*, 1997), grape (Torregrosa and Bouquet, 1996), citrus (Balch and Alejo, 1997), apple (James *et al.*, 1984), almond (Mehra and Mehra, 1974), pear (Chevreau *et al.*, 1989), olive (Canas and Benbadis, 1988), cranberry (Marcotrigiano *et al.*,1996), Kiwi (Lionakis *et al.*, 1997), Lime and mandarin (Balch and Alejo, 1997).

Graham *et al.* (1997) reported regeneration from eight different *Rubus* genotypes, using leaf sections and internode segments, but at different efficiencies, depending on the genotype. They found that a minimum level of thidiazuron (TDZ), 4.5µM, is required for regeneration. The optimum level of TDZ varied (9-22.5µM) among genotypes investigated. Zeatin was not generally as effective as TDZ in inducing regeneration, with only sporadic incidences of regeneration, and the only

significant effect was at a level of 22.8μM. When TDZ supplemented with a range of individual auxins was investigated, it was found that IBA at 0.49μM in combination with 9μM TDZ was the optimal level, while increasing the level of TDZ to 13.5μM in the combination, resulted in a complete inhibition of regeneration. Similarly, the presence of 2,4-D at 0.45 or 0.9μM, in combination with TDZ levels, inhibited the regeneration capacity. However, NAA proved to be a better auxin source, where it was applicable across a wide range of cultivars at relatively low levels. This auxin was found to be complementary to TDZ and levels of regeneration from a number of lines were stimulated. Fiola *et al.* (1990) reported organogenesis from *Rubus* leaves and cotyledons. Shoots were obtained on a medium containing MS salts and sugar, Staba vitamins, casien hydrolysate (100mg/l) and 10μM TDZ. Explants were kept in the dark for 2 weeks before being placed in a 16/8-hr light/dark regime. Preconditioning of source shoots with 1.5μM TDZ enhanced organogenic potential of detached rubus leaves (Swartz *et al.*, 1990).

The most efficient protocols for regeneration of *Rubus* (Black berry) plantlets were published by Hassan *et al.* (1993). They used either leaf petiols or stem internode segments. Maximum shoot regeneration (99-100%) was induced on MS medium supplemented with 0.09 mM sucrose,

0.2mM adenine sulfate, 0.55mM inositol, 0.14mM ascorbic acid, 100mg/l of casien hydrolysate, Staba vitamins, 5µM TDZ and 0.5µM IBA. Cytokinins were necessary for organogenesis, but auxins were not.

Very recently, high levels of regeneration were obtained from young leaves excised from axillary shoots in proliferating nodal cultures of several *Vitis* x *muscadinia* hybrids. Best results were obtained when the explants were cultured on solidified half-strength MS medium supplemented with 8-9µM Benzyl adenine (BA) and 0.05µM NAA. Though variations were observed among the hybrids, the procedure used dose not seems to be genotype-specific as all the hybrids cultivars tested could regenerate (Torregrosa and Bouquet, 1996). Adventitious buds were formed from cultured internodes of grape cultivars when the explants were grown in agitated liquid Nitsch medium supplemented with 1 µM BA and 5µM 2,4-D or a mixture of 5µM 2,4-D and 5µM NAA (Rajaskaran and Mullins, 1981). Direct shoot organogenesis from leaves of grape (*Vitis* spp.) has also been reported. Stamp *et al.* (1990a) obtained adventitious shoots at the petiolar stub and occasionally from wounded laminar tissue, when leaf explants were cultured for 4 weeks on MS or Nitsch and Nitsch basal medium supplemented with 1, 2 or 4mg/l BAP. The highest frequency of shoot

organogenesis was obtained with 2mg/l BAP. Shoots are rooted easily in sterile soil and plantlets are morphologically identicated to their parents.

Very recent, a method for regeneration from leaf explants in two cultivars of cranberry was described by Marcotrigiano *et al* (1996). They used a modified Anderson's medium (1984a) supplemented with combinations of thidiazuron (TDZ) with or without 1μM NAA to optimize shoot regeneration. Maximum regeneration was obtained, in the light, in the presence of 10μM TDZ and 1μM NAA, but this medium was suitable for leaf regeneration explants obtained from shoot cultures, while regeneration did not occur from leaves collected from greenhouse-grown plants.

Plantlets and shoot regeneration from calli derived from unburst lateral Kiwi fruit have been reported. Lionakis *et al.* (1997) obtained callus from lateral buds of Kiwi fruit cv. Hayward using 2mg/l BA for 4 weeks of culture in an initiation medium. When BA concentration in the medium was reduced to 1 mg/l, plantlet regeneration occurred and complete plants with shoots and roots were obtained. By keeping the concentration of BA at 2mg/l, only shoots were regenerated from the callus. Similarly, Famiani *et al.* (1997) achieved organogenesis and callus production from leaf, petiole, stem and root explants of Kiwi fruit cultivars Hayward, Tomuri and Katiuscia and the interspecific hybrid *A. deliciosa* x *A. orguta*. They found

that genotype and explant type, as well as medium composition, strongly affected organogenesis and callogenetic ability. Of the cultivars, Katiuscia showed the best results, followed by Tomuri, Hayward and the hybrid. Leaf, petiole and stem explants generally gave better results than root explants. A medium containing a high cytokinin: auxin ratio produced the best results in terms of callus and shoot production.

Balch and Alejo (1997) reported an efficient protocol for *in vitro* organogenesis of Mexican lime (*Citrus aurantifolia*) *reticulata*) cv. Monica using seedlings internodal stem segments. They indicated that the best results were obtained when the wounded edges of internodal stem segments cut longitudinally were placed downward on the surface of the culture medium. The optimal culture medium for both species was Murashige and Skoog (MS) with vitamins from B5 medium, 5% sucrose, 33.3μM BA and 5.4μM NAA. The best response was obtained when the segments were incubated at 25 ± 2 °C for 21 days in the dark, followed by 29 days on a 16 / 8 h light/dark cycle at 54 mol m^{-2} s^{-1}.

In the best regeneration system tested, adventitious shoots were obtained from 96 and 88% of the explants in Mexican lime and mandarin, respectively. In Mexican lime, an average of 7-8 well differentiated shoots

per explants were obtained, and in mandarin the yield was 5.1 shoots per explant.

James *et al.* (1984) reported organogenesis in callus derived from stem and leaf tissue of apple rootstocks. Callus was initiated from stem internodes of M.25 and M.27 on 2-10mg/l NAA based medium and the calli were then transferred to corresponding media lacking NAA where regeneration of shoot occurred. Organogenesis was also obtained from leaf discs of M.27 on medium containing BAP at 5.0mg/l and 2,4-D at 0.1mg/l. The key factor in this work was exposing the explants to dark conditions for 4 weeks to induce callus. Culture directly exposed to low light did not undergo shoot formation. Adventitious shoot formation from "Red Delicious" apple cotyledons was achieved by Kouider *et al.* (1984). Embryo axis-free cotyledons produced callus and adventitious shoots when cultured on modified Nitsch and Nitsch medium supplemented with 4 mg/l BAP. Explanted cotyledons were placed in the dark for 4 days and then moved to the light, where callus differentiated into shoots within three weeks. Kouider *et al.* (1985) also used a similar protocol for the production of shoots from an intact and sectioned embryo axes of apple seeds.

Yepesm and Aldwinckle (1994) examined several factors affecting the frequency of organogenesis in apple leaf explants of different species.

They found that the main factors affecting morphogenesis were benzyladenine (BA) concentration, basal medium, leaf explant origin and maturity and photosynthetic photon flux. Depending on the genotype, optimal regeneration was obtained using either 22.2 or 31.1μM BA and N6 basal medium, with the exception of cv. Golden Delicious, which regenerated better on an MS medium. After 6 weeks, the average number of shoots per segment varied from 5 to 16, and the percentage of regeneration ranged between 70 and 100%, depending on the genotype tested and the maturity of the explant. Regeneration capacity increased dramatically from the tip towards the base of the leaf, and was higher from the middle to the proximal end. Similarly, Bartish and Korkhovoi (1997) proved that shoot formation by apple leaf explants depends on the composition of the nutrient medium. They reported that optimizing the nutrient medium, by changing BA concentration (5-10 mg/l), increasing NAA concentration up to 1-1.5 mg/l, and diluting Murashige and Skoog salts up to 3 folds, enhanced regeneration frequency to 67% in *Nezalezhnist* cultivar, 72% in *Idared* cultivar, 80% in *Florina* cultivar and 100% in clone RF1.

In almond (*Prunus amygdalus*), different plant parts, i.e., leaves, cotyledons and embryos were stimulated to form callus. Their calli showed optimal growth on MS basal medium supplemented with 5mg/l NAA, and

10% coconut water. Differentiation of shoots and whole plantlets took place when the MS basal medium was supplemented with1g/l of casein hydrolysate and either zeatin (0.25-0.5 mg/l), BAP (0.25-0.5mg/l) or kinetin (0.5-1.0 mg/l), with differentiation being most frequent on kinetin medium (Mehra and Mehra, 1974).

Chevreau *et al.* (1989) developed an efficient protocol to regenerate adventitious shoots from leaf tissue of several pear (*Pyrus sp.*) cultivars. Half-leaves, taken from *in vitro* grown shoots were cultured on MS medium supplemented with different combinations of cytokinin and auxin. TDZ combined with NAA was the most efficient medium for stimulation of adventitious shoots. Shoot regeneration was observed over a wide range of TDZ and NAA concentrations (0.5-5.0µM and 2.5-13µM, respectively). The optimum level of TDZ was 3 µM. Murashige and Skoog at 1/2 and 1/4 strength salts were the most effective. Sucrose concentration (10-50 g/l) had a linear and significant effect on shoot regeneration of only one out of three tested cultivars.

In vitro plant regeneration from cotyledon fragments of olive (*Olea europea* L.) was reported by Canas and Benbadis (1988). Calli were induced from cotyledon segments on olive medium supplemented with high auxin (5mg/l IBA) and low cytokinin, 0.2-0.5mg/l isopentyl adenine (2iP)

or zeatin riboside concentrations. Shoot induction mainly occurs from calli of cotyledon fragments proximal to embryo axis and on medium supplemented with 4mg/l 2iP. Recently, Mencuccini and Rugini (1993) investigated the morphogenic capacity of petiole, leaf discs and midribs of several olive cultivars, collected from 1-year-old potted greenhouse plants, 10-year-old field - grown trees and *in vitro* cultured shoots. Explants from field-grown and greenhouse plants, and leaf disc and midrib explants from *in vitro* shoots in cultivars Moraiolo, Dolce Agogia and Halkidikis, on MS medium and on modified olive medium only in the dark. The highest regeneration was achieved directly from the proximal part of the petioles after 2-3 weeks in media containing 5-40μM thidiazuron, or with both 10μM 2-isopentyl adenine + 2.2μM BA with or without low auxin concentration ($\leq$2.5μM IBA). Adventitious shoots were regenerated from 2-5% of calli when transferred from auxin + cytokinin media to cytokinin only medium. The regeneration potential was higher in petioles collected from apical nodes than from basal nodes (40 and 5%, respectively). The adventitious shoots were transferred to solid half-strength MS medium supplemented with 4.5μM zeatin for further development. Several regenerated shoots were rooted and the plantlets hardened off in the greenhouse. No apparent morphological differences were observed among

the regenerated plantlets or with those obtained by stimulation of axillary buds.

Very recently, organogenesis from date palm shoot tip cultures was reported (Bekheet and Saker, 1998). They stated that both direct and indirect shoot proliferation from Egyptian date palm cultivar "Zaghlool" were obtained. They established shoot tip culture on Murashige and Skoog medium supplemented with 100mg/l myo-inositol, 50 mg/l adenine sulfate, 1-5g/l activated charcoal, 2mg/l 2iP and 0.1mg/l NAA. Direct shoot proliferation was noticed after two subcultures onto MS medium supplemented with 4mg/l 2iP, 4mg/l BAP and 0.5mg/l NAA. Subculturing the proliferated shoots onto MS medium contained 1mg/l NAA led to root formation.

Chabane and Bougedoura (1998) reported direct organogenesis from two date palm cultivars (Takerbouchet and Deglet Nour) occured from young leaves on media supplemented with low ratio of 2,4-D / 2ip. Male and female inflorescences of date palm were used to establish a micropropagation system (Loutfi, 1998). He reported that young inflorescences formed many shoot primordia when cultured on Gamborg and Evelegh (1968) and Gresshoff and Doy (1972a) mineral salt solutions containing 2.6µM NAA, 8.8µM BA, 6.1µM 2iP. Initiated shoots were

multiplicated on the same medium. Most of these shoots rooted spontaneously on a medium containing 10.7µM NAA and 4.4µM BA or 6.1µM 2ip. Histological analysis revealed that buds were originated from the petal primordia.

El-Henawey and Salom (1982) reported root and shoot differentiation from date palm shoot tip *in vitro*, using MS salts mixture with the addition of 1mg/l of both NAA and kinetin, 25mg/l adenine and 200mg/l myo-inositol. They also reported that environmental conditions seemed critical especially incubation temperature (25-32°c). Their histological study indicated that morphological differentiation including explants with a degree of cell division, differentiation and dedifferentiation had occurred.

Khan *et al.* (1982), cultured different date palm tissues including shoot apex, primordial leaves, axillary buds, and roots on medium containing 3% sucrose, 1% agar, 100mg/l myo-inositol, and 0.4mg/l thiamin-HCl. IAA, NAA, IBA and cytokinin (Kinetin) were added to the basal medium at varying concentrations. NAA at levels from 1-10mg/l with Kinetin induced callus growth and root initiation. IAA concentration was found to be unsuitable for offshoot tissue. They regenerated plantlets by reculturing shoot apex on a medium containing NAA at 0.1mg/l and Kinetin at 0.1mg/l.

Different types of explants excised from date palm seedling, offshoots and adult trees were investigated to establish a regeneration

system. Plant regeneration was attained in excised cotyledonary sheath in NAA and 2iP containing medium. Leaf segments initiated either shoot only, or callus followed by asexual embryogenesis. Offshoots shoot tips initiated numerous adventitious buds directly and without intermediary callus. Multiplication of shoot tips was achieved in NAA medium, which were rooted individually (Omar, 1988).

Starting from soft tissue or young leaves, and from shoot tips or axillary buds, Beauchesne (1982) and Rhiss (1980) reported the production of many thousands of new plantlets from offshoots of date palm. However, in each tested cultivar, i.e., Bou Feggous, Bouskri, Bou Stammi noir, often needs modifications in media composition was necessary.

Tisserat (1984) cultured shoot tips, 0.5mm in length, of date palm on MS inorganic salts and 30g sucrose/l, 0.4mg thiamine-HCl, 100mg/l myo-inositol, 10mg/l NAA, 3g/l charcoal, and 8 g agar. After 8 weeks tips were proliferated through axillary bud outgrowths on a liquid medium devoid of charcoal containing 0.1mg/l NAA and 10mg/l BA. These additional shoots then could be rooted by recultured on agar medium devoid of BAP, containing 0.1mg/l NAA and successfully transferred to soil.

Adventitious plantlets were obtained from lateral buds, shoot tips, and stem pieces of date palm cultured on modified Murashige and Skoog

medium containing 3mg/l 2iP, 0.1-100mg/l NAA or 2,4-D and 3g/l activated charcoal. Additions of auxins were necessary to induce explants to produce callus, adventitious plantlets, and roots. Plantlets were obtained from explants cultured 3-4 months *in vitro*. No difference in growth responses between male and female explants was observed during culture. Data also proved that activated charcoal fostered satisfactory growth by reducing the browning and inhibition of growth of explants. (Tisserat, 1979a).

Date palm plantlets have been produced through callus phase by Tisserat (1982) through culturing shoot tip and lateral buds on media containing MS inorganic salts, 30 g/l sucrose, 100mg/l myo-inositol , 0.4mg/l thiamine-HCl, 8g/l phytagar, 3.0 g/l activated charcoal, in addition to 100mg/l 2,4-D and 3.0mg/l 2iP. The resulted callus was friable yellow-white in color and was composed of minute nodules, that initiated 2-4 months after explant introduction to callus induction medium. The researcher also pointed out that to obtain a large number of plantlets from date palm callus, individual plantlets should be isolated from calli masses and planted separately to facilitate their development.

Vegetative propagation of several date palms through shoot tip culture and plant differentiation was investigated by Gabr and Tisserat

(1985). They studied the influence of explant size, medium composition and physical environment required establishing date palm shoot tips *in vitro*. They pointed out that satisfactory date palm shoot tip growth and proliferation was obtained from explants that were 3 mm in length, consisting of the apical meristem region and 2-5 adjacent leaf primordia. Optimum shoot tip development and axillary budding was obtained by initially establishing explants on agar medium for 2 weeks, then transferring them to a liquid medium containing 0.0-1.0mg/l NAA and 0.0-15.0mg/l benzyladenine or 2iP. When shoot tips of date palm were cultured on MS media containing 100mg/l 2,4-D, 3mg/l 2iP and 3g/l activated charcoal or to determine their morphogenetic response *in vitro*, explants initiated callus and complete plantlets were produced after 4-8 months in culture. But when shoot tips of several date palm cultivars were cultured on MS medium that was supplemented with 1mg/l NAA and 3.0g/l charcoal, they developed into plantlets with well-developed leaves and adventitious roots within 2-6 months from the time of planting.

Anjarne and Zaid (1993) reported that organogenesis of date palm shoots from the young bottom leaves of offshoots was inhibited on media with a low concentration of auxins. Shoot tip explants consisted of the apical meristem (dome) with two to four leaf primordia and varied in size

from 0.5 to 1 mm^3. Meristems and tips were cultured on modified MS medium containing 3g/l activated charcoal 0.1-300mg/l NAA, 2,4-D, IAA, IBA, BA, Kinetin or 2iP. They reported that best consistent shoot regeneration occurred on nutrient media containing NAA. Best rooting was achieved with 0.1mg/l NAA with 63% of the shoots initiating adventitious roots after the first culture passage (Zaid and Tesserat, 1983).

Starting from bottom of young leaves, soft tissue, shoot tips or axillary buds of date palm offshoots, Beauchesne *et al.* (1986) reported the obtaining of well formed and vigorous plantlets which were able to be successfully transferred in greenhouse. The media used were Murashige and Skoog half-strength or Beauchesne medium supplemented with various growth regulators depending on the stage of development. Initiation medium contained NOA at 1-5mg/l, NAA at 1.0mg/l, IAA at 1mg/l and the cytokinin 2iP at 0.1-3mg/l. In the multiplication stage, medium was supplemented with NOA at 2 mg/l, NAA 1mg/l, IAA 1 mg/l, BAP at 0.5mg/l, 2iP 1mg/l and kinetin at 1-5 mg/l. In the elongation stage, medium was supplemented with NAA at 1mg/l, BAP at 0.5mg/l, Kinetin at 0.5mg/l and Gibberellin at 1-3mg/l. Finally, the swelling medium was the same as the multiplication medium except that the sucrose level was 100-150g/l. Buds were initiated after six months of *in vitro* culture using previous steps

and media. Rhiss *et al.* (1979) pointed out that buds and roots were obtained from tissues taken up form soft part of offshoots of adult date palm and cultured on MS medium, supplemented with the auxins: IAA, IBA, or NAA at 0.5 to 5mg/l, and with the cytokinins: 2iP, BAP, and Kinetin at 0.1 to 1mg/l.

2.3 Embryogenesis

Somatic embryogenesis is the initiation and development of embryos from somatic tissue of plants rather than from zygotes. This was first reported in cultures of carrot (*Daucus carota*) by Steward *et al.* (1958). Somatic embryogenesis may be initiated from a single cell, or from several cells at the surface or from the interior of tissues in culture. During development, somatic embryos progressed through globular, heart and torpedo stages (Kohlenbach, 1978).

Sharp *et al.* (1980) suggested that somatic embryogenesis may be initiated directly in the absence of any callus proliferation and this occurs through pre-embryogenic determined cells, e.g., nuclear cells of polyemryogenesis that require some callus proliferation and this can occur in differentiated varieties of *Citrus* and epidermal cells of the hypocotyle of wild carrot. In direct embryogenesis that requires some callus proliferation and this can occur in differentiated non-embryogenic cells including

secondary phloem of domestic carrot and inner hypocotyl tissue of wild carrot.

The most important factors involved in the induction of somatic embryogenesis are the exogenous auxin content of the medium and the composition of nitrogen compounds added as nutrients. The induction of embryogenesis requires the presence of auxin or auxin-like substances. After induction, lowering auxin concentration or its absence fosters embryo maturation (Steward *et al.*1967). Subsequent development of embryogenic cells is restricted by the presence of auxin (Halperin and Jensen, 1967). The presence of reduced nitrogen appears to stimulate embryogenesis, and it is important for initiation or maturation steps (Ammirato and Steward, 1971). Thus the basic protocol for embryogenesis requires a primary medium with an auxin source and a secondary medium devoid of growth regulators, both containing a reduced nitrogen source. The role of cytokinin in embryogenesis is not clear because of conflicting results. For example, the addition of 0.1μM of zeatin stimulated embryogenesis in carrot cell suspension in auxin free medium, but the addition of kinetin or BA inhibited embryogenesis (Fujimura and Komamine, 1975). Recently, there have been many reports of somatic embryogenesis in fruit crops. Embryos have been obtained from citrus, strawberry, apple, pear, grape, walnut, *Rubus*, chinese date, papaya, *Prunus*, and date palm.

Regan *et al.* (1968) obtained embryos and plantlets form zygotic *Citrus* embryos excised 100-120 days after pollination, corresponding to an early heart-shaped stage. Embryogenesis was obtained in MS medium supplemented with 0.5mg/l NAA, in the absence of cytokinin, but in the presence of 500mg/l malt extract and 500mg/l casein hydrolysate. Adventive embryos were obtained on the initiation medium without subculture.

Carimi *et al.* (1994) induced somatic embryos from *Citrus* styles treated with different growth regulators. Styles and Stigma were dissected from flowers and cultured on MS basal medium supplemented with 4.52µM 2,4-D and 13.3µM benzyladenine (BA). Callus was induced from the style base 2 weeks after the treatement initiation and embryos appeared 2 months later.

Somatic embryos were obtained from nucellar-endosperm explants of calamansi (*Citrofortunella mitis*) two and a half months after inoculation (with one monthly subculture) on to Schenk and Hildebrandt (SH) medium with 0.05mg/l picloram. Embryos germinated and grown on picloram-free for shoot and root growth. Similar work was done in pummelo (*Citrus maxima*) but using only nucellar tissue. The resulted plantlets were identical to type and virus -free plants (Patena *et al.* 1996).

Very recently, Ling Tian (1997) obtained somatic embryos from immature seeds of eight *Citrus* relatives including *Aegle marmelos*, *Atalantia ceylanica*, *Citropsis gabunensis*, *Clausena excavata*, *Glycosmis pentaphylla*, *Microcitrus anstralasica*, *Murraya paniculata* and *Severinia buxifolia* on MT medium supplemented with 0.05mg/l 2,4-D, 0.05mg BA and 400 mg malt extract/l. Approximately 20% of somatic embryos from six of the genera underwent callogenesis during subculture on the same medium. After at least six successive subcultures on MT medium containing 10 mg/l BAP callus became growth regulator independent. Somatic embryos from growth regulator independent callus were obtained with MT medium containing 5% lactose. Plantlets were regenerated from somatic embryos on MT medium containing 0.02 mg/l NAA and established in soil.

In strawberry, somatic embryogenesis and plant regeneration was obtained by culturing immature zygotic embryos on MS basal medium supplemented with 2,4-D and BA. The percentage embryogenesis was highest in the presence of 5mg/l 2,4-D, 0.5mg/l BA and 500mg/l casein hydrolysate. Morphologically normal plants were obtained from somatic embryos that were transferred to MS medium supplemented with 1mg/l GA3 or 0-5mg BA plus 0.1mg/l NAA.

Kouder *et al.* (1984) reported embryogenesis from immature apple embryos cultured on Nitsh and Nitsh medium supplemented with 4mg/l BA, 400mg/l malt extract/l. and 40ml/l coconut milk. The immature embryos were grown in the dark for 4 days and then moved to the light under 16 hr days with 100 m Mol m^{-2} Sec^{-1} of cool white fluorescent tubes at about 23°C. Also, Liu *et al.* (1983) reported adventitive embryos from leaf explant of apple after 3 weeks incubation on MS medium supplemented with 10mg/l BA and 3mg/l NAA.

Da-KeDong *et al.* (1996) reported the induction of embryogenic cells from *in vitro*-derived leaves of Apple cv. Gala after 7 days in the dark on MS medium supplemented with 1.0 mg BA, 5.0 mg NAA, 0.5mg 2,4-D and 20g sucrose/l. The somatic embryo regeneration rate was 65% when leaves were transferred to MS medium supplemented with 1.0 mg BA and 20g sucrose/l in the dark for 40 days. The regenerated embryos germinated and developed into shoots on the same medium.

Daigny *et al.* (1996) developed a procedure for regeneration of apple (*Malus punica*) plants through secondary somatic embryogenesis in cv. Gloster 69. Primary somatic embryogenesis was produced from cotyledon-derived cultures of immature zygotic embryos. These somatic embryos were multiplied by secondary somatic embryos (SSE) on media with

different plant growth regulator combinations. The highest SSE rate (55.5%) was obtained with a combination of NAA (5.3µM), BAP (0.9µM), and Kinetin (0.9µM) or with thidiazuron (TDZ) alone (10µM). Optimum SSE (> 73%) was obtained by culturing large somatic embryos or cotyledon-like structures on medium containing a combination of NAA/BAP/Kin or TDZ alone, maltose (175mM) and phytagel (2.8g/liter).

In pear, somatic embryogenesis was obtained from nucellar tissue on MS basal medium containing 1g/l casien hydrolysate, 40ml/l coconut water and without added growth regulators (Janick, 1982).

Mullins and Srinivasan (1976) reported embryogenesis *in vitro* using unfertilized ovules. Ovules were cultured first on liquid medium containing 1-125mg/l BA for three weeks, and then subcultured to medium containing 1.0mg/l BA and 0.9mg/l naphthoxyacetic acid (NOA), for two weeks. Finally they were transferred to reduced BA (0.56mg/l) and NOA (0.47 mg/l) for proembryo differentiation. A subsequent study indicated that somatic embryos could be developed on hormone-free media after the above initiation steps (Srinivasan and Mullins, 1980).

Very recently, Kukusove *et al.* (1997) were able to regenerate plants of *Vitis vinifera* cv. Podark Magaracha from leaf explants through somatic embryogenesis. Chromose counts of root tips was used for screening of

regenerated plants. Among 242 studied plants, six (2.5%) tetraploids (2n=4x=76) were identified, all others were diploid (2n=2x=38). Neither chimeral, nor aneuploid plants were observed. Gamma-irradiation (5-100 GY) increased tetraploid plant formation frequency of primary (7%) and embryogenic calli (7.6%) and some aneuploid plants were also found. Colchicine treatment was not effective for the production of tertaploid plants. Variability among regenerated plants was also found after field-testing.

In walnut, immature cotyledons of different cultivars were excised and used to induce somatic embryogenesis. Explants were cultured on a conditioning medium containing 1 mg/l BA, 0.01mg/l IBA, 20 mg/l Kinetin and 250mg/l alpha-glutamine added to the MS basal medium. The development sequence for normal somatic embryos included the globular, heart and cotyledonary many stages, followed by cotyledon expansion and thickening to give a mature somatic embryo. Embryogenic lines were maintained for more than 18 months by culturing somatic embryos on MS media. Adventitive somatic embryos arising from somatic embryos (repetitive somatic embryogenesis) appear to have a similar direct origin, without intervening callus growth (Tulecke and Mogranahan , 1995).

Fiola and Swarts (1986) succeeded in obtaining embryos from *Rubus* zygotic embryos. Ovules and embryos from controlled interspesific crosses were aseptically excised at various stages of maturity. Initiation media contained MS basal salts, staba vitamins, 30g/l sucrose, 200 to 500 mg/l casien hydrolysate, 0.1mg/l 2,4-D and 0.1 or 1.0mg/l BA. Embryogenesis was observed from immature ovules and mature embryos after limited callus development, but shoot regeneration only occurred from mature embryos.

A high frequency of somatic embryogenesis in Pawpaw cultivar Kapoho, Sunset, Sunrise and Waimanala tissue culture was achieved by culturing hypocotyl sections from 10-day-old seedlings on 1/2 strength MS medium containing vitamins, 2.3-112.5μM 2,4-D, 400mg/l glutamine and 6% sucrose (Fitch, 1993). The four hermaphroditic Hawaiian cultivars produced embryogenic callus after 10-14 weeks of culture at 27°C in the dark. The genotypes differed in embryogenic response efficiently in the order: Kapoho > Sunset > Sunrise > Waimanala. The frequency of embryogenesis in the induction medium containing 4.5μM 2,4-D was lowest with 3% sucrose and highest with 7% sucrose. Somatic embryos developed directly from embryogenic calli on induction medium, or more after, differentiated from calli subcultured on medium devoid of growth

regulators. Between 50 to 500 somatic embryos were produced from each 2mm - hypocotyl section after at least 2 months on induction medium, and 2 months on maturation medium. The somatic embryos subsequently developed into normal-looking plants on MS medium. Shoot cuttings from germinated somatic embryos and micropropagated plants were rooted *in vitro* with 5.0 µM IBA, grown in the greenhouse, and transferred to the field.

March *et al.* (1993) pointed out that immature zygotic embryos of *Prunus avium* were collected and sorted into 2 classes (C1: 2.5-3.5 and C2: 3.6-4.5 mm), received different experimental treatments. In C1 class, 2.5% of cotyledons expressed direct somatic embryogenesis when cultured for 10 days on an inductive medium containing 18.1 µM 2,4-D and 9.3 µM kinetin before transferring to fresh medium without growth regulators. C2 class cotyledons were less responsive. The replacement of half of 2,4-D amount presented in the inductive media by the same amount of NAA, reduced the incidence of somatic embryogenesis. Conversely, a rhizogenic response was strongly enhanced. When cultured on an inductive medium containing IAA and zeatin without any subcultures for 3 months, C1 cotyledons were the most morphogenic and developed leaves and cotyledon-like structures.

Recently, Mitrofanova *et al.* (1997) succeeded to establish a procedure for the induction of somatic embryogenesis and plant regeneration from the Chinese date *(Zizyphus jujuba) in vitro*. The highest number of somatic embryos was obtained on 1/2 strength MS medium supplemented with 2.2µM 2,4-D. In darkness, at 26 °C, the frequency of embryogenesis was 90%. During culture, secondary embryoids were formed on the developed non-zygotic embryos. Plant regeneration was conducted on modified Pierik medium without hormones, and plantlets were adapted *in vitro*.

Embryogenic callus and morphogenetic responses of date palm tissues have been induced by many investigators (Tisserat,1979a, 1981, 1982; Dass *et al.*, 1989; Anjarne and Zaid, 1993; Vernamend and Navarro, 1996; Bekheet and Saker 1998). Auxins were found to be the most critical component in the media used for the embryogenic induction callus. Some investigators obtained embryogenic callus on media contained only low concentrations of 2,4-D did not exceeded 2mg/l (Drira *et al.*, 1983; Zaid M., 1989). Other investigators found that concentration and type of hormone, especially auxins were the most critical factor in stimulating embryogenic callus production and subsequently embryogenesis process (El-Bellaj *et al.*, 1998).

Bekheet and Saker (1998) pointed out that date palm embryogenic callus produced from shoot tip explants cultured on MS medium supplemented with 10 mg/l 2,4-D, differentiated plantlets after 3 subcultures on medium containing 3 mg/l 2iP and 1mg/l NAA, with one month intervals between subcultures. Also Sharon and Shankar (1998) indicated that callus was induced from zygotic embryos of date palm on MS medium supplemented with 10mg/l 2,4-D, 3mg/l Kin, 0.5mg/l 2iP, 3% sucrose and 0.5% activated charcoal in dark at 28°C, which when transferred to light became nodular. Further transfer to MS devoid of 2,4-D, but supplemented with glutamine, it developed into somatic embryos. They also pointed out that during somatic embryos to plantlet formation, four stages developed each required different light intensity and growth regulators supplement.

Somatic embryogenesis was obtained from callus especially nodular callus when sections of shoot apical meristems were cultured on MS medium supplemented with 2mg/l 2,4-D, and 3mg/l 2iP for callus initiation and proliferation. Embryogenesis callus produced somatic embryos on phytohormone free medium. Somatic embryos were converted to plants (Chabane and Bougedoura, 1998).

Several reports indicated that callus of date palm embryo explants was produced with 0.01mg/l NAA and kinetin. Also, it was recorded that 100 mg/l 2,4-D or NAA and 3 mg/l 2iP gave the best embryogenic callus when lateral buds and apical meristems were cultured on MS medium (Gabr and Tisserat, 1985). Shoot tip and leaf-bud fragments were removed from offshoots of date palms and cultured on MS inorganic salts 453-µM 2,4-D, 14.8µM 2iP and 3g/l activated charcoal to develop nodular callus after 8 months of culture. Callus was cultured in agar - solidified and stationary or shaken liquid media containing half-strength MS inorganic salts, 3g/l activated charcoal and different sucrose concentrations. The best condition for embryo development were culturing in liquid medium shaken at 100 rpm for a period of 2 weeks without sucrose, followed by a culture on a 3% sucrose (Vernamendi and Navarro, 1996).

Dass *et al.*, (1989) reported that embryogenic cultures were induced from shoot tips of date palm (cv. Muscat) on nutrient medium containing MS basal salts along with NAA, 2,4-D, BAP and PVP under dark condition at 28°C cellular embryos further differentiated into well-developed nodular-shaped embryos on medium supplemented with NaH_2PO_4 and Kinetin and on increasing KH_2PO_4 to 200mg/l concentration. Somatic embryos

germinated into complete plants by first initiating root and then shoot at 0.1mg/l NAA.

Calli were induced from shoot tip, bud, leaf, stem and root explants from bearing trees, offshoots, seedlings, and asexual plantlets of date palm by culturing on modified MS nutrient medium containing 3g/l activated charcoal, 100mg/l 2,4-D, 3mg/l 2iP. Differential morphogenetic responses were obtained from calli dependent on the explant type and parent source. Subcultured shoot tips and leafy lateral buds callus on nutrient media devoid of charcoal and supplemented with 0.1mg/l NAA produced adventitious plantlets. Subcultured leaf calli produced roots only. Root callus failed to exhibit any morphogenic response upon subculturing.

Undifferentiated non-leafy buds and stem tissues did not give rise to callus regardless of the parent source. Generally, the best callus and embryogenic responses from explants were obtained from seedling and plantlet parent sources. Similarly, organogenetic responses such as root formation and development from shoot tips cultured on media containing 10mg/l NAA were also related to the parent explant source (Zaid and Tisserat, 1983).

Growth of explants from excised embryos cultured *in vitro* on RM-1965 medium was stimulated by 1mg/l IAA and 0.5mg/l Kinetin. However,

rooted seedlings were obtained only when 10mg/l NAA was added to the medium. In most cases differentiation took place directly from the thin layers of callus produced on the cut surface of the explants. However, in the absence of NAA there was no differentiation except when the explants were recultured (Khalil *et al.*1982).

Mater (1986) cultured shoot tip explants excised from date offshoots of *Phoenix dactylifera*, cv. Barhee and Hallawi on nutrient media supplemented with 2 mg/l of BA and Kinetin, and the auxins NAA or 2,4-D at five levels (0.0, 0.1, 1.0, 10.0, 100.0mg/l). Vigorous callus growth occurred in explants cultured on media containing high levels of auxin (10 and 100mg/l). The callus had yellowish aggregated type, and could be subcultured on high auxin containing media to produce callus only, or to low auxin (0.0 and 0.1mg/l) to produce organs only. When subcultured on a medium devoid of auxin, the aggregates gave rise to leaves only, while it produced roots only when subcultured on NAA containing medium. No embryoid differentiation was observed in this type of callus. Following repeated subcultures on media containing high levels of auxins, white colonies of embryogenic nodular callus, which proved later to be precursors of asexual embryos and originating from small single meristematic cells in the callus tissue were observed.

2.4 Factors affecting shoot organogenesis or regeneration.

Several factors other than growth regulator have been reported to affect regeneration *in vitro*. These factors include plant genotype, explant source, addition of charcoal to the medium, the use of antibiotics and light and temperature. Each of these factors and their relationship to regeneration will be discussed in detail in this section.

The effect of genotype regeneration has been studied extensively in many species including *Vicia* (Phillips, 1983), *Coronilla* (Mariotti and Arcioni, 1983) and strawberry (Nehra *et al.*, 1989). In strawberry, ten different genotype were tested for regeneration from leaf disks. Regeneration frequency varied considerably from 4.4 to 91%. The differences in genotype response was unclear, however, there is evidence suggesting that a few genes may have had an effect on the regeneration capacity of plant tissues. Reisch and Birgham (1980) suggested that at least two dominant genes control differentiation in *alfa alfa* callus.

Explant tissue is another important factor affecting regeneration. In some species, only certain parts of the plant can respond in culture. In these plants, regions of actively diving cells seem to respond most readily, e.g. immature embryos have been extremely useful (Wang *et al* 1984). However, in some other plants such as *Daucus carota*, embryogenesis can

be induced almost for any cell or part of the plant including excised embryos, hypocotyls, young roots, petioles, peduncles and protoplasts (Ammirato, 1983). In several cases, younger tissue have been found to produce more uniform regeneration from dedifferentiated cells (DeJong and Custers, 1986; Hanisch *et al.*, 1986).

Culture medium effects on regeneration have been demonstrated by several studies. Evans *et al.* (1981) surveyed somatic embryogenesis in crop plants and found that 70% of explants were cultured on MS medium or a modification of MS. This medium satisfies the nitrogen requirement in case of carrot somatic embryos because of the presence of high concentrations of inorganic nitrate (Reinert *et al.*, 1967). In contrast, White's medium (White, 1963), which is low in nitrate nitrogen and without reduced nitrogen, needs to be supplemented to support continued somatic embryogeny.

The addition of charcoal to the medium has been very useful. Callus of *Hedera helix* from mature stems gave rise to embryos only on medium containing activated charcoal. When charcoal was omitted, only undifferentiated callus growth occurred (Banks, 1979). Activated charcoal was also useful in anther culture (Fraser and Harvey, 1986). This was due to the fact that charcoal absorbed inhibitors that would prevent growth and

reduced the level of growth promoters that would cause continued proliferation (Fridborg *et al.* 1978).

The use of antibiotics in culture media has become more widespread with the emergence of antibiotic resistance genes as selectable markers in transformation experiments and in transformation systems. In addition to cultivation of *Agrobacterium tumefaciens* requires the use of an antibiotic to kill the bacteria. The antibiotic Kanamycin has been found to be inhibitory to cell or tissue growths at comparatively low concentrations. Fiola *et al.* (1990) indicated that the addition of 110mg/l or higher kanamycin sulfate to *Rubis* cotyledon regeneration medium drastically reduced the growth and organogenesis of explants. The use of rifampicin at 50mg/l in tissue culture medium effectively controlled bacterial contamination without affecting the growth of explant cultures of *Helianthus tuberosus* (Phillips *et al.*1981). Mathias and Mukasa (1987) investigated the effect of cefotaxine on callus initiated from immature embryos of four barley cultivars. Callus growth was up to 45% greater on cefotaxine than on control medium and the frequency of regenerating calli was increased by up to 80%.

Several studies demonstrated the effect the light on regeneration. *Heloniolisis orientals* callus cultured in the dark produced shoots when transferred to light (Kato, 1978). Also, continue darkness reduced the

number of shoots produced in cultures of *Asparagus* (Hasegawa *et al.*, 1973).

Information about the effect of temperature on organogenesis is limited. In *Citrus* nucellus-derived cultures, embryogenic potential was reduced as the temperature was lowered from 27°C to 12°C (Esan, 1973). In contrast, cold treatment of *Actinidia* another increased somatic embryogenesis (Fraser and Harvey, 1986).

In summary, the ability of explant tissues to regenerate Plantlets is primarily related to the growth regulator composition of the culture medium, although the other factors, which have been discussed in this section, should be considered in development of protocols for plant regeneration. Care should be given to be the choice of explant material.

CHAPTER III

MATERIALS AND METHODS

CHAPTER 3.

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 establishment of efficient regeneration systems from excised date palm tissues and to assess the effect of seasonal variations on regeneration capacity.

3.1 Plant Materials

The conducted experiments included the use of "Khnazi" date palm (*Phoenix dactylifera* L.) offshoots, a well-known cultivar throughout the UAE. The offshoots were collected from good reputation farms in Al-Ain City and transferred to the laboratory at Al-Oha region. The offshoots were 3-4 years old, collected from healthy, disease-free mother palms (Fig.1), and weighted approximately 7-10 kg per each bulb offshoot. The offshoots were cleaned by running water and the outer large leaves and fibers were removed carefully and gradually by sharp knife until the appearance of

shoot tip zone. Special care was taken not to injure the meristematic region. Shoot tips were then isolated carefully using a sharp knife and pruned to approximately 5-7 cm in length and 3-5 cm in width (Fig.2).

3.2 Shoot tip sterilization

The excised shoot tips were washed by distilled water then subjected to sterilization procedure to make sure that the tissue is free from any contamination. The procedure should be done carefully and accurately because contamination problem is considered one of the obstacles, which are standing against the successful culture for plant tissue culture *in vitro*, especially in date palm tissue culture. The excised shoot tips were subjected to two consecutive sterilization steps to minimize contamination (Fig.3). Firstly, the isolated shoot tips were sterilized by soaking them in a fungicide solution, Benlate at a concentration of 5 g/l or Benomelo at a concentration of 2 mg/l, for 20 minutes. Secondly, the shoot tips were resterilized by dipping in 33% commercial Clorox solution (5% sodium hypochlrite) for 20-25 minutes. The explants were then rinsed three times with sterilized distilled water, each for 5 minutes under the laminar airflow hood, to remove any residual disinfectant before culturing.

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

Fig.2. Shoot-tip isolation.

Fig.3. Shoot-tip sterilization.

Fig.4 Sterilized explants soaked in an anti-oxidant solution.

3.3 Treating explants with an antioxidant solution

The sterilized explants were then soaked in an antioxidant solution (Fig.4) to minimize production of phenols (causing the browning), and to protect them from drought. The antioxidant solution consisted of 2 g/l polyvinyl pyrolydon (PVP, Mw = 40,000), 100mg/l sodium diethyldithiocarbonate AR (Mw=225.30), and 200 mg/l caffeine anhydrous (Mw=194.2). The shoot tips were kept in this solution until culture time.

3.4 Culture procedure of shoot tips

Isolated shoot tips were taken from the antioxidant solution and placed in a sterilized petri dish containing some of antioxidant solution to avoid browning and drought of explants during culturing. The primary xylem and bases of leaves were then cut off from the shoot tips using sterilized surgical blades (Fig.5, 6). The rest of each explant was cut in half at right angles around the apical dome. The apical domes were 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 20ml initiation medium in 24x200 mm culture tubes, capped with aluminum foil and Nalagin plastic cover.

Fig. 5. Primary xylem and leaf base cutting from the shoot-tip

Fig.6. Dividing apical dome to several smaller explants.

3.5 Initiation stage

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

The initiation medium was supplemented with different hormonal combinations as presented in Experiments 1 and 2. The initiation medium included activated charcoal for 2 months, then the explants were subcultured on the same media, but without charcoal until the end of the experiments. During the 4 months initiation stage, the cultures were kept in complete darkness, at a temperature of 28°C ± 1 inside special growth room.

3.6 Multiplication stage

After 4 months on 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 30g/l

sucrose instead of 40g/l as in the initiation medium (Fig.8). The growth regulators added to the multiplication medium were indol acetic acid (IAA) at 0.4mg/l, naphthaline acetic acid (NAA) at 0.1mg/l, kinetin (KIN) at 0.1mg/l, and isopentyl purin (2iP) at 1.5mg/l. All growth hormones 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 subjected to subculture every one month.

3.7 Elongation stage

Multibuds formed on explants in the multiplication medium were isolated and separated individually, 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 30g/l sucrose. The cultures were kept for one month under a 16/8-hr photoperiod regime, at 30µmol m^{-2} sec^{-1}. Cultures were maintained under this condition for one month before transferring them to the rooting stage (Fig.9).

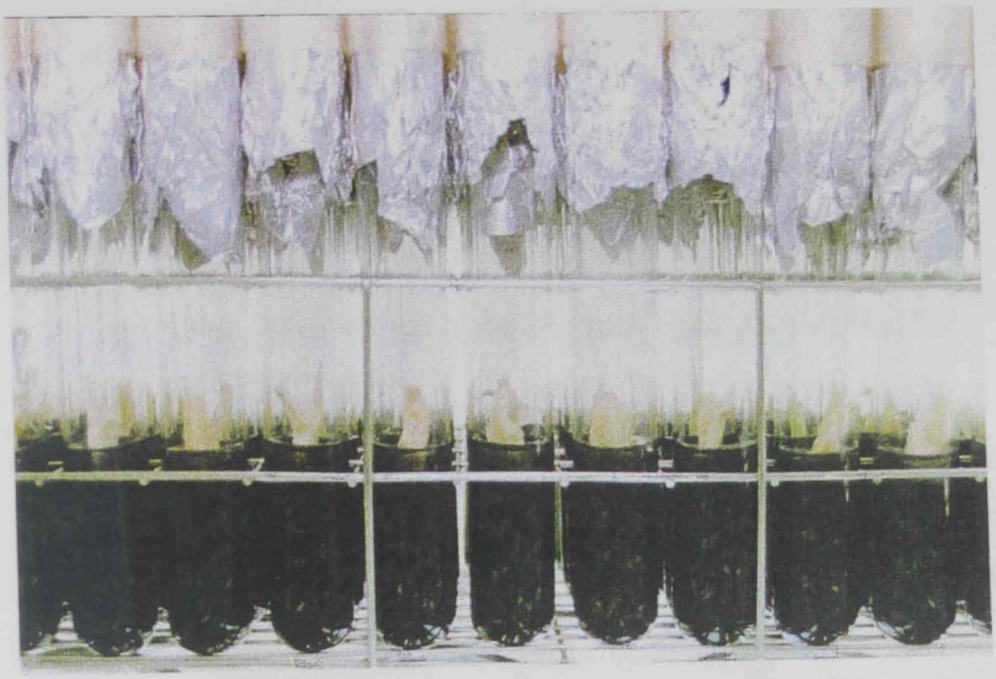


Fig.7. The initiation stage.

Fig.8 The multiplication stage.

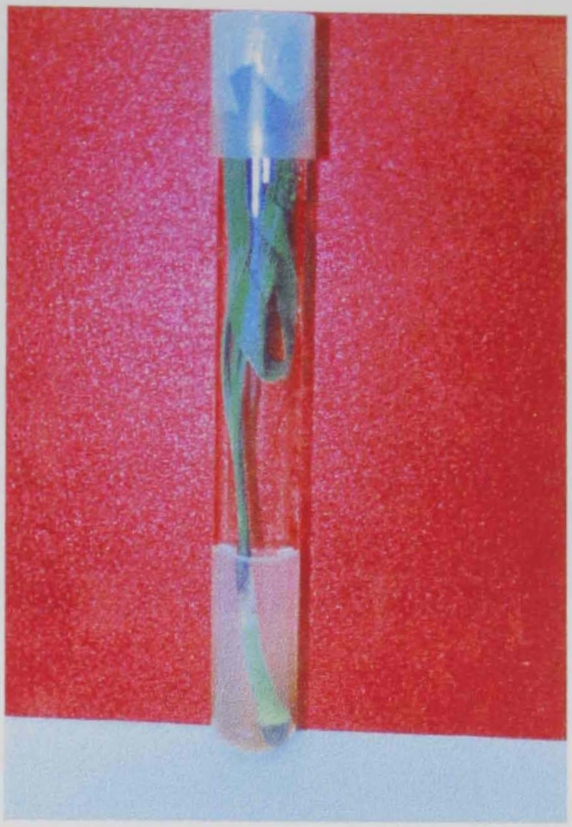


Fig.9. The elongation stage.

Fig.10. The rooting stage.

(a)

(b)

Fig.11.a,b. Transfer of the seedling to the Green House.

3.8 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 maintained in multiplication and elongation stages, where they became ready to transfer to greenhouse (Fig.10, 11a,b).

3.9 Experimental Procedures

3.9.1 Experiment 1:

Effect of different auxin and concentration on percentage explants produced bud generative tissues and number of differentiated buds per explant.

Effect of 18 different media developed from various combinations of auxin types and concentrations, in addition to control (free hormones medium) in the initiation stage on bud regeneration from shoot-tips, was investigated. In this experiment, the initiation medium was supplemented with two different auxins namely indole 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 6.4mg/l. In addition, five media were developed from an equal

combination of IAA + NAA at 0.1mg/l each, 0.4mg/l each, 0.8mg/l each, 1.6mg/l each, and 3.2mg/l each. NAA was added to the medium before autoclaving, whereas IAA was supplemented after autoclaving and when the medium temperature was about 55°C through a 22µm Millipore sterilized filter. In addition to the tested auxins, naphthoxy acetic acid, BA, Kin and 2iP were added to all media at a fixed rate of 4mg/l, 0.4mg/l, 0.4mg/l, and 0.4mg/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 least significant difference (LSD) test (Gomez and Gomez, 1984). Contaminated cultures were not included in the analysis.

3.9.2 Experiment 2:

Effect of different cytokinin and concentration on percentage explants produced bud generative tissue and number of differentiated buds per explant.

Effect of 23 different media developed from various combinations of cytokinin types and concentrations in the initiation stage on bud regeneration from shoot-tip, was investigated. In this experiment, 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 6.4mg/l. In addition, five media were developed from an equal combination of Kin, BAP and 2iP at 0.1mg/l each, 0.4mg/l each, 0.8mg/l each, 1.6mg/l each, and 3.2mg/l each. All auxins, IAA, NAA, and NOA were added to the media at a fixed rate of 0.4mg/l. The statistical design and analysis followed the same procedure explained in experiment 1.

3.9.3 Experiment 3:

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

Effect of culturing time on shoot bud generative tissue and shoot bud regeneration from shoot tips of Khnazi date palm cultivar was investigated. In this experiment, the two best hormonal combinations were selected based on the results of experiments 1 and 2.These combinations were as follows:

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

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

Culture media in this experiment were the same as in the initiation medium in both experiment 1 and 2, 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 beginning from September 5, 1996 and continued for 12 months, with one-month intervals. The last culture was conducted in August 5, 1997. The cultures were maintained under the same physical conditions as in experiment 1 and 2. The experiment had 32 replications (test 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, with means separated by the least significant difference (LSD) test. Contaminated cultures were not included in the analysis.

3.10 Collected Data

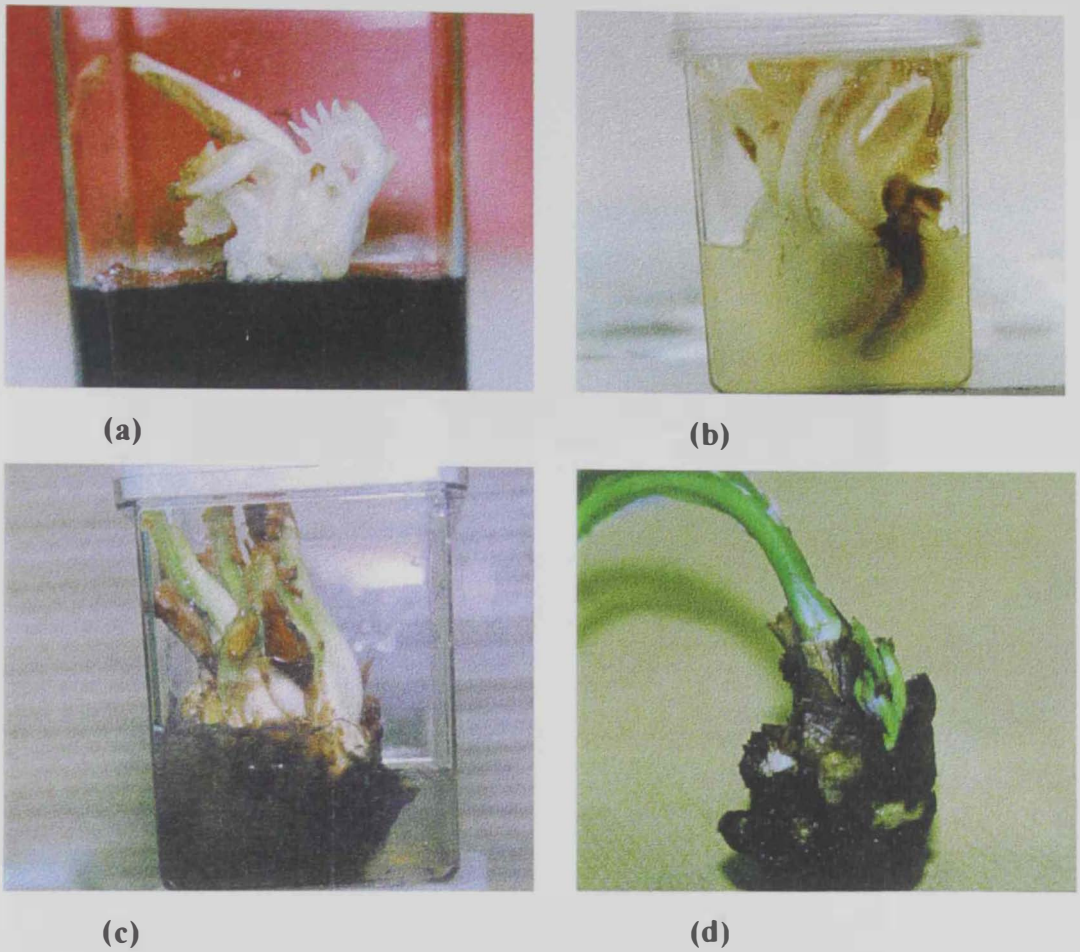
The following data were recorded in the three experiments after 4 months in initiation culture:

(1) Percentage of explants formed apical buds. (Fig.12.a,b,c,d).

(2) Percentage of explants formed roots (Fig.13.a,b,c).

(3) Percentage of explants formed bud generative tissues after 4, 5, 6, and 7 months on initiation medium (Fig.14.a,b,c).

Also Number of differentiated buds per explant were recorded after 5, 6, and 7 months from culture initiation. (Fig.15.a,b).

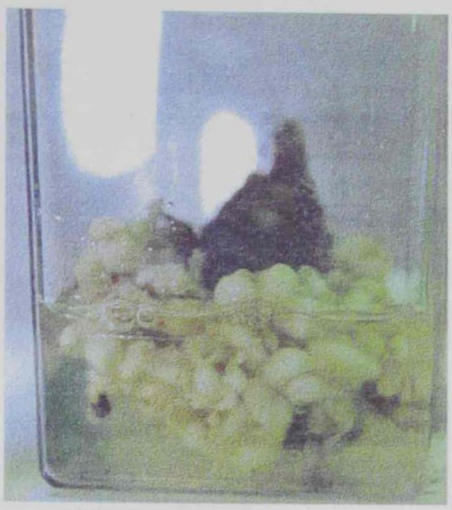


(a) (b)

(c) (d)

Fig.12.a,b,c Apical bud formation at various stages of development.

(a)



(b)

(c)

Fig.13.a,b,c. Root formation on the explants at various stages of development.

(b)

(b)

(c)

Fig.14.a,b,c Differentiation of bud generative tissue.

(a)

(b)

Fig.15.a,b. Regeneration of shoots from differentiated buds.

CHAPTER IV

RESULTS AND DISCUSSIONS

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Experiment 1

Effect of different auxin and concentration on percentage explants produced bud generative tissue and number of differentiated buds per explant.

4.1.1: Part 1. Effect of different auxin and its concentration on percent explants forming apical buds and roots from cultured shoot tips of Khnazi date palm cultivar.

The illustrated results indicated that the presence of auxin in culture medium was not an essential requirement for the formation of apical buds from the shoot tips, however, it was essential for the rooting of explants (Fig.16). Increasing the level of either tested auxin (IAA) or (NAA) to 1.6mg/l resulted in an increase in the number of explants formed apical buds (Fgi16.a). However, increasing IAA concentration to 3.2 or 6.4mg/l resulted in a reduction on percentage of explants that formed apical buds. Similarly, the auxin NAA behaved in a similar

manner like the auxin IAA and the best percentage of apical bud formation resulted when NAA was used at 1.6 mg/l.

Combining IAA and NAA together at an equal concentration of 0.1mg/l resulted in a significant increase in the percentage of explants that formed apical buds over the control treatment, 0.4mg/l of IAA or NAA and the combination of IAA and NAA, each at 3.2mg/l (Fig.16.b). It was also more effective than all other tested auxin treatments, but not to the level of significance.

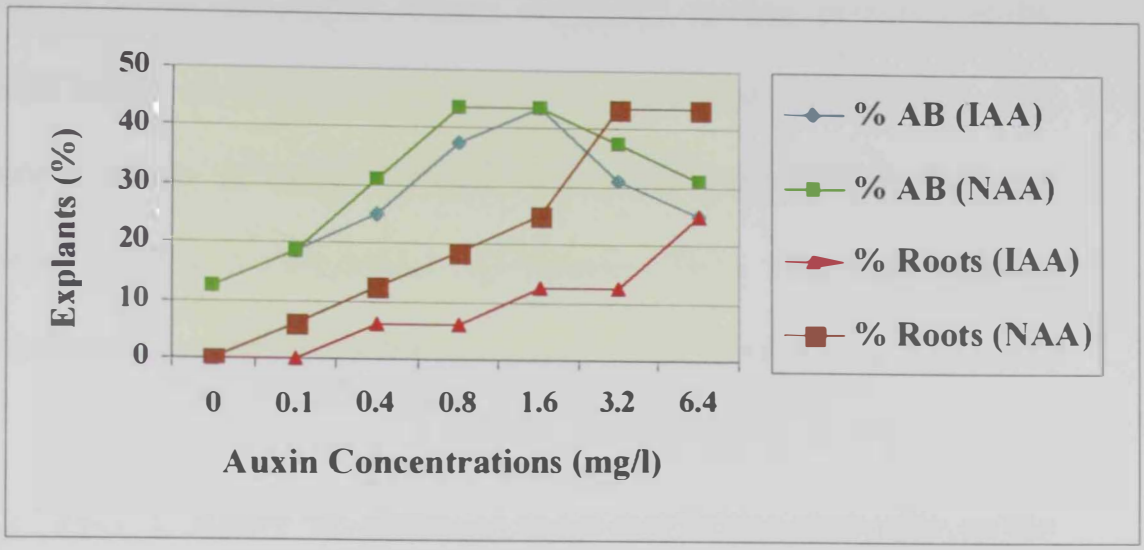
The obtained results indicated that the presence of enough endogenous auxin in the cultured explant tissues was enough to enhance the regeneration of apical buds, but it was not enough to induce the maximum apical bud regeneration capacity. Maximum numbers of apical buds were obtained when IAA or NAA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

that apical buds grew and were able to proliferate shoots at 0.01mg/l NAA, but maximum number of shoots proliferated from apical buds was achieved when NAA was used at 1mg/l.

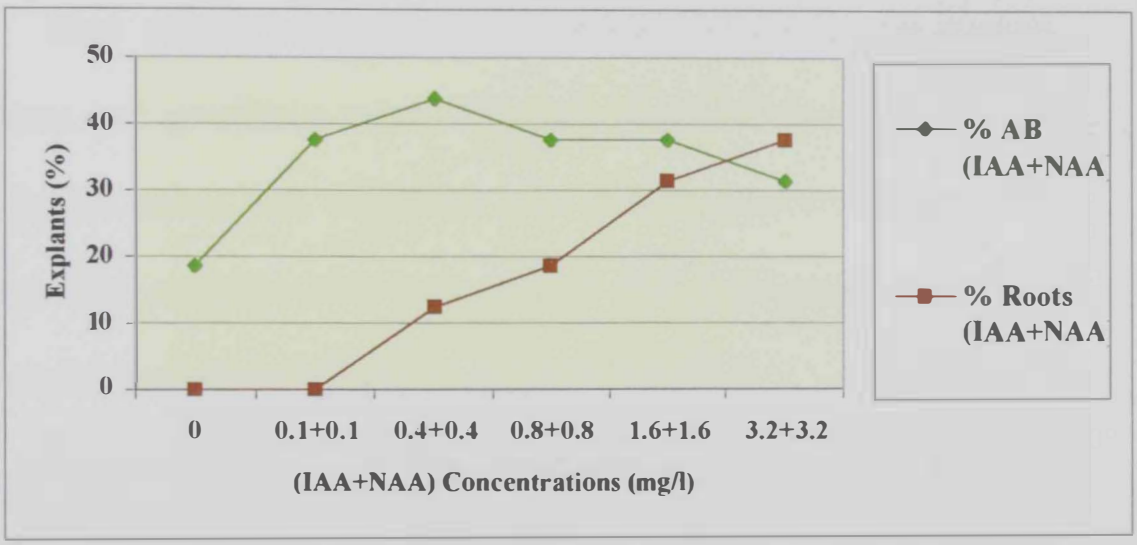
Concerning the effect of different concentrations of auxin on percentage explant formed roots, data are illustrated in (Fig.16.a,b). Data showed that increasing the level of auxin, regardless of the different of auxin, was associated with the increase in percentage explants formed roots.

Culturing explants on medium containing NAA at 3.2 or 6.4mg/l resulted in producing 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 (6.4mg/l) or the highest concentration of auxins in the combination (IAA at 3.2mg/l NAA at 3.2mg/l). Data in (Fig.16.a) also revealed that 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, where the medium contained only cytokinins. 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



(a)



(b)

Fig.16.a,b. Effect of different auxin and concentration on the percentage of explants that formed apical buds and roots from cultured shoot tip of Khnazi date palm (*Phoenix dactylifera* L.) cultivar.AB (Apical Bud).

formation (Friedman *et al.*, 1985). The obtained results were correlated to the previous information where maximum rooting occurred at the highest tested auxin level, 6.4mg/l IAA or 3.2 and 6.4mg/l NAA. The achieved results are supported by the research of Omar (1988), Zaid and Tisserat (1983) and Vernamendi and Navarro (1996). They indicated that relatively high auxin concentration is required to obtain enough roots.

4.1.2. Part 2. Effect of different auxin and its concentration on the percentage of explants that formed bud generative tissue after 4, 5, 6 and 7 months of incubation.

Data pointed out that auxin was essential to stimulate the explants to form bud generative tissue. While the absence of auxin in any tested incubation period was associated with no formation of bud generative tissue. The excessive IAA concentration (6.4mg/l) also did not induce the explant to form bud generative tissues (Fig.17.a).

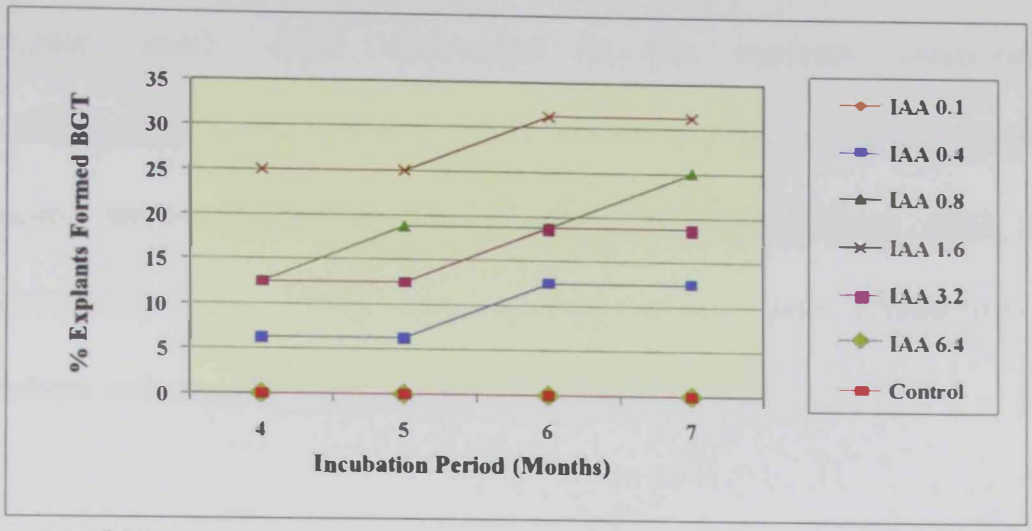
Data also indicated that there was a gradual increase in the percentage of explants that produced bud generative tissues with the increase of IAA concentration from 0.4mg/l to 0.8mg/l and then to 1.6 mg/l. Increasing the level of auxin in the medium to 3.2mg/l or higher (6.4mg/l) resulted in a reduction in the formation of bud generative tissues. However, the concentration of 3.2mg/l proved to be better than 0.4mg/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 we compared number of generative tissue after 4, and 5, months 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 0.1mg/l to 1.6mg/l resulted also in a gradual increase in the percentage of explants that formed bud generative tissue (Fig.17.b). The NAA concentration above 1.6mg/l caused a reduction in the tested parameter and no bud generative tissues were formed when the level of NAA was increased to 6.2mg/l. The rate of increase in percentage bud generative tissues was associated with the increase in IAA level from 0.4 to 1.6mg/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.

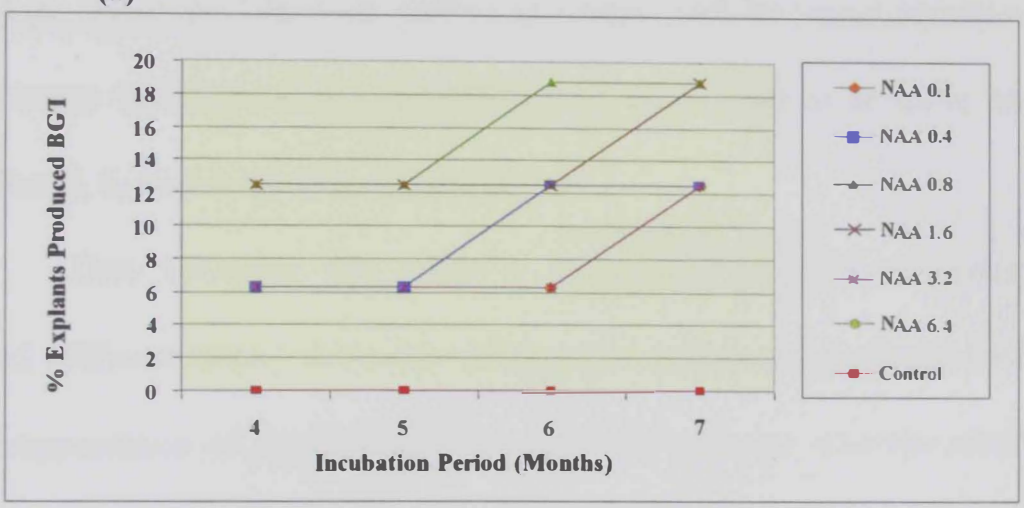
The combination of both NAA and IAA behaved in the same manner as any of the auxin alone (Fig.17.c). The combination (0.4mg/l IAA and 0.4mg/l NAA) was the most effective in stimulating the production of bud generative tissues. There was a reduction in percentage of explants formed bud generative tissues, happened when

the concentration of both auxin in the combination reached 1.6mg/l. Further more data indicated that the most significant and promotive treatments for increasing percentage bud generative tissues were IAA alone at a concentration of 1.6mg/l and the combination of (IAA at 0.4mg/l + NAA at 0.4mg/l). This was true all over the different tested incubation periods.

The process of regeneration of bud generative tissue includes two steps, the first is cell dedifferentiation where cells lose their identity to specific tissues, and the second is the redifferentiation of these cells into meristemoids or centers of meristematic cells (George. 1993). The differentiation of meristemoid into shoots or roots is controlled by the balance of growth regulators added to regeneration medium. High concentration of cytokinin and low level of auxin containing medium would result in the regeneration of shoots, while high auxin and low cytokinin medium would enhance root formation (George 1993). The obtained results agreed with the previous mentioned conclusion, where using auxin at level of 0.8mg/l, regardless of auxin type or 0.4 mg/l of both auxins in combination resulted in the highest and significant percentage of explants that formed bud generative tissue. Increasing the used auxin level to a higher concentration reduced significantly the percentage bud generative tissue formation. This is due to balance of cytokinins to auxins ratio will be to the side of auxin. Increasing the time



(a)



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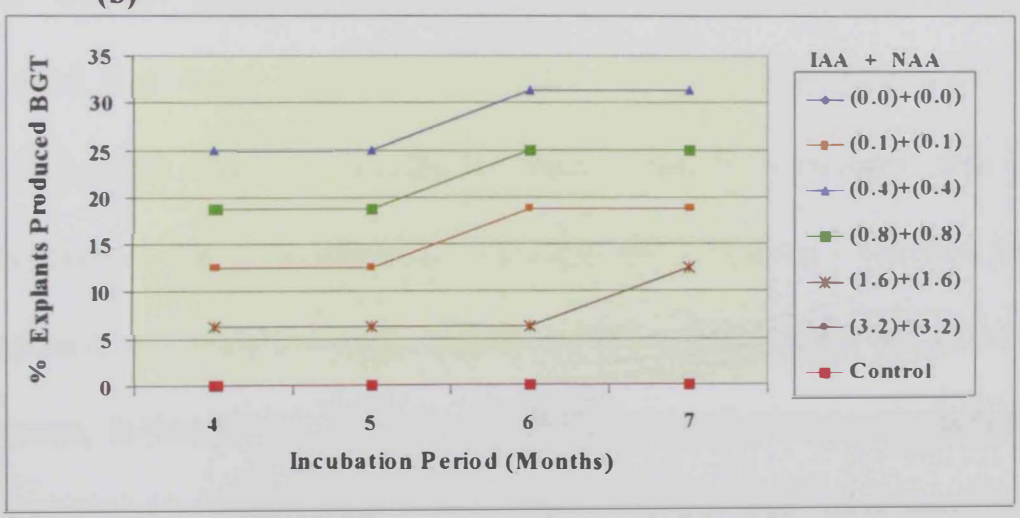


(c)

Fig.17.a,b,c. Effect of different auxin and concentration on percentage explants produced bud generative tissues after 4, 5, 6 and 7 months of incubation resulted from cultured shoot tip of Khnazi date palm (*Phoenix dactylifera* L.) cultivar. BGT (Bud generative tissue).

was associated with the increase in percentage tissue generated buds because more cells responded to the medium components dedifferentiated, then redifferentiated into bud generative tissue. Similar results were reported by (Mater 1986, Zaid and Tisserat 1983, and Beauchesne *et al.* 1986), they reported that low auxin concentration in medium enhanced the formation of bud generative tissue.

4.1.3. Part 3. Effect of different auxin and its concentration on average number of regenerated buds from bud generative tissue after 5, 6, and 7 months of incubation.

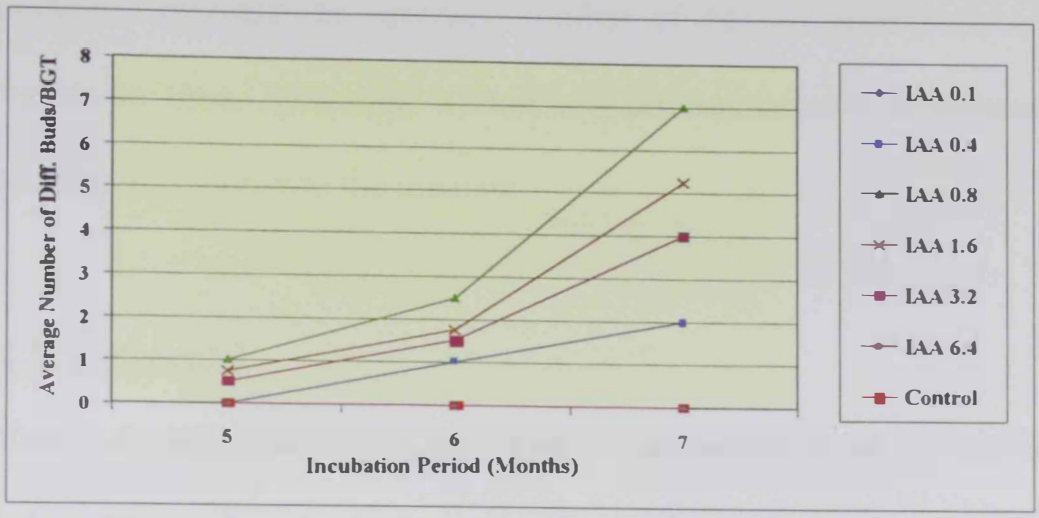
Data indicated that auxin is a necessary medium component for bud differentiation, since the absence of auxin was associated with the disappearance of buds from bud generative tissues. Also the results of the number of differentiated buds after 5 months incubation period proved that even the low concentration of exogenous IAA, i.e., 0.1mg/l and 0.4mg/l was not enough to induce bud differentiation (Fig.18.a). Increasing the concentration of IAA up to 0.8mg/l resulted in the regeneration of significantly highest number of buds from bud generative tissues, followed by 1.6mg/l of IAA. However, increasing the level of IAA to a concentration higher than 0.8mg/l was associated with a significant reduction in the number of buds that formed from merestemoid. In addition, if the concentration of used IAA reached

6.4mg/l, no bud differentiation occurrs at all. Similarly, NAA at a low concentration (Fig.18.b), (0.1 and 0.4mg/l) did not stimulate bud regeneration similar to IAA. The addition of NAA at a concentration of 0.8mg/l resulted in a significant increase in number of bud regenerated from tissues, and any increase in NAA level above 0.8mg/l was accompanied by a reduction in number of regenerated buds, and a complete absence of buds at a concentration of 3.2mg/l or 6.4mg/l. The most significant combinations of auxins were those containing IAA and NAA at concentration of 0.4mg/l or 0.8mg/l for each (Fig.18,c). Any other tested combinations of IAA and NAA, resulted in a significant reduction in number of differentiated buds. After 6 and 7 months of incubation, it was quite clear that IAA at 0.8mg/l was the most effective and significant treatment in increasing number of differentiated buds from bud generative tissues. The low concentration of IAA, i.e. 0.1 and 0.4mg/l showed positive increase of bud regenerated from tissue after 6 or 7 months of incubation but they were at the least in terms of significance. Increasing the level of IAA to above 1.6mg/l caused a significant reduction in number of differentiated buds after 6 and 7 months of incubation. Similarly behaved the auxin NAA, where data in (Fig.18.b) showed that the best and most effective concentration of NAA was 0.8mg/l. After 6 months of incubation, 0.4mg/l NAA was equal in its effect to 0.8mg/l, but was less effective compared to 0.8mg/l after 7

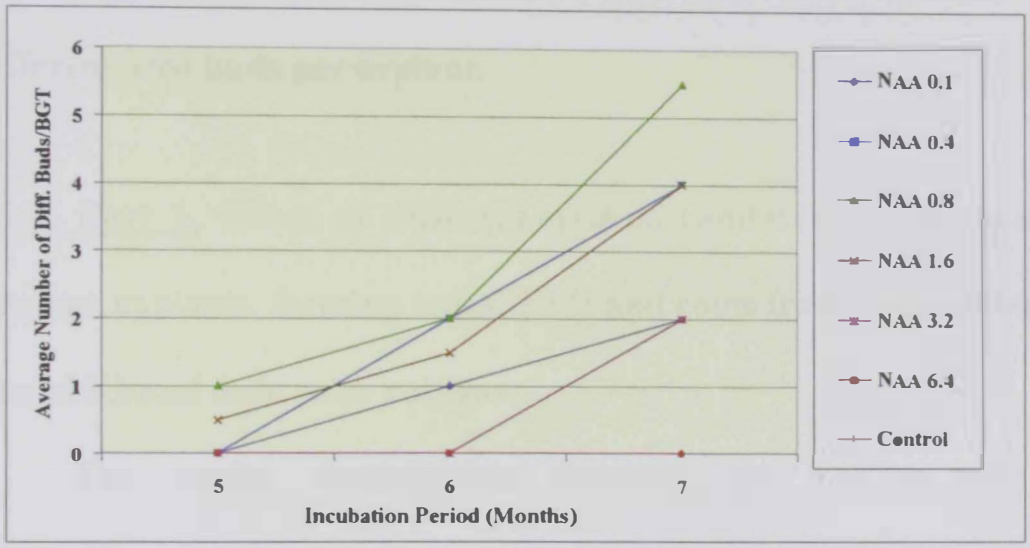
months of incubation. Increasing the level of NAA to a concentration higher than 0.8mg/l significantly reduced number of regenerated buds per bud generative tissue. IAA at 0.4mg/l + 0.4mg/l NAA were the most effective and significant combination that improved number of differentiated buds from bud generative tissue 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.18), was that the number of differentiated buds from bud generated tissue increased with the increase of incubation period.

The demonstrated data about the number of differentiated buds behaved similarly to percentage formed bud generative tissue, where both required low auxins and high cytokinins. The increase in auxin concentration to above 0.8mg/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 generally should be higher in balance, where high concentration of auxin will promote either undifferentiated callus or root formation.

The results were correlated to those obtained by (Omar, 1988; Gabr and Tisserat, 1985; and Zaid and Tisserat, 1983). Also there was a



(a)



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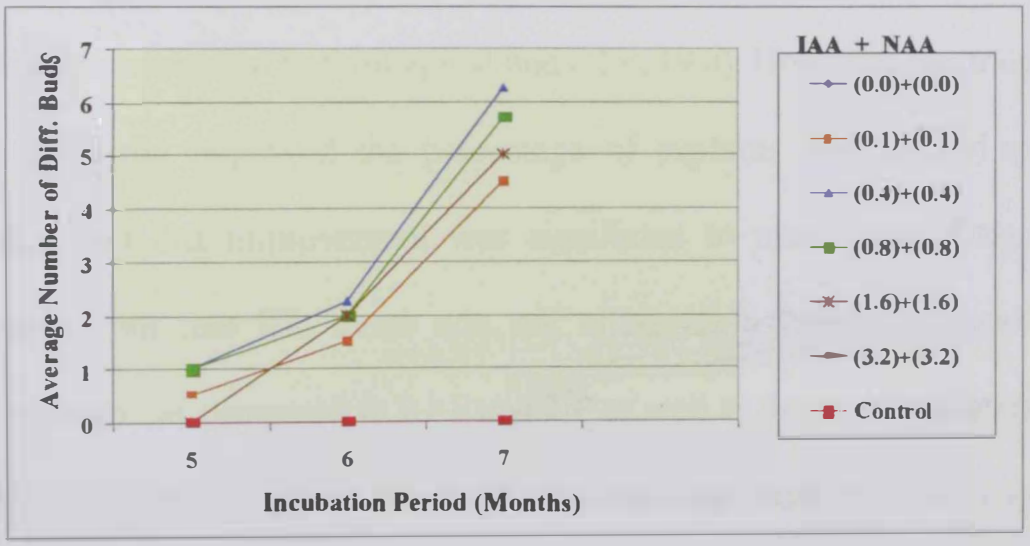


(c)

Fig.18.a,b,c. Effect of different auxin and concentration on average number of differentiated buds per bud generative tissue resulted from cultured shoot tip of Khnazi date palm (*Phoenix dactylifera* L.) cultivar.

significant increase in average number of differentiated buds with progress in time, indicating a successful differentiation of meristemoid into buds in response to the medium.

4.2 Experiment 2

Effect of different cytokinin and concentration on percentage explants produced bud generative tissue and number of differentiated buds per explant.

4.2.1. Part 1. Effect of different cytokinin and its concentration on percent explants forming apical buds and roots from cultured shoot tips of Khnazi date palm cultivar.

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 (Fig.19.a). However, the addition of cytokinin improved the percentage of explants that formed apical buds, and this improvement was significant in most cases. Data also pointed out that Kin alone was not effective in increasing apical bud formation as compared to BAP and 2iP as well as the combination of all three cytokinins, where the highest percentage explants formed apical buds in the case of Kin treatments was (37.5%), which was achieved at a level of 0.4mg/l, compared to 50% apical bud formation that resulted

from BAP at 1.6mg/l., 2iP at 3.2mg/l, and the combination of (Kin + BAP + 2iP) each at 1.6mg/l (Fig.19.b) It was also quite clear from the data that the low concentration of cytokinin (0.1mg/l), as well as the high concentration (6.4mg/l) reduced the percentage of explant formed apical buds, even in the case of combination of the three cytokinins. The most effective concentration varied depending on the type of cytokinin, it was 0.4mg/l in the case of Kin, 1.6mg/l in the case of BAP, 3.2mg/l with regard to 2iP and the combination of Kin + BAP + 2iP at 1.6mg/l each.

The results which indicated the ability of control explants to form apical buds may be attributed to the presence of enough endogenous level of 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), they stated that in the tissues of *Oxalis dispar*, cell division proceeds with the addition of cytokinin to culture medium. Similarly, Skoog *et al.* (1973) succeeded to isolate three natural cytokinins from a cytokinin-independent strain of tobacco callus.

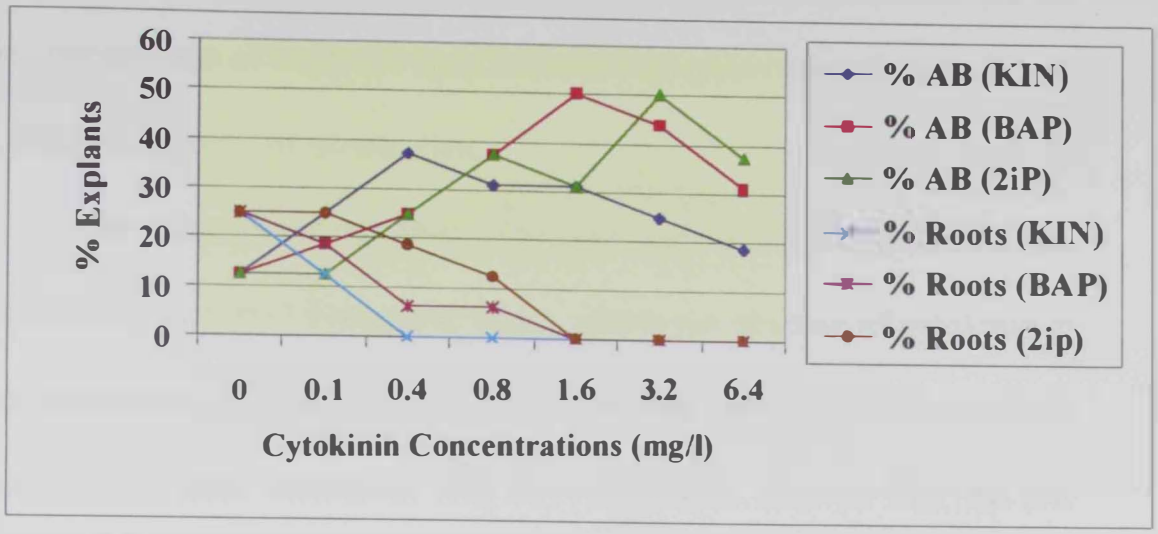
Since different cytokinin types differ in their effectiveness, different concentrations of tested cytokinins, i.e. Kin, BAP, 2iP and their combination resulted in maximum apical bud formation. However, the results showed that using the highest concentration of cytokinin led to reduction in percentage apical bud formation. The reason for this phenomenon is 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 regulant is usually required to initiated growth or differentiation in tissue culture. So, high concentration of cytokinin might have unbalanced ratio to auxin that could cause a reduction in percentage 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 tissue, 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.

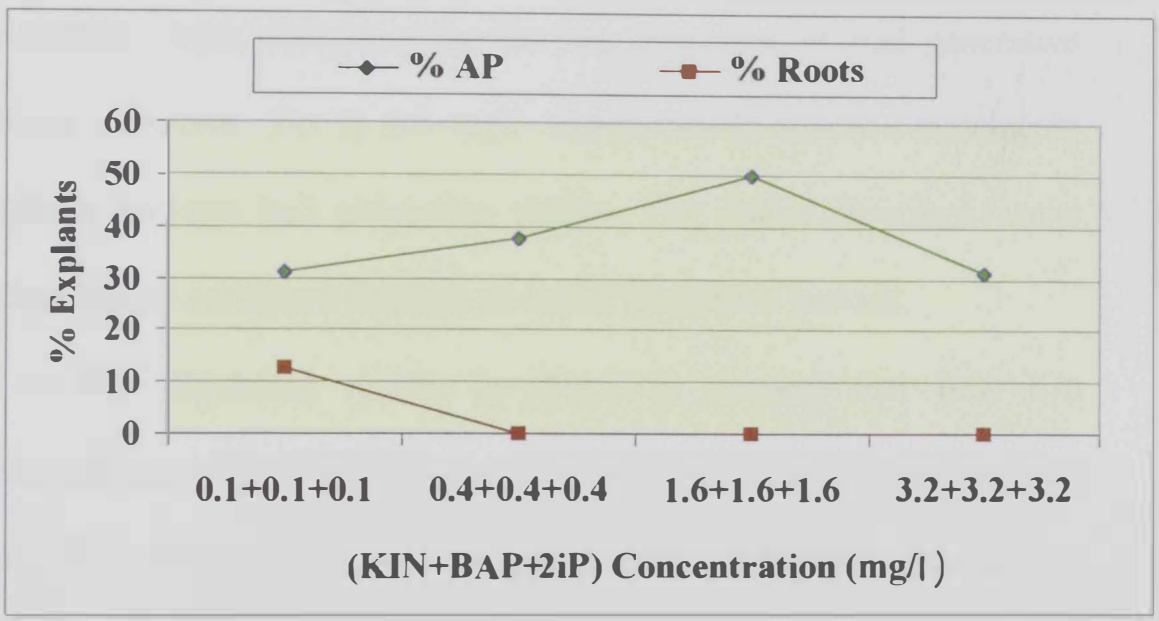
Concerning percentage of explants formed roots, data illustrated in (Fig.19.a,b) indicated that there was an opposite relationship between percentage explant formed roots and the increase in cytokinin concentrations, where increasing the level of cytokinin above 0.1 mg/l in the case of kin (Fig.19.a) or the combination of the three cytokinin (Fig.19.b) at 0.1mg/l resulted in an inhibition of root formation. Also, in the case of BAP and 2iP, data pointed out that increasing the concentration from 0.1mg/l to 0.8mg/l or higher was associated with a significant reduction in percentage root formation, and any increase in cytokinin concentration above 0.8mg/l was accompanied with complete

inhibition 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 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 very low level (0.1mg/l 2iP). These results were 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 cytokinin can sometimes induce or promote root growth. Also, Boxus and Terzi (1988) advocated the addition of 0.5mg/l Kin and auxin to the rooting medium for strawberries and several woody plants, finding that the cytokinin had a bacteriostatic effect and rooting was not impared.



(a)



(b)

Fig.19.a,b Effect of different Cytokinin and concentration on percentage explants formed apical buds and roots from cultured shoot tip of Khnazi date palm (*Phoenix dactylifera* L.) cultivar.

4.2.2. Part 2. Effect of different cytokinin and its concentration on the percentage of explants that formed bud generative tissue after 4, 5, 6 and 7 months of incubation.

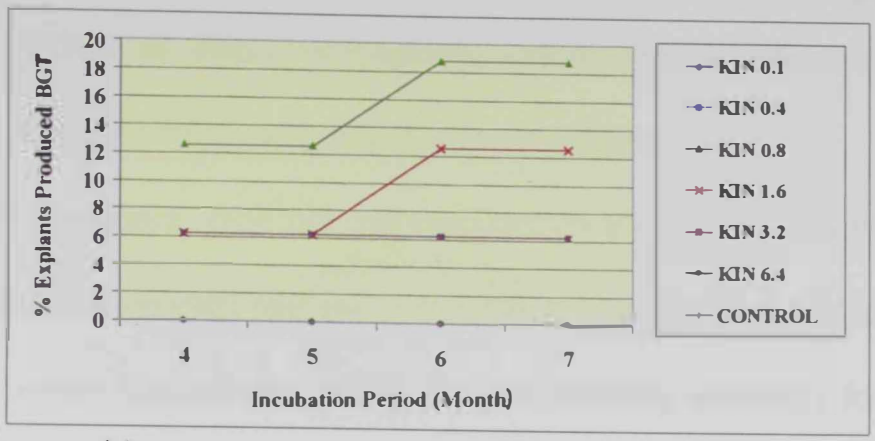
The results proved that cytokinin was essential to induce the explants to form bud generative tissue, where the absence of cytokinin at any incubation period was associated with the complete absence of bud generative tissues formation (Fig.20). Data also showed that the low concentration of cytokinins (0.1mg/l of Kin or BAP and 0.1 and 0.4mg/l of 2iP) or the high concentration (6.4mg/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 above described results were true and consistent through all tested incubation periods.

The illustrated results in (Fig.20.a) showed that best Kin concentration was 0.8mg/l, where it induced higher percentage explants formed bud generative tissue compared to other Kin treatments, and this was true after 4, 5, 6 and 7 months of incubation. In the case of BAP tested concentration, the level of 0.8mg/l proved to be effective in improving percentage explants produced bud generative tissues. However, the concentration of 1.6mg/l BAP was even better than 0.8mg/l BAP in all tested incubation period. Also, BAP at 3.2mg/l was able to induce explants to produce bud generative tissue, but it was less

effective than 0.8mg/l and 1.6mg/l, especially after 6 and 7 months of incubation. Data also showed (Fig.20.c) that 2iP at 3.2mg/l was more effective than other tested 2iP concentration in increasing percentage explants produced bud generative tissues, followed by 1.6mg/l 2iP. Increasing the concentration of 2iP to 6.4mg/l was associated with a reduction in percentage explants formed bud generative tissues and was similar in its effect to that of 2iP at 0.8mg/l. 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 1.6mg/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 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 can 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).



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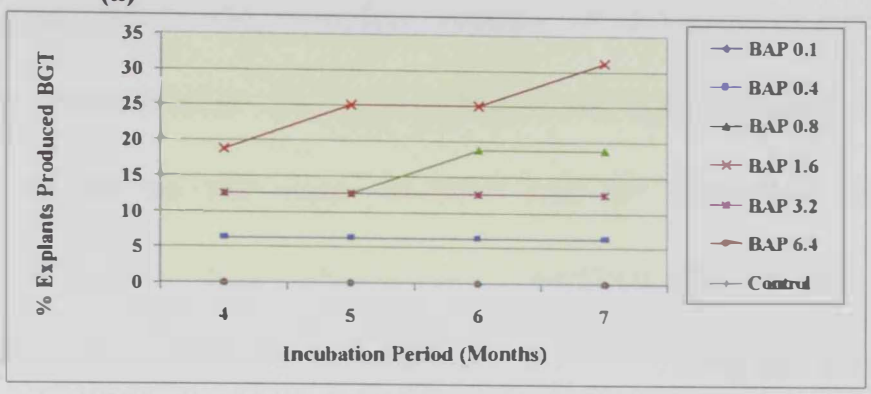

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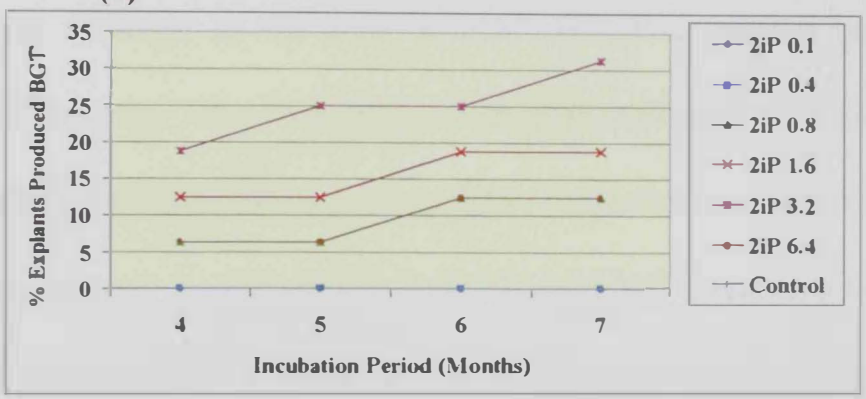

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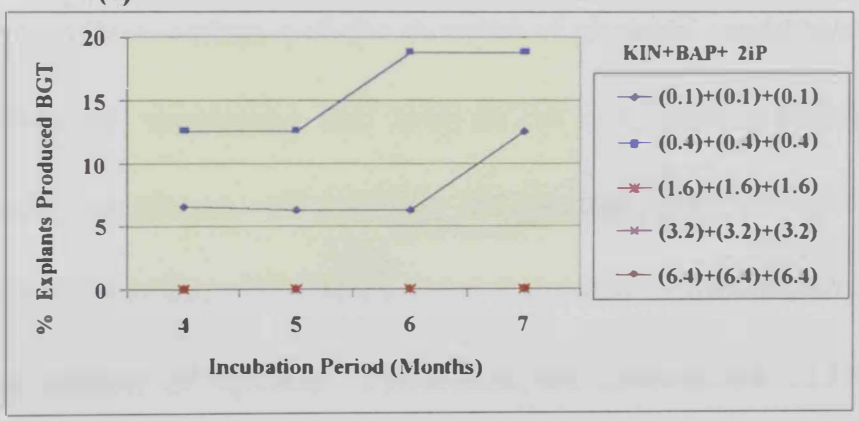


(d)

Fig.20.a,b,c,d. Effect of differnt cytokinin and concentration on percentage explants produced bud generative tissue after 4, 5, 6, and 7 months of incubation time, from cultured shoot tip of Khnazi date palm (Phoenix dactylifera L.) cultivar.

4.2.3. Part 3. Effect of different cytokinin and its concentration on the buds differentiated from each bud generative tissue.

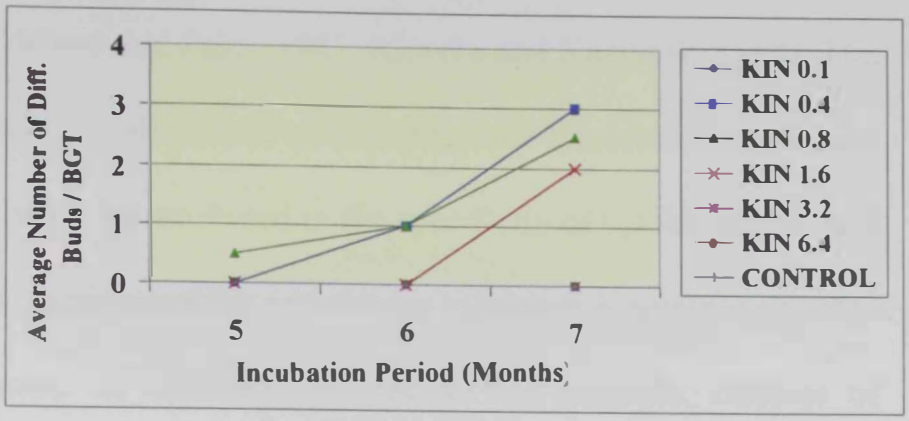
Effect of cytokinin type and concentration on average number of buds differentiated from each bud generative tissue was demonstrated in (Fig.21). The results showed that cytokinins are absolutely necessary for bud differentiation, where the complete absence of cytokinin or even using it at low concentration (0.1 and 0.4mg/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 after 6 and 7 months incubation, but not after 5 months (Fig.21.a). Increasing the level of cytokinin to 1.6mg/l in the case of BAP or 2iP increased bud differentiation frequency after 5 months of incubation (Fig.21.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 1.6mg/l resulted in the absence of bud differentiation and the increase of phenolic components production. However, increasing the level of 2iP to 3.2mg/l or 6.4mg/l did not enhance oxidation of phenolic components and, therefore, stimulated bud regeneration (Fig.21.c). After 6 months of incubation, or after the accumulation of uptaken 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 0.8mg/l.

Increasing the level of Kin to more than 0.8mg/l inhibited the regeneration of buds, but increased bud regeneration in the case of BAP and 2iP, especially at 1.6mg/l. Again, increasing the level of 2iP to 3.2 or 6.4mg/l caused a non significant reduction in number of differentiated buds, compared to the level of 1.6mg/l. After seven months of incubations there was a quite 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 level. Increasing the level of Kin above 1.6mg/l inhibited bud differentiation. In the case of BAP (Fig.21.b), the induction levels were from 0.8mg/l to 3.2mg/l. 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. In fact, these two treatments of 2iP (3.2 and 1.6mg/l)

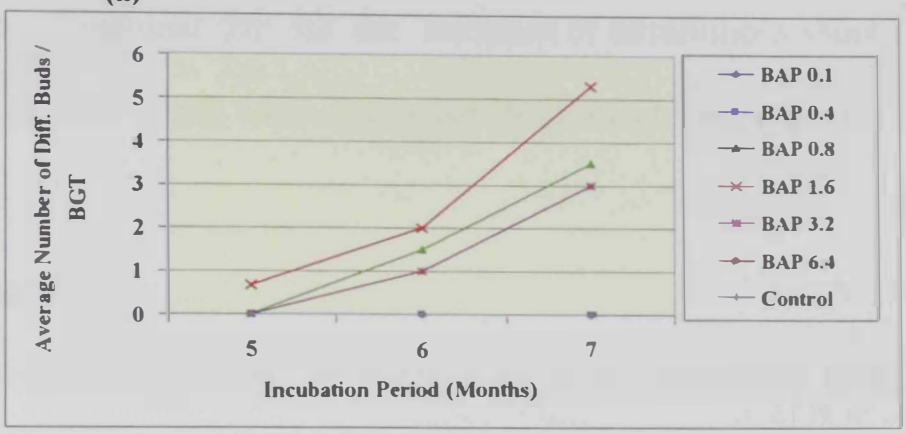
were significantly better than all other tested hormonal concentrations, regardless of the cytokinin type.

The combinations of cytokinins were significantly less effective in inducing bud regeneration compared to separate cytokinin treatments (Fig.21.d). Only the combination of Kin + BAP + 2iP at 0.4mg/l each, resulted in bud differentiation, but at a significantly lower level compare to the other discussed cytokinin treatments. The data also showed that increasing incubation period was associated with an increase in number of differentiation buds / bud generative tissue.

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 tissue was achieved when the cytokinin was 2iP used at 1.6 or 3.2mg/l, followed by BAP at 1.6mg/l. Decreasing the level of cytokinin to less than 1.6mg/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 6.4mg/l resulted in a reduction in number of regenerated buds / bud generative tissue, and this phenomenon may be due to the activity of cytokinin oxidase which is enhanced by the high exogenous application



(a)



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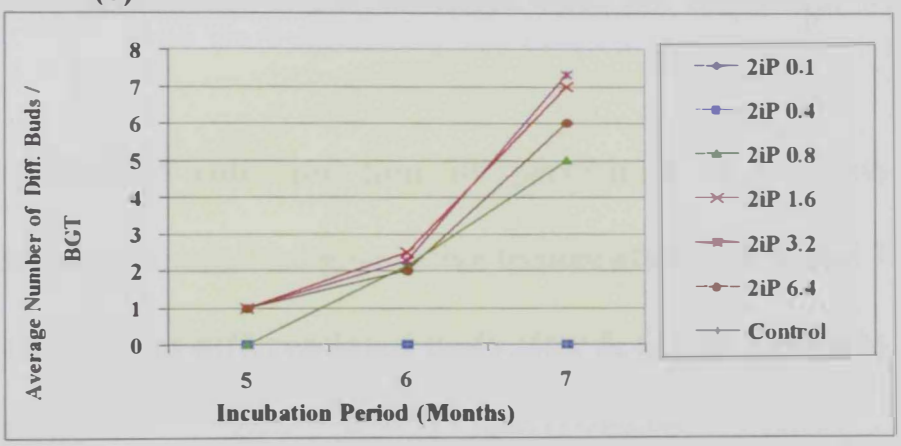

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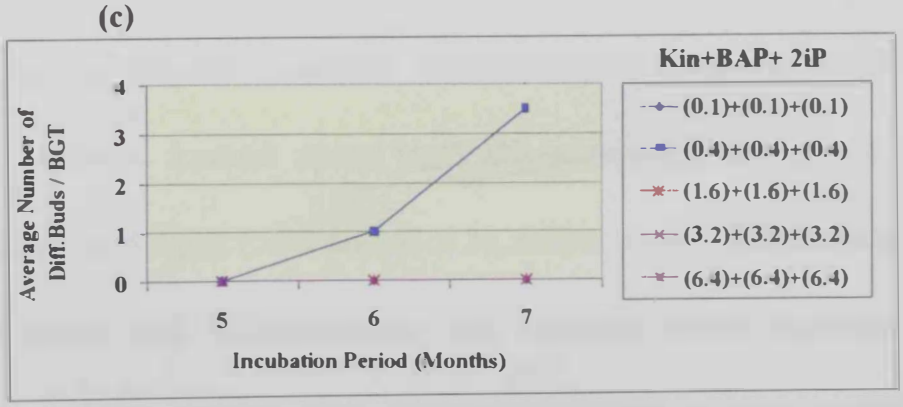

(c)

Fig.21.a,b,c,d. Effect of differnt cytokinin and concentration on average number of differentiated buds per bud generative tissue resulted from cultured shoot tip of Khnazi date palm (*Phoenix dactylifera* L.) cultivar.

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).

4.3. Experiment 3:

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

4.3.1 Part 1. Effect of culturing time on percentage of explants 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.22) revealed that the highest, and significant percentage of explants 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.22) also indicated that highest significant percentage of explants 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 formed roots at all during August and December. The ratio of explants formed roots was significantly low during July, September, November and January. The rest of culturing months had a similar moderate effect on percentage explant formed roots ranging from 4.9 to 7.8 %.

Culturing the isolated shoot tips in March resulted in production of most significant and highest percentage of explants formed bud generative tissue. The results were true and 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 tissue (Fig.22).

Number of buds differentiated from bud generative tissue is presented in (Fig.23). The results pointed out that culturing the isolated shoot tips during April resulted in a significant improve in number of buds regenerated from bud generative tissue. The same improving in number of buds regenerated per bud generative tissue that occurred in

October and April, then it came in order the months of February and March. This was true 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 tissue. 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 tissue. 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 percentage of explants 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 effetely high temperature in summer or by the low temperature in winter and got maximized during spring. The results are in accordance to those of Nissen and Sutter (1990) who pointed out that IAA auxin 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 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 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 adjusted periodically. 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 inhibitor level within explanted tissues (Quoirin *et al.*, 1975). Shoot formation on *Nicotiana glanca* internode fragments was promoted by the addition of only a cytokinin (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

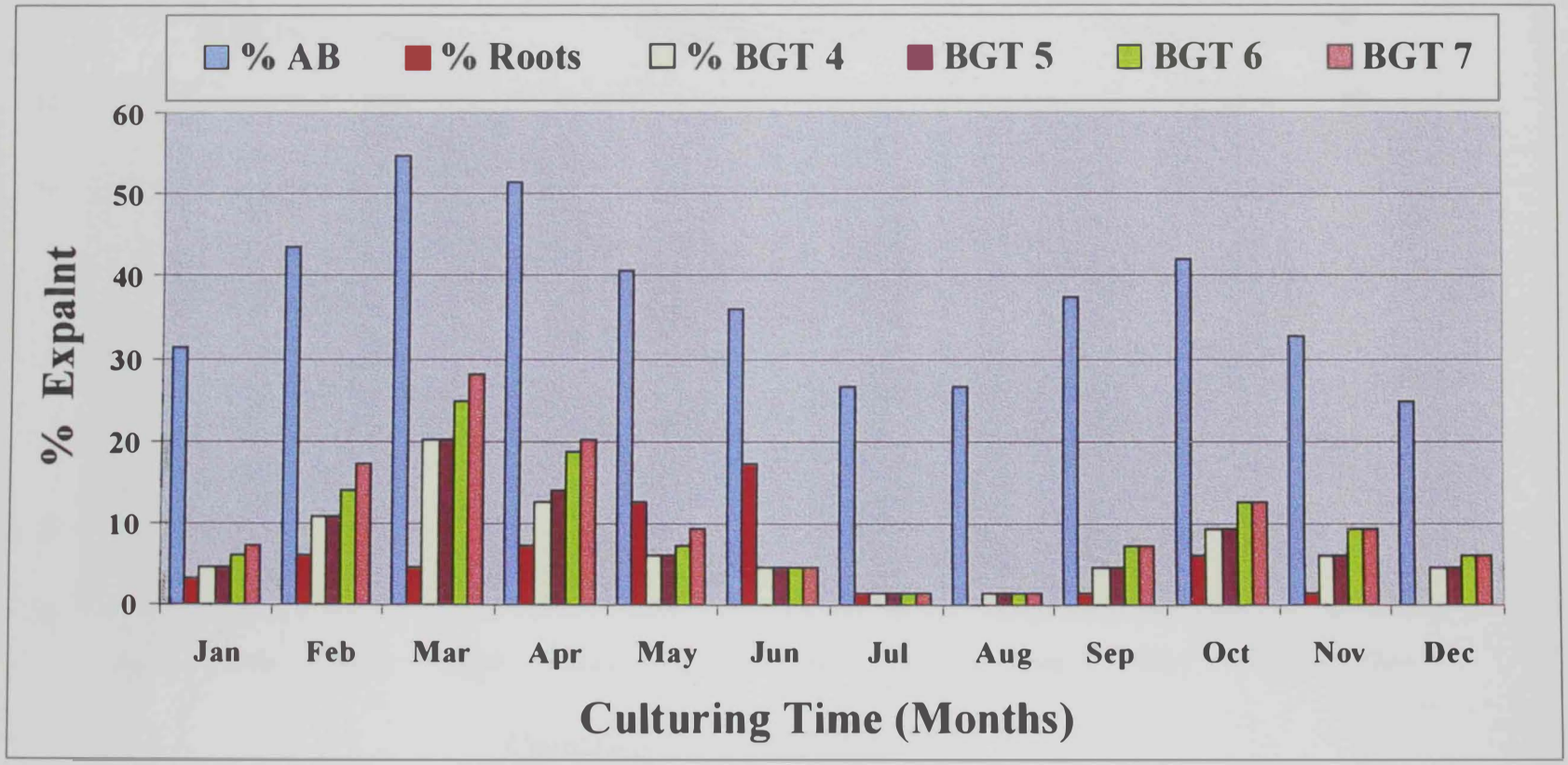

Fig.22. Effect of culturing time on percentage of apical buds, roots, and bud generative tissue after 4, 5, 6, and 7 months of incubation, resulted from cultured shoot-tips of Khnazi cultivar. AB (Apical Bud), BGT (Bud generative tissue after 4, 5, 6, 7 months).)

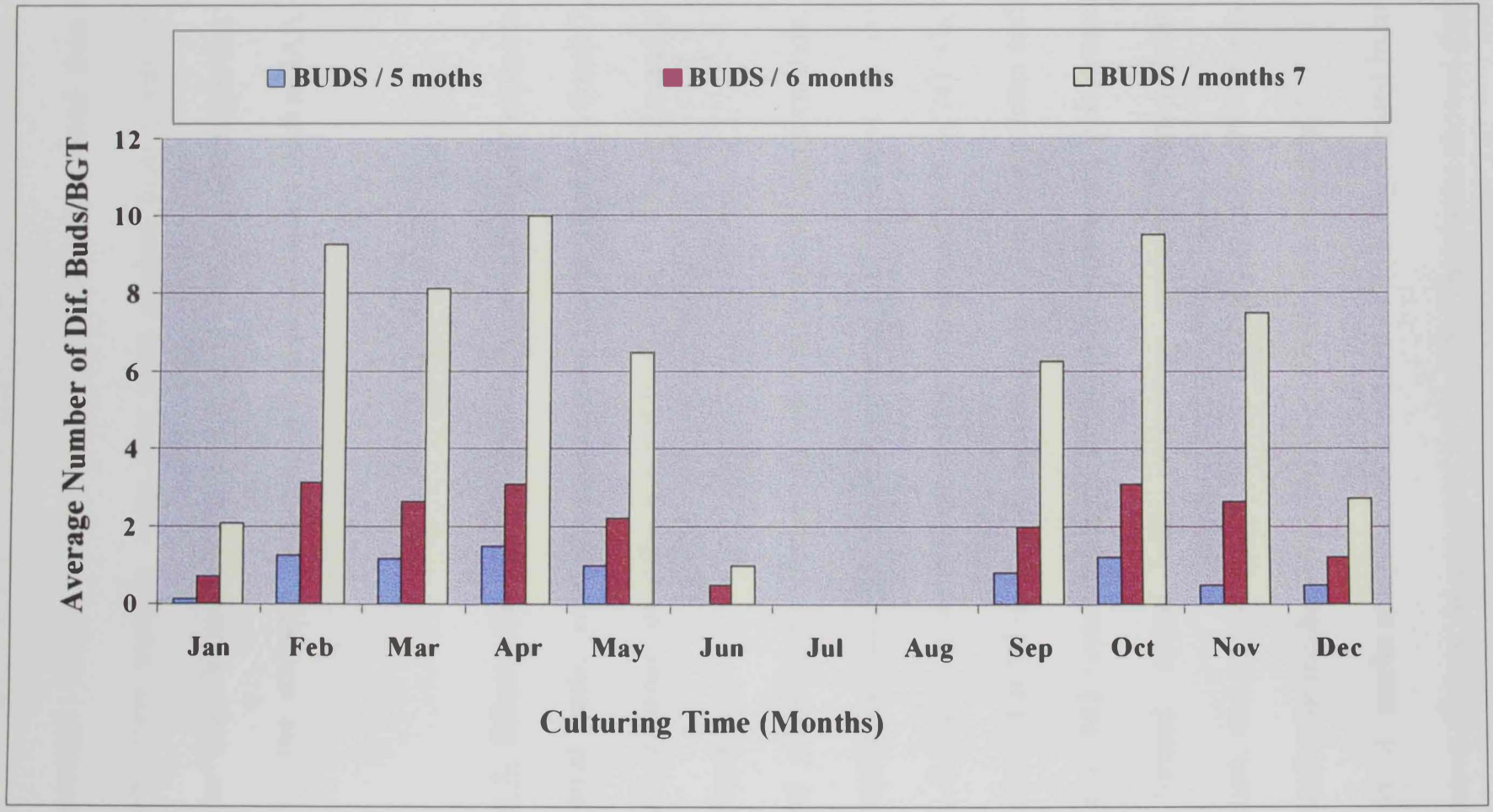

Fig.23. Effect of culturing time on average number of differentiated buds regenerated after 5, 6, 7 months per bud generative tissue, resulted from cultured shoot-tips of Khnazi cultivar.

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 ABA.

4.3.2 Part 2. Effect of media components on percentage of explants 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.

Effect of media components on percentage apical buds formation, root differentiation, and percentage formation bud generative tissues after 4, 5, 6 and 7 months of incubation is presented in (Fig. 24). The results showed that Ml (auxin rich medium) was significantly better and effective than M2 (cytokinin rich medium) in increasing percentage explants formed apical buds, percentage explants formed roots differentiation, and percentage of explants formed bud generative tissues after the 4 different incubation periods, i.e. 4, 5, 6, and 7 months.

Effect of media components on number of buds regenerated from bud generative tissues, is presented in (Fig.25). The results showed that after 5 months of incubation, Ml was better than M2 in increasing

number of regenerated buds per bud generative tissue. But with the progressing of time, M2 started to be more effective than MI, where after 6 months, M2 was more effective, than M1. After 7 months of incubation, it was quite clear that M2 enhanced significantly the regeneration of more buds generative tissues compared to M1.

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 percentage bud generative tissue formation. Percentage of explants 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

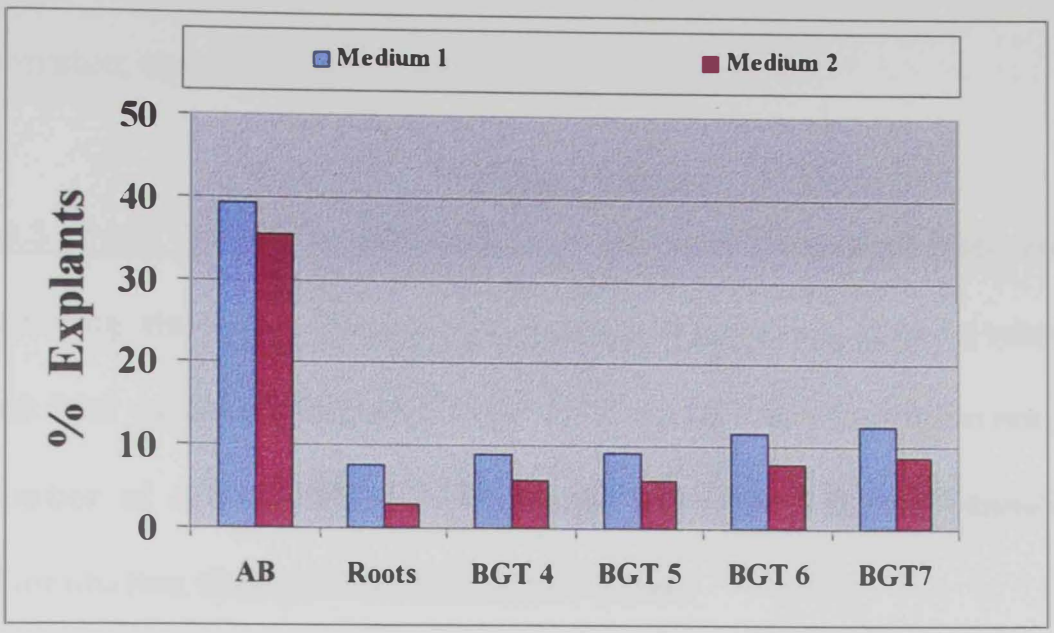

Fig. 24. Effect of media components on percentage apical buds, roots, bud generative tissue after 4, 5, 6, and 7 months, resulted from cultured shoot-tips of Khnazi cultivar. AB (Apical bud), BGT (Bud generative tissue).

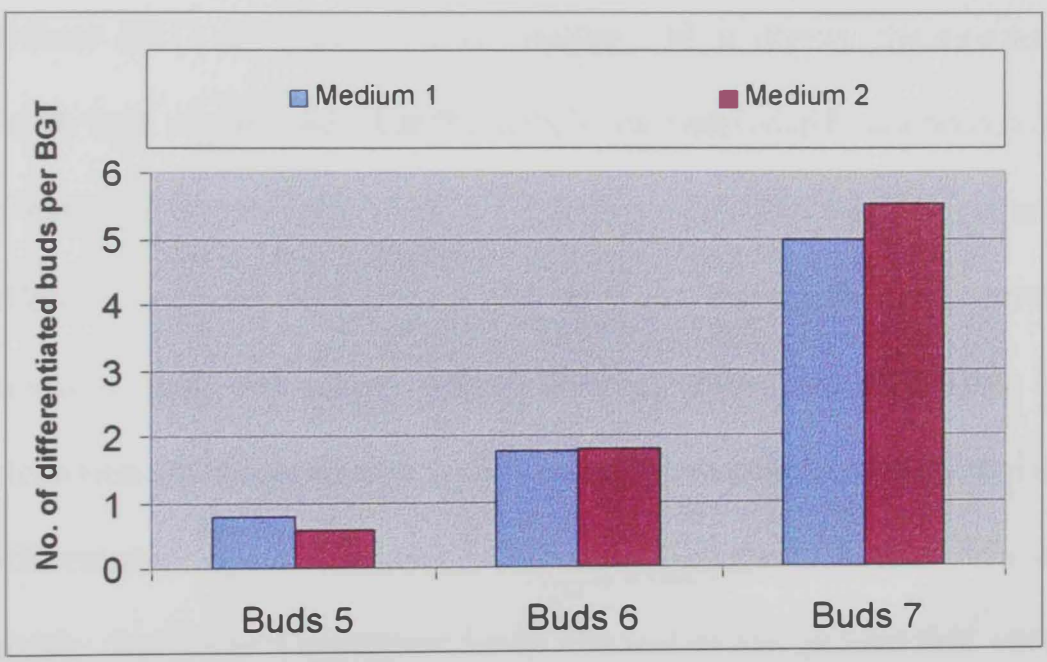

Fig.25. Effect of media components on average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissue, resulted from cultured shoot-tips of Khnazi cultivar.

the tissues. The medium M2 proved to be better in stimulating shoot formation, especially after 7 months of incubation.

4.3.3 Part 3. Effect of interaction of medium components and culturing time on percentage of explants formed 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 tissue.

Effect of interaction of medium components and culturing time on percentage explants formed apical buds, and roots is presented in (Fig.26). 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 interaction with February (as a culturing time) the interaction of M1 x February month improved the percentage of explants 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 percentage explants formed apical buds, regardless of its 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.

Regarding the percentage of explants that differentiated bud generative tissues (Fig.27), the presented data pointed out that the highest and most significant percentages of explants formed bud generative tissue 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. Also, it had been noticed that interactions

during the hot weather of July and August with M2 inhibited the production of bud generative tissue from shoot tip explants, while the interaction of July and August with M1 reduced significantly, 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 percentage explants formed bud generative tissues. Except in a few countable cases, when the interaction of months with M1 or M2 has 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 percentage explants formed bud generative tissue. The concluded notices were true and 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.28). 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 reduced significantly started May and continued during the hot months of summer (June, July and August). The number of buds were significantly improved in October, especially when M2 was used, but it was reduced

again during the winter months (November, December and January). Maximum number of buds was produced 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 absent totally during the hot months of summer and started to show up again during October. The maximum number of buds was achieved when explants were cultured during October on M2 medium. The same trend was observed again after 7 months of incubation, but was 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 (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 tissue, 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 relatively high auxin concentration, rooting was almost inhibited

during winter and summer months because of the absence or unavailability of auxins. The use of auxin rich medium was not even 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 hormone level available in mother tissues during spring. Number of regenerated buds/bud generative tissue 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 relatively high concentration of cytokinin (George 1993) so the naturally endogenous balanced auxins and cytokinins were supported with the exogenous supplement in M2, and resulted in a significant increase in bud differentiation. In contrast, the high temperature in winter months reduced, but did not inhibit, bud regeneration. This observation indicated that cytokinins are not completely inactivated by low temperature as in the case of auxin.

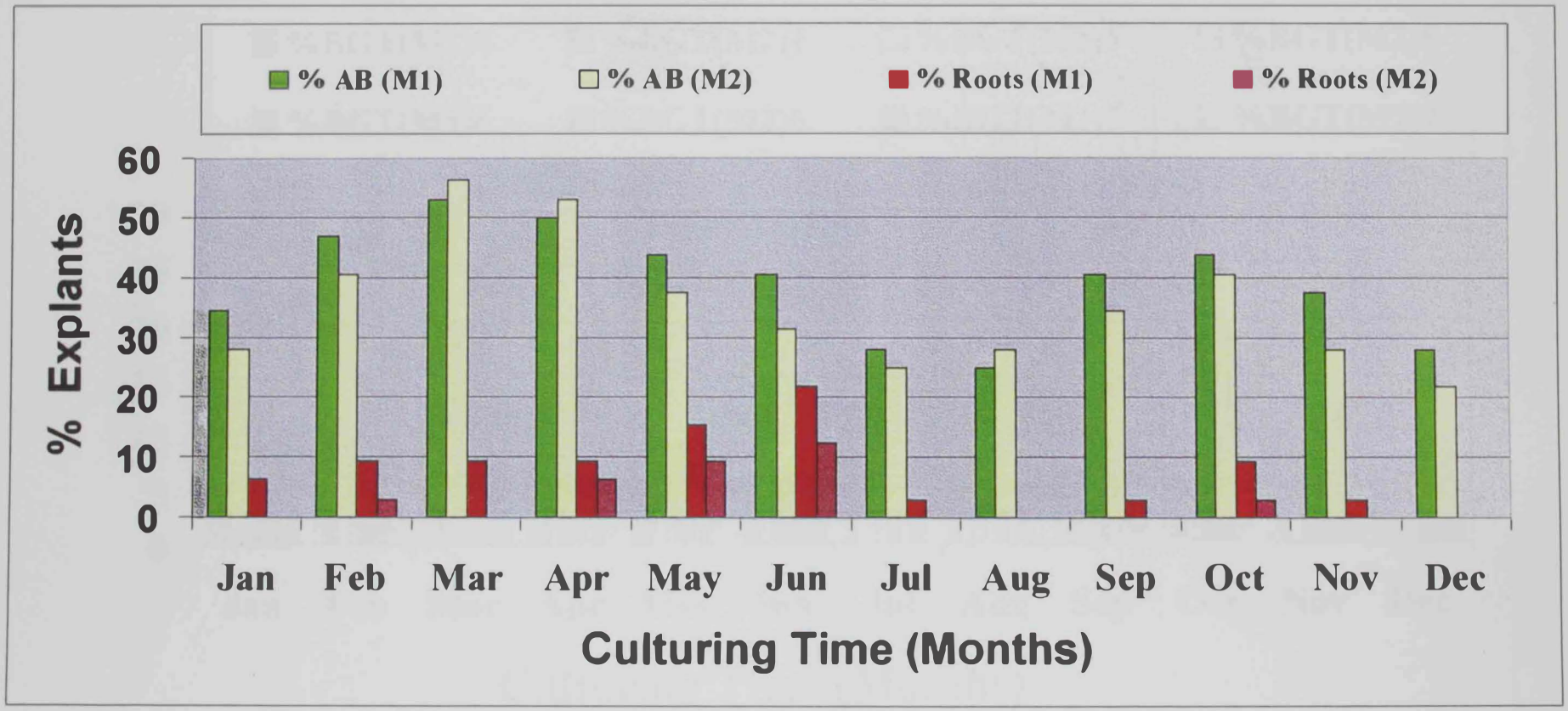

Fig 26. Effect of the interaction of medium components and culturing month on apical buds and roots after 4, 5, 6 and 7 months, resulted from cultured shoot-tips of Khnazi cultivar.

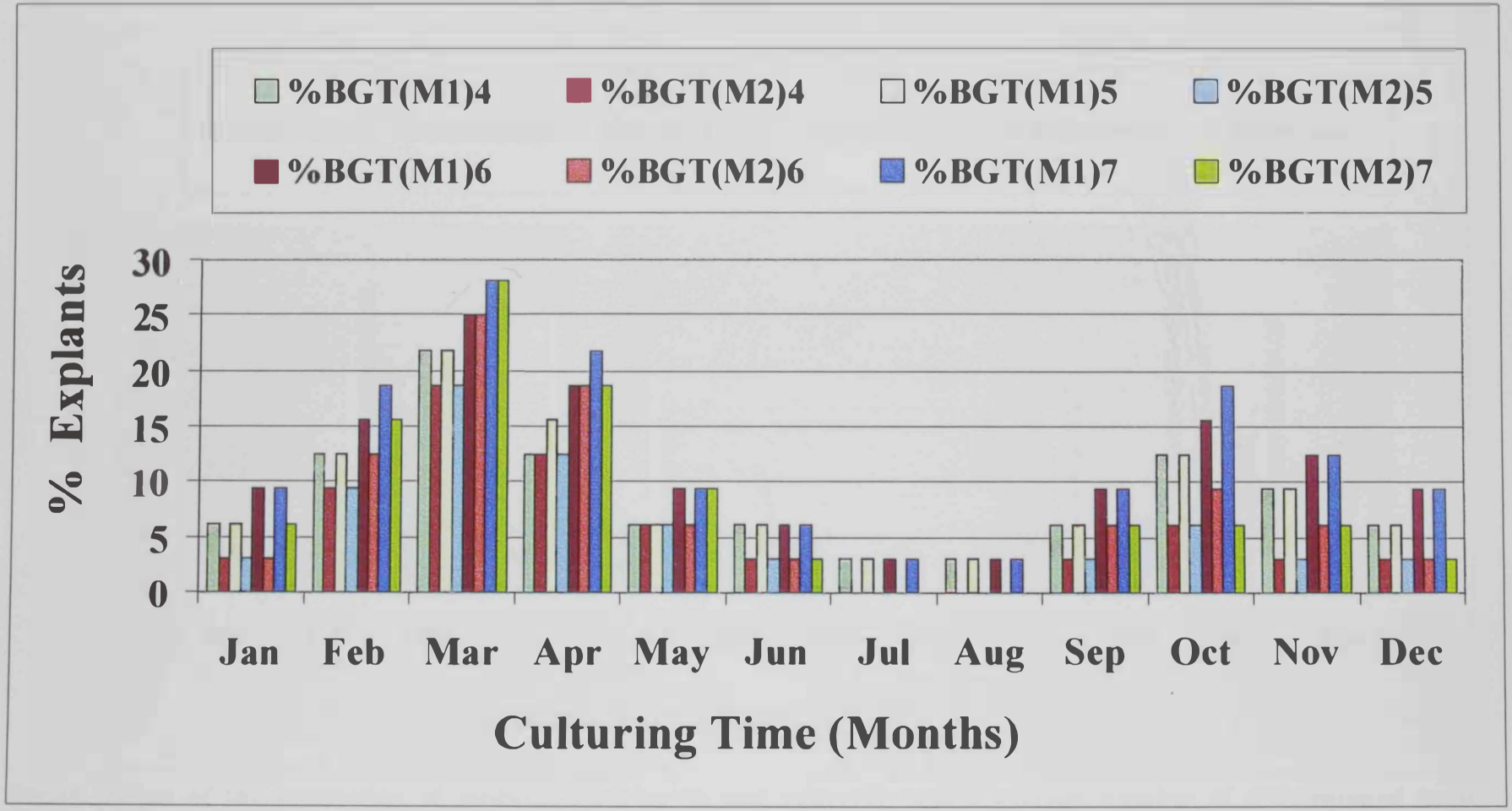

Fig.27. Effect of the interaction of medium components and culturing month on bud generative tissue after 4, 5, 6 and 7 months resulted, from cultured shoot-tips of Khnazi cultivar.

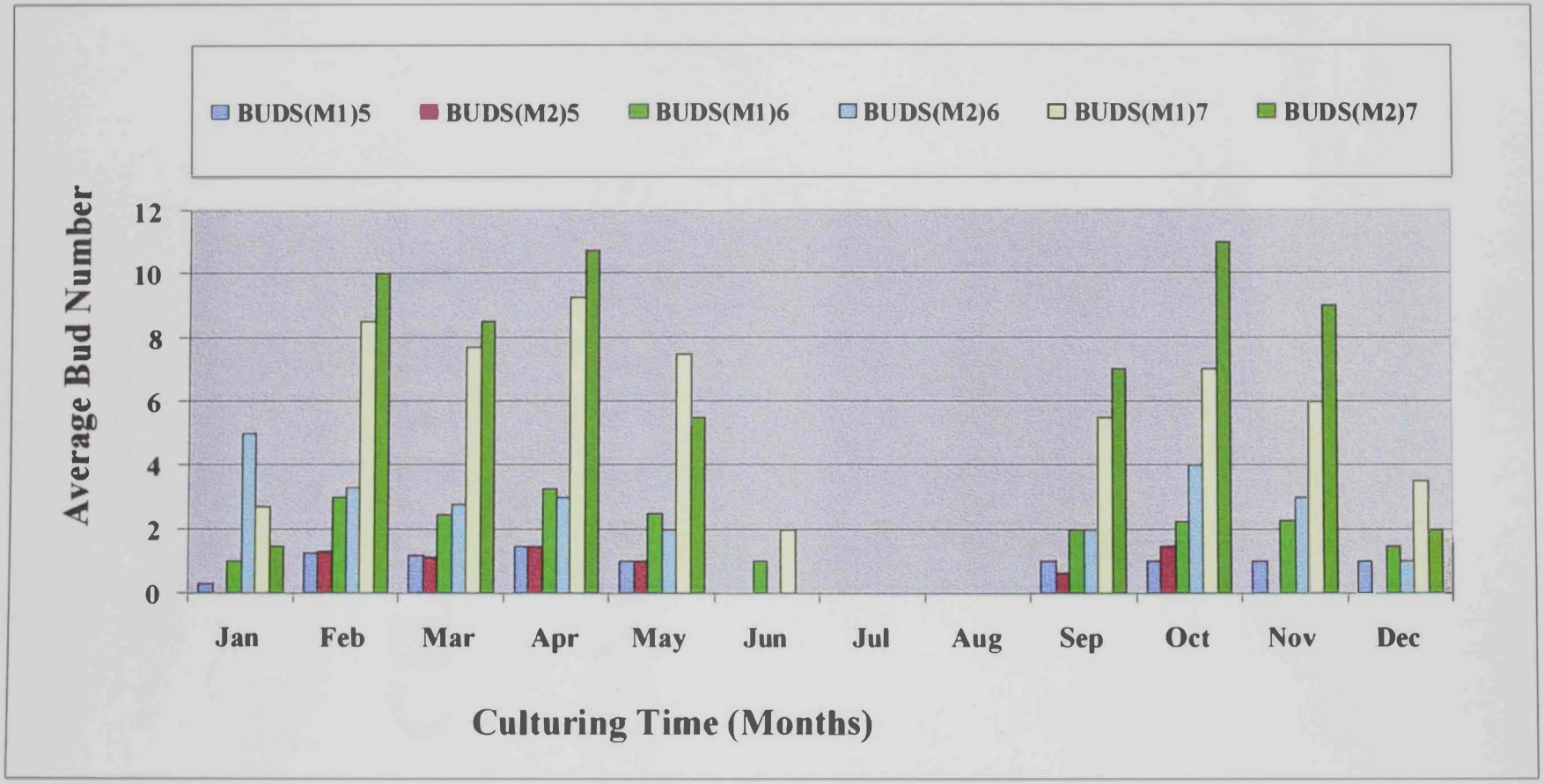

Fig.28. Effect of the interaction of medium components and culturing month average number of differentiated buds regenerated after 5, 6, 7 months per bud generative tissue, resulted from cultured shoot-tips of Khnazi cultivar.

CHAPTER V

CONCLUSIONS

CHAPTER 5

CONCLUSIONS

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

From the results of the thesis it can be concluded that:

5.1 Experiment 1:

5.1.1 Part 1

The presence of auxin in 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 1.6mg/l resulted in an increase in the number of explants formed apical buds. Increasing IAA concentration to 3.2 or 6.4mg/l resulted in a reduction on percentage of explants that formed apical buds. Similarly, the auxin NAA behaved in a similar manner like the auxin IAA. Combining IAA and NAA together at an equal concentration of

0.1mg/l resulted in a significant increase in the percentage of explants that formed apical buds over the control treatment.

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

Increasing the level of auxin, regardless of the type of auxin, was associated with the increase in percentage explants formed roots. 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.

5.1.2 Part 2

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 mg/l to 0.8mg/l and then to 1.6 mg/l. Increasing the level of auxin

in the medium to 3.2mg/l or higher (6.4mg/l) resulted in a reduction in the formation of bud generative tissues.

The combination of both NAA and IAA behaved in the same manner as any of the auxin alone, with the combination (0.4mg/l IAA and 0.4mg/l NAA) 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. This is due to the balance of cytokinins to auxins ratio will be to the side of auxin.

5.1.3 Part 3

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., 0.1mg/l and 0.4 mg/l were not enough to induce bud differentiation. Increasing the concentration of IAA up to 0.8mg/l resulted in the regeneration of significantly highest number of buds from bud generative tissues, followed by 1.6mg/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.

5.2 Experiment 2

5.2.1 Part 1

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 0.4mg/l in the case of Kin, 1.6mg/l in the case of BAP, 3.2 mg/l with regard to 2iP and the combination of Kin + BAP + 2iP at 1.6mg/l each.
In addition, the ability of control explants to form apical buds may be attributed to the presence of enough endogenous level of cytokinin 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 percentage 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 very low level (0.1mg/l 2iP).

5.2.2. Part 2

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 1.6mg/l and 2iP at 3.2mg/l. Those 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.

5.2.3 Part 3:

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 0.4mg/l) did not stimulate bud differentiation at all in the case of BAP and 2iP

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

5.3 Experiment 3:

5.3.1 Part 1:

Data showed that the highest, and significant percentage of explants 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 months 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 formed bud generative tissue. 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 tissue. 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 effetely high temperature in summer or by the low temperature in winter and got maximized during spring.

5.3.2 Part 2:

The results showed that M1 (auxin rich medium) was significantly better and effective than M2 (cytokinin rich medium) in increasing percentage explants formed apical buds, percentage root differentiation, and 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 number of buds regenerated from bud generative tissues, the results showed that after 5 months of incubation, M1 was better than M2 in increasing number of regenerated buds generative tissue. But with the advancing of time, M2 started to be more effective than M1, whereas after 6 months, M2 was more effective, than M1. After 7 months of incubation, it was quite clear that M2 enhanced significantly 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, which contained higher cytokinin concentration compared to M1. 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.

5.3.3 Part 3:

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 absent totally during the hot months of summer and started to show up 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 auxin-rich medium during hot summer months or cold winter months would result in an improvement of percentage explants forming bud generative tissue, compared to the interaction with cytokinin-rich medium. Rooting was almost inhibited during winter and summer months because of the absence or unavailability of auxins. The use of auxin-rich medium was not even enough to induce rooting. The results are in agreement with the fact that shoot bud differentiation requires relatively high concentration of cytokinin so the naturally endogenous balanced auxins and cytokinins were supported with the exogenous supplement, and resulted in a significant increase in bud differentiation. In contrast the high temperature in winter months reduced, bud did not inhibit, bud regeneration. This notice indicated that cytokinins are not completely inactivated by low temperature as in auxin case.

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APPENDIX

Table 1. Effect of different auxin and concentration on the percentage of explants that formed apical buds and roots, resulted from cultured shoot tip of Khnazi date palm (*Phoenix dactylifera* L.) 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 hormons.

Table 2. Effect of different auxin and concentration on percentage of explants that produced bud generative tissues after 4,5,6 and 7 months of incubation resulted from cultured shoot tip of Khnazi date palm (*Phoenix dactylifera* L.) 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	0.0
IAA (0.1), NAA (0.1)	12.5	12.5	18.75	18.75
IAA (0.4), NAA (0.4)	25.0	25.0	31.25	31.25
IAA (0.8), NAA (0.8)	18.75	18.75	25.0	25.0
IAA (1.6), NAA (1.6)	6.25	6.25	6.25	12.5
IAA (3.2), NAA (3.2)	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 auxin and concentration on the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissue, resulted from cultured shoot ip of Khnazi date palm (*Phoenix dactylifera* L.) 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	0.0
IAA (0.1), NAA (0.1)	0.50	1.5	4.5
IAA (0.4), NAA (0.4)	1.0	2.25	6.25
IAA (0.8), NAA (0.8)	1.0	2.0	5.70
IAA (1.6), NAA (1.6)	0.0	2.0	5.0
IAA (3.2), NAA (3.2)	0.0	0.0	0.0
LSD (5%)	0.2242	0.5776	1.328

Table 4. Effect of different cytokinin and concentration on the percentage of explants that formed apical buds and roots resulted from cultured shoot tip of Khnazi date palm (*Phoenix dactylifera* L.) 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 (0.1)	31.25	12.5
KIN (0.4), BAP (0.4), 2iP (0.4)	37.5	0.0
KIN (1.6), BAP (1.6), 2iP (1.6)	50.0	0.0
KIN (3.2), BAP (3.2), 2iP (3.2)	31.25	0.0
LSD (5%)	16.01	14.75

Table 5. Effect of different cytokinin and concentration on the percentage of explants that produced bud generative tissue after 4, 5, 6 and 7 months of incubation resulted from cultured shoot tip of Khnazi date palm (*Phoenix dactylifera* L.) 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 (0.1)	6.25	6.25	6.25	12.5
KIN (0.4), BAP (0.4), 2iP (0.4)	12.5	12.5	18.75	18.75
KIN (1.6), BAP (1.6), 2iP (1.6)	0.00	0.00	0.00	0.00
KIN (3.2), BAP (3.2), 2iP (3.2)	0.00	0.00	0.00	0.00
LSD Value (5%)	13.2	14.75	18.66	21.55

Table 6. Effect of different cytokinin and concentration on the average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissue, resulted from cultured shoot tip of Khnazi date palm (*Phoenix dactylifera* L.) 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 (0.1)	0.0	0.0	0.0
KIN (0.4), BAP (0.4), 2iP (0.4)	0.0	1.0	3.5
KIN (1.6), BAP (1.6), 2iP (1.6)	0.0	0.0	0.0
KIN (3.2), BAP (3.2), 2iP (3.2)	0.0	0.0	0.0
LSD Value (5%)	0.3125	0.5665	0.8340

Table 7. Effect of culturing time on percentage explants formed apical buds, roots, bud generative tissue after 4, 5, 6, and 7 months and average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissue, resulted from cultured shoot tips of Khnazi 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 8. Effect of media components on percentage explants formed apical buds, roots, bud generative tissues after 4, 5, 6, and 7 months and average number of differentiated buds regenerated after 5, 6, and 7 months per bud generative tissue, resulted from cultured shoot tips of Khnazi cultivar.

Media	% AB	% Roots	% BGT4	% BGT5	% BGT6	% BGT7	# Buds5	# Buds6	# Buds7
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	0.0363

M1: medium 1 (Auxin rich medium)
M2: medium 2 (Cytokinin rich medium).

Table 9. Effect of the interaction of medium components and culturing time on percentage of explants formed apical buds, roots, and bud generative tissue after 4, 5, 6, and 7 months, resulted from cultured shoot tips of Khnazi 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	34.38	28.13	6.25	0.0	6.25	3.12	6.25	3.12	9.37	3.12	9.375	6.25
February	46.88	40.63	9.37	3.12	12.5	9.37	12.5	9.37	15.63	12.5	18.75	15.63
March	53.13	56.25	9.37	0.0	21.88	18.75	21.88	18.75	25.0	25.0	28.13	28.13
April	50.0	53.13	9.37	6.25	12.5	12.5	15.63	12.5	18.75	18.75	21.88	18.75
May	43.7	37.5	15.63	9.375	6.25	6.25	6.25	6.25	9.37	6.25	9.37	9.375
June	40.63	31.25	21.88	12.5	6.25	3.12	6.25	3.12	6.25	3.12	6.25	3.125
July	28.13	25.0	3.12	0.0	3.125	0.0	3.12	0.0	3.12	0.0	3.12	0.0
August	25.0	28.13	0.0	0.0	3.125	0.0	3.12	0.0	3.125	0.0	3.12	0.0
September	40.63	34.38	3.12	0.0	6.25	3.12	6.25	3.12	9.37	6.25	9.37	6.25
October	43.75	40.63	9.37	3.12	12.5	6.25	12.5	6.25	15.63	9.37	18.75	6.25
November	37.5	28.13	3.12	0.0	9.375	3.12	9.375	3.12	12.6	6.25	12.5	6.25
December	28.13	21.88	0.0	0.0	6.25	3.12	6.25	3.12	9.37	3.12	9.375	3.125
LSD	10.86		10.6		8.21		8.35		10.3		11.72	

Table 10. 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 tissue, resulted from cultured shoot tips of Khnazi 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 11. 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.

ARABIC SUMMARY

كذلك قد تبين من هذه التجربة أن أشهر الصيف قد ثبطت تكون النسيج المولد للبراعم والبراعم المتكشفة كما أن في هذه الأشهر زادت ظاهرة اسمرار الأجزاء النباتية كنتيجة لزيادة تراكم المركبات الفينولية.

و تتركب أوساط الإدخال من الأملاح المعدنية لوسط موراشيج وسكوغ MS مزودة بـ ١٠٠ ملغ/لتر ميو-إينيستول، ٠,٥ ملغ/لتر نيكوتينك أسيد ، ٠,٥ ملغ/لتر بيرودوكسين ، ٠,١ ملغ/لتر ثيامين-HCL ، ٢ ملغ/لتر جليسين ، ٤٠ ملغ/لتر أدنين سلفيت، ٢ غ/لتر بولي فينيل بيروليدون ، ٣ ملغ/لتر فحم نباتي نشط ، ٤٠ غ/لتر سكر.

الجزء الثاني:

تضمن دراسة عدد ٢٣ وسط غذائي يتكون من تراكيز مختلفة من ٣ أنواع من السيتوكينينات (كاينتين ، بنزيل أمينو بيورين ، أيزوبنتيل بيورين) على أنسجة النخيل المستأصلة من منطقة القمة النامية (المرستيم) لصنف "خنيزي" لتوليد البراعم الأولية القابلة للإكثار.

نتائج الدراسة:

أعلى نسبة من الأجزاء النباتية لتي كونت نسيج مولد للبراعم تشكلت من إضافة ٣,٢ ملغ/لتر من أيزوبنتيل أدنين أو ١,٦ ملغ/لتر بنزيل أمينو بيورين. أما أعلى عدد من البراعم المتكشفة من النسيج المولد للبراعم فقد نتجت من إضافة ٣,٢ ملغ/لتر من أيزوبنتيل أدنين في أوساط الإدخال.

تبين من التجربتين السابقتين أن كلا من الأكسينات والسيتوكينينات أثبتت أنها مهمة وضرورية لتحفيز الأنسجة لمولدة للبراعم ثم لتكشف البراعم من الأجزاء المزروعة.

الجزء الثالث:

في هذا الجزء تم زراعة الأجزاء المستأصلة من الصنف تحت الدراسة شهريا إبتداءا من شهر سبتمبر ولمدة مدة ١٢ شهرا على وسطين غذائيين مختلفين هما.

وسط (١): ويتكون من إضافة (٠,٤ ملغ/لتر IAA ، ٠,٤ ملغ/لتر NAA، ٤ ملغ/لتر NOA، ٠,٤ ملغ/لتر Kin، ٠,٤ ملغ/لتر BAP ، ٠,٤ ملغ/لتر 2iP).

وسط (٢): ويتكون من إضافة (٠,٤ ملغ/لتر لكل من IAA ، NAA ، NOA ، ٣,٢ ملغ/لتر 2iP).

نتائج الدراسة:

أعلى نسبة من الأجزاء النباتية و لتي كونت نسيج مولد للبراعم قد تحققت في فصل الربيع وبالأخص في شهر مارس. كذلك أعلى عدد من البراعم لكل نسيج مولد قد تكون خلال أشهر الربيع وبالأخص خلال شهر إبريل بغض النظر عن الوسط الغذائي المستخدم.

الخلاصـــــــــــــة

تعتبر نخلة التمر *Phoenix dactylifera L.* من أهم أشجار الفاكهة في منطقة الخليج العربي بشكل عام و في دولة الإمارات العربية المتحدة بشكل خاص. وهي شجرة ثنائية المسكن وحيدة الفلقة تتبع العائلة النخيلية Palmaceae .

تتكاثر نخلة التمر إما جنسيا عن طريق استخدام البذور أو خضريا عن طريق استئصال وزراعة الفسائل Offshoots. ويعتبر الإكثار الجنسي طريقة غير مرغوبة لإكثار النخيل وذلك لأنه ينتج أنعزالات وراثية مختلفة ينتج عنها شتلات مختلفة عن الصنف الأم المراد إكثاره. أما طريقة الإكثار الخضري باستخدام الفسائل فإنها طريقة تضمن مطابقة الصنف إلا أنها بطيئة ولا تفي باحتياجات السوق من الأصناف ذات الجودة العالية وخاصة النادرة منها. فلذلك يبدو من المهم استخدام تقنيات زراعة الأنسجة النباتية لإكثار و إنتاج شتلات نخيل التمر.

تهدف الدراسة الحالية إلى تحديد أفضل الأوساط الغذائية ملاءمة لنمو وتطور نسيج نخلة التمر للوصول إلى تكوين البراعم القابلة للإكثار ، بالإضافة إلى دراسة تأثير وقت الزراعة لتحديد أفضل أشهر السنة ملاءمة لزراعة أنسجة النخيل معمليا في دولة الإمارات العربية المتحدة.

الدراسة المقدمة تضمنت ثلاثة أجزاء تمت خلال ثلاثة فصول متتالية (١٩٩٦-١٩٩٨) على النحو التالي:

الجزء الأول:

تضمن دراسة تأثير ١٨ وسط غذائي يتكون من تراكيز مختلفة من نوعين من الأوكسينات إندول حامض الخليك (IAA) ونفثالين حامض الخليك (NAA) بالإضافة إلى وسط غذائي مقارن خالي تماما من الهرمونات ، على أنسجة النخيل المستأصلة من منطقة القمة النامية (المرستيم) لصنف "خنيزي" لتوليد البراعم الأولية القابلة للإكثار.

نتائج الدراسة:

أعلى نسبة من الأجزاء النباتية والتي كونت نسيج مولد للبراعم نتجت من إضافة إندول حامض الخليك بتركيز ١,٦ ملغ/لتر أو من إضافة كلا من إندول حامض الخليك ونفثالين حامض الخليك بتركيز ٠,٤ ملغ/لتر ، ٠,٤ملغ/لتر على الترتيب في أوساط الإدخال. أما أعلى عدد من البراعم المتكشفة من النسيج المولد للبراعم قد نتجت من إضافة إندول حامض الخليك بتركيز ٠,٨ ملغ/لتر في أوساط الإدخال.

تأثير وقت الزراعة والخليط الهرموني على إستنساخ نخلة التمر صنف "خنيزي" معمليا

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رسالة مقدمة إلى
كلية العلوم - جامعة الإمارات العربية المتحدة
لإستكمال متطلبات الحصول على درجة الماجستير في
علوم البيئة

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