J. Agric. Sci. Mansoura Univ., 34 (3): 1629 - 1644, 2009

MICROPROPAGATION OF DATE PALM CV. MALAKABY THROUGH SOMATIC EMBRYOGENESIS
3- EFFECT OF TRYPTONE, YEAST EXTRACT, CASEIN HYDROLYSATE AND PINEAPPLE EXTRACT

Hegazy, A.; M. I. Nasr; I. A. Ibrahim and H. H. El-Bastawissy
Genetic Engineering and Biotechnology Research Institute (GEBRI), Plant Biotech. Deparment., Minufiya University, Sadat City, Egypt

ABSTRACT

Date palm (*Phoenix dactylifera.* L.) dry cultivar, i.e. Malakaby repetitive somatic embryos clusters cultured on MS (Murashige and Skooge, 1962) modified medium (MMS) supplemented with NAA (0.1 mg/l), kin (0.5), activated charcoal (1.5 g/l) and gelrite (1.5 g/l) in addition to tryptone at the concentration of 1.0 g/l recorded higher significant number of embryos and multiplication rate, also higher fresh weight and growth value. However, addition of yeast extract at the concentration of 1.5 g/l recorded higher significant number of embryos as well as higher embryos multiplication rate, fresh weight and growth value. Also, addition of casein hydrolysate at the concentration of 1.5 g/l recorded higher significant number of embryos as well as higher embryos multiplication rate, fresh weight and growth value. While, addition of pineapple extract at the concentration of 9 g/l recorded higher significant value of all growth characters as compared with the control.

Resulted shootlets were cultured individually on MS basal medium supplemented with NAA 0.5 mg/l and IBA 0.5 mg/l in addition to the same chemical compounds concentrations previously tested and solidified with 6 g/l phyto agar. Well- rooted plantlets obtained were transferred *ex vitro* for acclimatization on soil type mixture of compost and perlite (1:1 v/v).

Addition of tryptone at the concentration i.e. 1, 1.5 and 2 g/l to the rooting medium decreased all morphological characters after 8 weeks of incubation and declined plantlets survival (26.67, 13.33 and 6.67 %) respectively compared to the control (40 %) after 3 months in acclimatization. However, addition of yeast extract at the concentration of 1.5 g/l to the rooting medium recorded higher no. of leaves, leaf length, no. of roots and raised survival in acclimatization (26.67 %) over the control. While, addition of casein hydrolysate at the concentration of 1.5 g/l to the rooting medium recorded higher significant values of number of roots and raised survival in acclimatization (13.33 %). On the other hand, addition of pineapple extract at the concentration of 9 g/l to the rooting medium recorded higher values of all growth characters and raised plantlets survival in acclimatization (33.33 %) over the control.

Keywords: *Phoenix dactylifera* L.,*in vitro*, embryogenesis, callus, complex, addenda.

INTRODUCTION

There is no available literature concerning the uses of complex addenda on *in vitro* growth and development of date palm somatic embryos. In this regard, some researchers studied the influence of tryptone; Fonesbech, (1972) *on Cymbidium*, Amaki and Higuchi (1989) on protocorm-like bodies of *Phalaenopsis*. Yeast extract; Guidin and Harada (1974) on artichoke explants, Maksoud (2007) on *Ocimum bacilicum*, Abd El-Aal (2008) on *Hyoscyamus muticus*. Pierik (1987) mentioned that casein hydrolysate is one of the most important natural product added to plant tissue culture medium. It is a mixture of compounds in particular amino acids manufactured from casein. Some natural products were added to nutrient medium i.e. casein hydrolysate (0.1-1.0 g/l), peptone (0.25- 3.0 g/l), tryptone (0.25- 2.0 g/l) and malt extract (0.5-1.0 g/l) .These mixtures are very complex and contain vitamins as well as amino acids. Yeast extract (0.25-2.0 g/l) is normally used because of its high contained of vitamins B.

Complex addenda produced amino acids (namely casein hydrolsate - yeast extract - pineapple - malt extract), peptides glutathione or amide glutamine can be added to plant media to satisfy the requirement of cultures for reduced nitrogen. The response to organic nitrogen depends on the ratio of NO3 to NH4. Amino acids provide plant cells with an immediately available source of nitrogen and uptake can be much more rapid than that of inorganic nitrogen in the same medium. Amino acids can also provide reduced nitrogen in culture media in place of NH4 and as a supplement to NO3.However they are usually employed as minor addition to media containing both NH4 and NO3. Uptake of amino acids into cultured tissues causes a decrease in the pH of the medium (George, 1993).

Somatic embryogenesis from date palm (*Phoenix dactylifera L.*) shoot tip explant derived callus has been viewed as the most appealing process for regeneration (Al-Khayri, 2001).

The aim of this work was to study the influence of some complex addenda i.e. Tryptone, yeast extract, casein hydrolysate and pineapple extract concentrations on *in vitro* growth of date palm (*Phoenix dactylifera.* L.) dry cultivar, i.e. Malakaby during repetitive somatic embryos cycle, plantlets formation and subsequently their effects on plantlets survival percentage during acclimatization.

MATERIALS AND METHODS

This work was carried out in the Plant Tissue Culture Laboratory, Plant Biotechnology Department of the Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Minufiya University Egypt, during the period 2005-2008.

The present work was carried out to study the influence of some complex addenda i.e. Tryptone, yeast extract, casein hydrolysate and pineapple extract on *in vitro* growth of date palm (*Phoenix dactylifera.* L.) dry cultivar, i.e. Malakaby during micropropagation stages:

1- Repetitive somatic embryos cycle.
2- Plantlets formation and acclimatization.

Plant materials:

In this work, regenerated somatic embryos of cv. Malakaby, obtained during the first part of this work (Nasr *et al.*, 2008) were utilized.

Medium composition:

The regenerated embryos clusters were cultured on MS (Murashige and Skooge, 1962) modified medium (MMS). The MMS medium (Nasr *et al.*, 2008), with the modification of such nutrients i.e. asparagen (100 mg/l), bioten (0.5 mg/l), thiamine-HCl (5 mg/l), Ca-pantothenate (2.5 mg/l), a ascorbic acid (75 mg/l), citric acid (75 mg/l), polyvinylpyrrolidone (1.5 g/l), NaH2PO4. 2H2O (170 mg/l asparagen (100 mg/l), bioten (0.5 mg/l), thiamine-HCl (5 mg/l), Ca-pantothenate (2.5 mg/l), a ascorbic acid (75 mg/l), citric acid (75 mg/l), polyvinylpyrrolidone (1.5 g/l), NaH2PO4. 2H2O (170 mg/l) and sucrose (40 g/l). The pH of all media was adjusted to 5.6 with 0.1 M KOH or 0.1 M HCl prior to addition of gelling agent. Media were dispensed either in a test tubes (20 x 2.5 cm) capped with Bellco caps containing 15 ml or in jars containing 50 ml and autoclaved at 121°C and 1.1 Kg/cm² for 20 min.

Effect of complex addenda (tryptone, yeast extract, casein hydrolysate and pineapple extract) treatments on:

A-Repetitive somatic embryos cycle.

Somatic embryos clusters were cultured on MMS medium supplemented with NAA (0.1 mg/l), kin (0.5), activated charcoal (1.5 g/l) and gelrite (1.5 g/l). The superior medium resulted from the first part of this study for induce repetitive embryos cycle. In addition to different concentrations of the following complex addenda [Duchefa Biochemicals Brand, (g/l)] treatments:-

Exp. 1: Tryptone (T1332) [0, 1, 1.5 and 2 g/l].
Exp. 2: Yeast extracts (Y1333) [0, 0.5, 1, 1.5 and 2 g/l].
Exp. 3: Casein hydrolysate (C1301) [0, 0.5, 1, 1.5 and 2 g/l].
Exp. 4: Pineapple extract (P1364) [0, 6, 9 and 12 g/l].

Cultures were incubated at 27 ± 1°C for 16-h photoperiod with light intensity of 1500 lux using fluorescent tubes. Nine culture jars (replicates) were used. After three weeks, the number, multiplication rate, fresh weight and growth value of embryos were recorded.

B- Plantlets formation and acclimatization.

Shootlets obtained from each previous treatment were subjected individually to the MS basal medium supplemented with NAA (0.5 mg/l) in combination with IBA (0.5 mg/l), the superior combination of the selected growth regulators, tested for rooting stage in the first part of this study. In addition to the same different concentrations of the complex addenda (g/l) previously mentioned.

Cultures were incubated under the same embryos formation conditions except raised light intensity to 3000 Lux. Nine culture tubes (replicates) were used. After eight weeks, the number of leaves, leaf length (cm), shootlet fresh weight (g) and growth value were recorded.

Acclimatization:

Healthy rooted plantlets produced after two months in the rooting medium were transplanted for acclimatization procedure. Fifteen Plantlets (replicates) from each treatment were acclimatized *ex vitro* on soil type contain a mixture of compost + perlite (1:1, v/v) for three months under similar conditions as previously mentioned for plantlets acclimatization in the first part of this study. Then survival percentage was recorded after 3 months.

Growth value:

Embryo and shootlet growth values were estimated according to the equation of Ziv (1992).

$$GV = \frac{Fw_F - Fw_i}{Fw_i}$$ Where

GV = Growth value. Fwf = Final explant fresh weight. Fwi = Initial explant fresh weight.

Statistical analysis:

Data of all the studied experiments were statistically analyzed by one factorial randomized complete design using the SAS (1988) package. The Least Significant Difference among levels of each treatment was compared using L.S.D test at 5%, according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Effect of complex addenda(tryptone, yeast extract, casein hydrolysate and pineapple extract) concentrations on:

A-Repetitive somatic embryos cycle.

Since the ultimate success of micropropagation, depends on the ability to transfer *in vitro* plantlets out to the *ex vitro* conditions. This was depending primarily upon the quality and type of materials produced in the previous stages. (Hegazy *et al.*, 2006). During transfer date palm plantlets dry cv. Malakaby from the *in vitro* to the *ex vitro* conditions, a high percentage of plantlets lost (Nasr *et al.*, 2008). This could be because of necessitating nutrients needed to satisfy the partial fulfillment of the requirements for micropropagation stages.

1- Tryptone

Concerning the effects of complex addenda on embryo growth characters during the repetitive embryo stage, the obtained data (Table 1) for tryptone treatments on Malakaby cultivar revealed that addition of tryptone at the concentration of 1.0 g/l to the repetitive embryo culture medium recorded significantly higher number of embryos and multiplication rate and higher fresh weight and growth value as compared with the other concentration treatments, i.e. 0.0, 1.5 and 2.0 g/l. From the obtained results, it could be noticed that all tryptone treatments recorded high growth value compared with the control. On the other hand, increase tryptone concentration gradually decreased growth value. This may be due to unsuitable types of protein

contained which accompanied by growth inhibition gradually with increased concentration. Contradictory, it could be suitable for another crop like orchid which affirmable that, its crop dependent. In this regard, Fonesbech (1972) reported that protocorms of *Cymbidium* (Orchidaceae) were grown on media containing amino acid mixtures casamino acids (casein hydrolysate) and tryptone increased growth while yeast extract was inhibitory and malt extract without effect. Optimal concentrations were 2 to 3 g · l^{-1} casamino acids and 3 to 4 g · l^{-1} tryptone. Amaki and Higuchi (1989) reported that protocorm-like bodies (PLB) obtained through *in vitro* culture of seedling leaf segments of *Phalaenopsis cv.* Surfrider X *(Phalaenopsis cv.* Joseph Hampton X *Doritaenopsis cv.* Kaala Gleam) were cultured on media containing peptone or tryptone at various concentrations. The addition of either protein to the culture medium promoted growth of PLB. Maximum stimulation was obtained when 2 g/l of tryptone was added.

Table (1): Effect of tryptone concentrations on multiplication rate, fresh weight (g) and growth value of date palm embryos cv. Malakaby cultured *in vitro* for 3 weeks.

Treatment	Growth character					
	No. of embryos		Multip. rate	Embryos F.wt (g)		Growth value
	Starting	Produced		Starting	Produced	
Tryptone (g/l)						
0	22.67 ^{a}	37.22 ^{d}	1.67 ^{d}	3.55 ^{a}	6.55 ^{c}	0.86 ^{b}
1	23.44 ^{a}	108.44 ^{a}	4.66 ^{a}	3.55 ^{a}	10.31 ^{a}	1.90 ^{a}
1.5	19.44 ^{b}	82.67 ^{b}	4.24 ^{b}	3.52 ^{a}	9.64 ^{ab}	1.73 ^{a}
2	21.33 ^{ab}	72.89 ^{c}	3.44 ^{c}	3.39 ^{a}	9.12 ^{b}	1.69 ^{a}

Means within each column followed by the same letter are not significantly different at P= 0.05 according to the LSD test.

2- Yeast extract

Data presented in Table (2) showed that addition of yeast extract at the concentration of 1.5 g/l to the repetitive embryo culture medium had significantly higher values of number of embryos as well as higher values of embryos multiplication rate, fresh weight and growth value as compared with the other yeast extract concentration treatments, i.e. 0.0, 0.5, 1.0 and 2.0 g/l. On the other hand, high yeast extract concentration 2.0 g/l significantly reduced number of embryos, embryos multiplication rate, fresh weight and its growth value. In this regard, Pierik (1987) mentioned that some natural products were added to nutrient medium. These mixtures are very complex and contain vitamins as well as amino acids. Yeast extract according to the LSD test.(0.25- 2.0 g/l) is normally used because of its high contained of vitamins B. The organic supplements (particularly amino acids) have been especially beneficial for growth or morphogenesis when cells or tissues were cultured on White's medium, which do not contain ammonium ions. When both NO3 and NH4 are present in the medium, the response to organic nitrogen depends on the ratio of these two ions (George, 1993). Also, Guidin and Harada (1974) reported that the presence of yeast extract in artichoke culture medium stimulated explants growth. However, Abd El-Aal (2008)

reported that addition of yeast extract had stimulatory effect on *Hyoscyamus muticus* callus growth and significantly improved the alkaloid content. In addition, Maksoud (2007) found that culture medium supplemented with yeast extract (5 mg/l) improved the growth of *Ocimum bacilicum* and increased rosmarinic acid content as more than three fold. On contrast, Fonesbech (1972) found that addition of yeast extract was inhibitory and malt extract without any effect in the protocorms of *Cymbidium* medium. Moreover, Vij *et al.* (1984) found that addition of casein hydrolysate enhanced the development of protocorm like bodies *of Rhynchostylis retusa* compared with yeast extract.

Table (2): Effect of yeast extract concentrations on multiplication rate, fresh weight (g) and growth value of date palm embryos cv. Malakaby cultured *in vitro* for 3 weeks.

Treatment	Growth character					
	No. of embryos		Multip. rate	Embryos F.wt (g)		Growth value
	Starting	Produced		Starting	Produced	
Yeast extract (g/l)						
0	22.67 ^{ab}	37.22 ^{c}	1.67 ^{c}	3.55 ^{ab}	6.55 ^{d}	0.86 ^{d}
0.5	19.56 ^{b}	38.89 ^{c}	2.01 ^{b}	3.40 ^{b}	7.97 ^{c}	1.35 ^{c}
1	22.67 ^{ab}	51.22 ^{b}	2.26 ^{ab}	3.49 ^{ab}	9.70 ^{ab}	1.79 ^{ab}
1.5	25.11 ^{a}	61.78 ^{a}	2.46 ^{a}	3.61 ^{ab}	10.52 ^{a}	1.92 ^{a}
2	20.67 ^{b}	31.22 ^{c}	1.56 ^{c}	3.66 ^{a}	9.07 ^{bc}	1.49 ^{bc}

Means within each column followed by the same letter are not significantly different at P= 0.05

3- Casein hydrolysate

Data presented in Table (3) indicated that addition of casein hydrolysate at the concentration of 1.5 g/l to the repetitive embryo culture medium recorded significantly high values of embryo number as well as high values of embryos multiplication rate, fresh weight and growth value as compared with the other casein hydrolysate concentration treatments, i.e. 0.0, 0.5, 1.0 and 2.0 g/l. On the other hand, high casein hydrolysate concentration 2.0 g/l gradually decreased number of embryos, embryos multiplication rate, fresh weight and its growth value. In this concern, (Pierik, 1987) reported that amino acids can be added to plant media to satisfy the requirement of cultures for reduced nitrogen, but as they are expensive to purchase, they will only be used in media for mass propagation. Casein hydrolysate is one of the most important natural products added to plant tissue culture medium. It is a mixture of compounds in particular amino acids manufactured from casein. Meanwile, Gebhardt and Friedrich (1987) illustrated that addition of casein hydrolysate (1.0 g/l) to shoot tips proliferation medium of *Calluna vulgaris* enhanced shoot proliferation and rooting. Also, Davis *et al.* (1977) found that, CH significantly enhanced carnation shoot growth at the concentration of 3 g/l. In addition, Fiola and Swartz (1986) found that, CH had a promotive effect on blackberry regenerated embryos. Addition of casein hydrolysate at the concentrations (400 or 600 mg/l) to the strawberry regeneration medium increased the percentage value of organogenesis and number of shoots per leaf section

(Hassen, 1996). El-Shamy (2000) found that addition of casein hydrolysate with 2 and 4 g/l to Bougainvillea shoot tips medium recorded the highest number of axillary shoots (3.8 and 3.9 shoots / explant, respectively. Vij *et al.* (1984) found that addition of casein hydrolysate enhanced the development of *Rhynchostylis retusa* protocorm like bodies. Ochatt (1991) reported that *Loncera nitida* explant when cultured on MS medium contained casein hydrolysate (250 mg/l) promoted rhizogenesis. Choudarry (1991) cultured carnation cv Scania Red shoot tips on B5 medium supplemented with casein hydrolysate (50 mg/l) and obtained the highest shoot number. Radojevic and Subotic (1992) published that embryogenic callus formation of *Iris retosa* could be induced on MS medium contained casein hydrolysate (1 mg/l). Zayed (2000) reported that *Spathephillum* culture medium contained casein hydrolysate at 200 mg/l or yeast extract at 100 mg/l recorded the highest number of shoots and leaves per explant. Most of the inorganic nitrogen supplied in culture media is converted by plant tissues to amino acids, which are then assimilated into proteins; it should be possible to culture plants on media on in which amino acids are the only nitrogen source. Amino acids provide according to the LSD test. plant cells with an immediately available source of nitrogen, and uptake can be much more rapid than that of inorganic nitrogen in the same medium (George, 1993).

4- Pineapple extract

Concerning the effects of pineapple on embryo growth characters during the repetitive embryo stage, the obtained data (Table 4) and (Fig. 1-A) revealed that addition of pineapple at the concentration of 9 g/l to culture medium recorded the highest number of embryos and multiplication rate and the highest values of fresh weight and growth value as compared with the other studied treatments. From the obtained results, it could be noticed that all pineapple treatments recorded the highest number of embryos, multiplication rate, fresh weight and growth value as compared with the control. Generally in both pineapple extract concentrations 6 and 9 g/l, it could be noticed that they produced the highest numbere of embryos, multiplication rate and highest fresh weight and consequently superiority of its growth value as compared with the other studied pineapple extract concentrations 0 and 12 g/l. Pineapple powder or juice is a natural products containing amino acid and vitamins. In this regard, Edenharder *et al.* (1994) studied *in vitro* effect of vegetable and fruit juices on the mutagenicity. They found that strong antimutagenic activities were detected in bananas, blackberries, blueberries, sweet and sour cherries, blackcurrants and redcurrants, pineapple according to the LSD test and watermelon. Moderate antimutagenic activities were detected in greengage, kiwi, mangos, honeydew melons and plums. Weak antimutagenic activities were detected in apple, apricot, pears, peaches and strawberries, whereas white and red grapes and raspberries were inactive, and gooseberries and citrus fruits in general possessed marginal or no antimutagenic activities. When fruit and vegetable juices were heated, a considerable reduction of antimutagenic potencies was seen with apple, apricot, kiwi, pineapple, beets, cabbage (Chinese, Savoy, red and white), cauliflower, leafy lettuce,

cucumber, onions, radish and rhubarb. Pineapple and celeriac juices inhibited the enzymatic system responsible for the activation of IQ, but had no desmutagenic activity. Peroxidase activity found to be present in broccoli, cauliflower, green beans and tomatoes may contribute to antimutagenic activities in these vegetables. In this concern, Duke (1985) reported that pineapple is rich in citric and malic acids; citric acid concentrations in some cultivars exceed 8%. The fruit also contains moderate amounts of ascorbic acid; 2 slices of pineapple contain ascorbic acid 100 mg. A steroidal component of the leaves possesses estrogenic activity, and a variety of aromatic compounds are found in the essential oil. The residue left after juice extraction contains large quantities of vitamin A and is used as a component of livestock feed. A crude, aqueous extract of pineapple, known as bromelain, is obtained from the stems and immature fruits; stem and fruit bromelains may be distinguished from each other. Bromelain comprises a complex mixture of sulfhydryl-containing proteolytic enzymes in addition to a number of nonspecific components such as phosphatases, glucosidases, peroxidases, cellulases, glycoproteins, and carbohydrates. The extract also contains a proteinase inhibitor consisting of 8 isoinhibitors. Each isoinhibitor has a 2-chain structure, and the amino acid sequence has been determined. In aqueous solution, bromelain rapidly deteriorates through self-digestion. Commercial bromelain preparations are evaluated according to their proteolytic activity.

Table (4): Effect of pineapple extract concentrations on multiplication rate, fresh weight (g) and growth value of date palm embryos cv. Malakaby cultured *in vitro* for 3 weeks.

Treatment	Growth characters					
	No. of embryos		Multip. rate	Embryos F.wt (g)		Growth value
	Starting	Produced		Starting	Produced	
Pineapple extract (g/l)						
0	22.67 ^{a}	37.22 ^{c}	1.67 ^{c}	3.55 ^{a}	6.55 ^{c}	0.86 ^{c}
6	18.56 ^{b}	68.11 ^{ab}	3.98 ^{ab}	3.40 ^{a}	13.53 ^{a}	2.96 ^{a}
9	17.44 ^{b}	76.22 ^{a}	4.33 ^{a}	3.43 ^{a}	14.43 ^{a}	3.21 ^{a}
12	16.11 ^{b}	64.00 ^{b}	3.75 ^{b}	3.39 ^{a}	11.85 ^{b}	2.50 ^{b}

Means within each column followed by the same letter are not significantly different at P= 0.05

Effect of complex addenda(tryptone, yeast extract, casein hydrolysate and pineapple extract) concentrations on:

B- Plantlets formation and acclimatization.

1- Tryptone

The results presented in Table (5) indicated that media free tryptone were recorded higher significant values of growth value and no. of roots as well as higher survival percentage in acclimatization as compared with all tryptone concentration treatments, i.e. 1.0, 1.5 and 2 g/l. Comparing the results of no. of leaves and shootlets fresh weight under all aforementioned treatments; it was found that no significant difference could be obtained among them, whereas a gradually decreased obtained in growth value and no. of roots as well as survival percentage in acclimatization specially with

tryptone at high concentration 2.0 g/l. Generally, it appears that increase the level of tryptone concentration decreased survival percentage comparing with those produced under the control, which reflect that there is no beneficial effect from tryptone addition treatments. This may be due to unsuitable types of protein contained which accompanied by growth inhibition before in the repetitive embryos stage gradually affected embryo growth and development and subsequently plantlets survival percentage in acclimatization and severed injury in high concentration. In Contradictory, it could be suitable for another crop. In this regard, Fonesbech, (1972) reported that protocorms of Cymbidium grown on media containing amino acid mixtures casamino acids (casein hydrolysate) and tryptone increased growth. Optimal concentrations were 2 to 3 g · l-1 casamino acids and 3 to 4 g · l-1 tryptone. In this concern, researchers trying to optimized those conditions. Amaki and Higuchi (1989) promoted growth of protocorm-like bodies (PLB) obtained through *in vitro* culture of seedling leaf segments of *Phalaenopsis cv.* Surfrider X *Phalaenopsis cv.* Joseph Hampton X *Doritaenopsis cv.* Kaala Gleam) using media containing peptone or tryptone at various concentrations. *In vitro* conditions so far reflect their effect on plantlets survival *ex vitro*. However, Al-Salih *et al.* (1986) suggested that, success or fail of transferred plantlets to greenhouse are dependent primarily upon the quality and type of materials produced in the previous stages of *in vitro* propagation. In this regard, Hogberg *et al.* (2003) suggested that a combined selection for somatic embryo plants with lateral roots and with an epicotyls length exceeding 8 mm resulted in taller plants and reduced intraclonal variation for 13 micropropagated *Picea abies* L. clones. Hegazy (2003) reported that the optimal conditions for successful transfer of *in vitro* regenerated date palm plantlets to *ex vitro* conditions; it was supposed to get firstly healthy plantlets with a good looking for root and shoot system, secondly, reduced the relative humidity in the culture medium during the later stage of plantlets formation could be beneficial for preparing mild stressful atmosphere for plantlets to be well adapted during acclimatization, thirdly, the soil culture type, where plantlets are cultivated.

Table (5): Effect of tryptone concentrations on growth and development as well as survival percentage in acclimatization of date palm shootlet cv. Malakaby cultured *in vitro* for 8 weeks

Treatment	Growth character						
	No. of leaves	Leaf length (cm)	Shootlet F. w (g)		Growth value	No. of roots	Survival % in acclimatization
			initial	Final			
Tryptone (g/l)							
0.0	2.56 ^{a}	8.44 ^{b}	0.38 ^{b}	1.66 ^{a}	3.39 ^{a}	2.00 ^{a}	40.00 ^{a}
1.0	2.78 ^{a}	11.22 ^{a}	0.93 ^{a}	2.16 ^{a}	1.43 ^{bc}	1.56 ^{b}	26.67 ^{ab}
1.5	3.67 ^{a}	10.44 ^{ab}	0.98 ^{a}	1.83 ^{a}	0.89 ^{c}	1.34 ^{b}	13.33 ^{ab}
2.0	3.56 ^{a}	9.22 ^{ab}	0.81 ^{a}	1.91 ^{a}	1.72 ^{b}	1.23 ^{b}	6.67 ^{b}

Means within each column followed by the same letter are not significantly different at P= 0.05 according to the LSD test.

Yeast extract

Data presented in Table (6) indicated that addition of yeast extract at the concentration of 1.5 g/l to the rooting medium had recorded higher no. of leaves, leaf length, no. of roots and the highest survival percentage in acclimatization as compared with other yeast extract concentration treatments, i.e. 0.0, 0.5, 1.0 and 2 g/l. On the other hand, there is no significant difference could be noticed among all yeast extract concentrations for shootlet fresh weight meanwhile; they recorded lower significant growth value as compared with the control. Generally, it appears that increase the level of yeast extract concentration up to 2.0 g/l was recorded the lowest survival percentage as compared with the other studied treatments. Yeast extract in culture medium stimulated the growth of explants. In this concern, Pierik (1987) mentioned that yeast extract is used because of the high quality of B vitamins. Guidin and Harada (1974) reported that the presence of yeast extract in culture medium stimulated artichoke explants growth. Maksoud (2007) found that culture medium supplemented with yeast extract (5 mg/l) improved the growth of *Ocimum bacilicum* and increased rosmarinic acid content as more than three fold.

Table (6): Effect of yeast extract concentrations on growth and development as well as survival percentage in acclimatization of date palm shootlet cv. Malakaby cultured *in vitro* for 8 weeks.

Treatment	Growth charachter						
	No. of leaves	Leaf length (cm)	Shootlet F.wt (g)		Growth value	No. of roots	Survival % in acclimatization
			Initial	Final			
Yeast extract (g/l)							
0	2.56 ^{b}	8.44 ^{ab}	0.38 ^{b}	1.66 ^{a}	3.39 ^{a}	2.00 ^{b}	40.00^{a}
0.5	2.22 ^{b}	8.11 ^{ab}	0.84 ^{ab}	1.96 ^{a}	1.45 ^{b}	2.56 ^{ab}	40.00^{a}
1	2.89 ^{ab}	8.89 ^{ab}	0.90 ^{a}	2.25 ^{a}	1.48 ^{b}	2.67 ^{ab}	53.33^{a}
1.5	3.56 ^{a}	10.33 ^{a}	0.91 ^{a}	2.15 ^{a}	1.74 ^{b}	3.22 ^{a}	66.67^{a}
2	2.56 ^{b}	7.33 ^{b}	0.87 ^{a}	1.82 ^{a}	1.37 ^{b}	2.22 ^{b}	33.33^{a}

Means within each column followed by the same letter are not significantly different at P= 0.05 according to the LSD test.

2- Casein hydrolysate

Data presented in Table (7) indicated that addition of casein hydrolysate at the concentration of 1.5 g/l to the rooting medium had recorded higher significant number of roots and the highest survival percentage in acclimatization as compared with other casein hydrolysate concentration treatments, i.e. 0.0, 0.5, 1.0 and 2 g/l. On the other hand, there is no significant difference could be noticed among all casein hydrolysate concentrations for shootlet fresh weight. Results indicated that casein hydrolysate application at low concentrations encourage number of leaves and leaf length. Generally, it appears that increase the level of casein hydrolysate concentration significantly decreased growth value as compared with the control. In this concern, casein hydrolysate can be used as a source of a mixture of amino acids. The organic supplements (particularly amino

acids) have been especially beneficial for growth or morphogenesis when cells or tissues were cultured on media such as White medium which do not contain ammonium ions. When both NO3 and NH4 are present in the medium, the response to organic nitrogen depends on the ratio of these two ions (George, 1993). Casein hydrolysate is one of the most important of natural product added to plant tissue culture medium. It is a mixture of compounds in particular amino acids manufactured from casein (Pierik, 1987). In addition, Davis *et al.* (1977) mentioned that CH significantly enhanced carnation shoot growth at 3 g/l. Also, Fiola and Swartz (1986) found that CH had a promotive effect on regeneration from blackberry embryos. The addition of casein hydrolysate at different concentrations (400 or 600 mg/l) to the regeneration medium increased the percentage of organogenesis and number of shoots per leaf section for strawberry plants (Hassen, 1996). El-Shamy (2000) found that addition of casein hydrolysate with 2 and 4 mg/l to Bougainvillea shoot tips medium recorded the highest number of axillary shoots (3.8 and 3.9 shoots / explant, respectively.

Table (7): Effect of casein hydrolysate concentrations on growth and development as well as survival percentage in acclimatization of date palm shootlet cv. Malakaby cultured *in vitro* for 8 weeks.

Treatment	Growth character						
	No. of leaves	Leaf length (cm)	Shootlet F. w (g)		Growth value	No. of roots	Survival % in acclimatization
			initial	Final			
Casein hydrolysate (g/l)							
0	2.56 ^{b}	8.44 ^{b}	0.38 ^{b}	1.66 ^{a}	3.39 ^{a}	2.00^{b}	40.00^{a}
0.5	2.56 ^{b}	10.00 ^{ab}	0.92 ^{a}	1.69 ^{a}	0.90 ^{b}	1.67^{bc}	26.67^{a}
1	3.89 ^{a}	12.33 ^{a}	0.86 ^{a}	2.01 ^{a}	1.40 ^{b}	1.89^{b}	46.67^{a}
1.5	2.44 ^{b}	8.56 ^{b}	0.95 ^{a}	1.91 ^{a}	1.67 ^{b}	2.44^{a}	53.33^{a}

Means within each column followed by the same letter are not significantly different at P= 0.05 according to the LSD test.

4- Pineapple extract

The results presented in Table (8) and Fig (1- B, C, D and E) indicated that addition of pineapple extract at the concentration of 9 g/l to the rooting medium were recorded higher number of leaves, leaf length, fresh weight, number of roots and the highest plantlets survival percentage in acclimatization as compared with the other pineapple concentration treatments, i.e. 0, 6 and 12 g/l. On the other hand, it appears that increase the level of pineapple concentration significantly decreased growth value as compared with the control. In this respect Edenharder *et al.* (1994) mentioned that pineapple juice inhibited the enzymatic system responsible for the activation of IQ, but had no desmutagenic activity. When they studied *in vitro* effect of vegetable and fruit juices on the mutagenicity of 2-amino-3-methylimidazo [4,5-f]quinoline,2-amino-3,4-dimethylimidazo[4,5-f]quinoline and 2-amino-3,8- dimethylimidazo[4,5-f]quinoxaline. It may be reduced somaclonal variation during date palm propagation *via* indirect embryogenesis.

Table (8): Effect of pineapple extract concentrations on growth and development as well as survival percentage in acclimatization of date palm shootlet cv. Malakaby cultured *in vitro* for 8 weeks

Treatment	Growth character						
	No.of leaves	Leaf length (cm)	Shootlet F.w (g)		Growth value	No. of roots	Survival % in acclimatization
			Initial	Final			
Pineapple extract (g/l)							
0	2.56 ^{b}	8.44 ^{a}	0.38 ^{b}	1.66 ^{a}	3.39 ^{a}	2.00 ^{b}	40.00^{a}
6	2.89 ^{ab}	7.56 ^{a}	0.92 ^{a}	1.84 ^{a}	1.98 ^{b}	2.56 ^{ab}	46.67^{a}
9	3.67 ^{a}	10.11 ^{a}	1.05 ^{a}	2.19 ^{a}	1.15 ^{b}	3.11 ^{a}	73.33^{a}
12	2.89 ^{ab}	8.94 ^{a}	1.03 ^{a}	1.86 ^{a}	0.77 ^{b}	3.11 ^{a}	60.00^{a}

Means within each column followed by the same letter are not significantly different at P= 0.05 according to the LSD test.

Complex addenda produced amino acids (namely casein hydrolsate - yeast extract - pineapple - malt extract), peptides glutathione or amide glutamine can be added to plant media to satisfy the requirement of cultures for reduced nitrogen. The response to organic nitrogen depends on the ratio of NO3 to NH4. Amino acids provide plant cells with an immediately available source of nitrogen and uptake can be much more rapid than that of inorganic nitrogen in the same medium. Amino acids can also provide reduced nitrogen in culture media in place of NH4 and as a supplement to NO3.However they are usually employed as minor addition to media containing both NH4 and NO3. Uptake of amino acids into cultured tissues causes a decrease in the pH of the medium (George, 1993).

Conclusion

Effect of complex addenda(tryptone, yeast extract, casein hydrolysate and pineapple extract) concentrations on:

1: Repetitive somatic embryos cycle.

Somatic embryo clusters cultured on MS (Murashige and Skooge, 1962) modified medium (MMS) supplemented with NAA (0.1 mg/l), kin (0.5), activated charcoal (1.5 g/l) and gelrite (1.5 g/l) in addition to tryptone at the concentration of 1.0 g/l recorded high significant number of embryos and multiplication rate, also high fresh weight and growth value. However, addition of yeast extract at the concentration of 1.5 g/l recorded higher significant number of embryos as well as high embryos multiplication rate, fresh weight and growth value. Also, addition of casein hydrolysate at the concentration of 1.5 g/l recorded high significant number of embryos as well as high embryos multiplication rate, fresh weight and growth value. While, addition of pineapple extract at the concentration of 9 g/l recorded high significant value of all growth characters as compared with the control. On the other hand, it recorded the highest no. of embryos, multiplication rate, fresh weight and growth value as compared with the other pineapple concentration treatments i.e. 6 and 12 g/l.

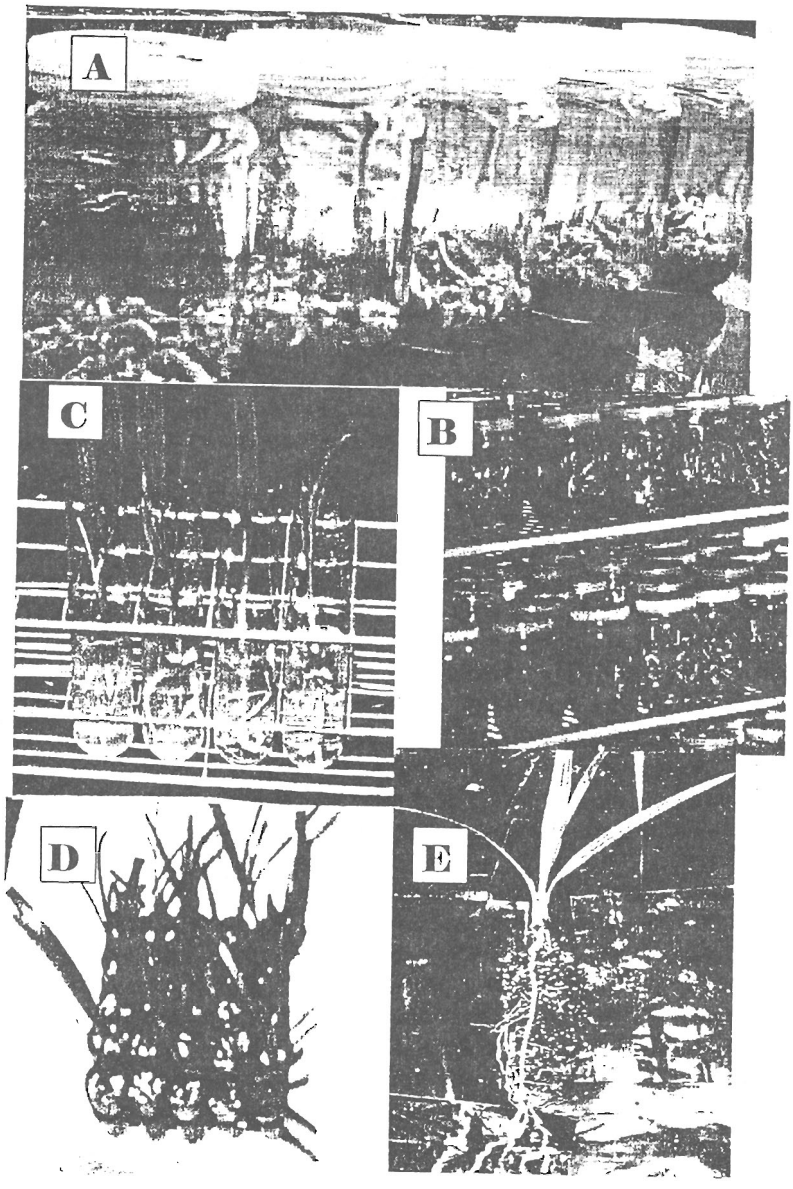

Fig (1): Developmental stages of date palm cv. Malakaby in micropropagation:

A - Repetitive somatic embryos during multiplication stage

B- Well developed shootlets during elongation stag.

C- Well-rooted plantlets in rooting stage.

D- Plantlets in soil mixture of compost + perlite (1:1, v/v) during acclimatization.

E- Plantlets with healthy obtained roots

2: Plantlets formation and acclimatization.

Shootlets obtained from each previous treatment were subjected individually to the MS basal medium supplemented with NAA (0.5 mg/l) in combination with IBA (0.5 mg/l) in addition to tryptone at the concentration i.e. 0, 1, 1.5 and 2 g/l decreased all morphological characters and declined plantlets survival (40, 26.67, 13.33 and 6.67 %) respectively. However, addition of yeast extract at the concentration of 1.5 g/l to the rooting medium recorded higher no. of leaves, leaf length, no. of roots and the highest survival in acclimatization (66.67 %). While, addition of casein hydrolysate at the concentration of 1.5 g/l to the rooting medium recorded higher significant values of no. of roots and the highest survival in acclimatization (53.33 %). On the other hand, addition of pineapple extract at the concentration of 9 g/l to the rooting medium recorded higher values of no. of leaves, leaf length, fresh weight, no. of roots and the highest plantlets survival in acclimatization (73.33 %).

REFERENCES

Abd El-Aal, A.A. (2008). Studies on production of some compounds from *Hyoscyamus muticus* using tissue culture technique. M Sc. Thesis. GEBRI. Minfiya Univ., Egypt.

Al-Khayri, J. M. and Al-Bahrany, A. M. (2001). Silver nitrate and 2-isopentyladenine promote somatic embryogenesis in date palm (*Phoenix dactylifera L.*). Sci. Hort., 89: 4, 291-298.

Al-Salih, A. A.; Bader, S. M.; Jarrah, A. Z. and Al-Qadi, M. T. (1986). A comparative morphological and anatomical study of seed and embryo culture-derived seedling of *Phoenix dactylifera L.* Date Palm J., 4: 153-162.

Amaki, W and Higuchi, H (1989). Effects of dividing on the growth and organogenesis of protocorm-like bodies in Doritaenopsis. Sci. Hort., 39(1): 63-72.

Choudhary, M. L. (1991). Rapid propagation of Indian breed rose cultivar *Prigadarshini* by axillary bud proliferation. Progressive Horti, 22:168-172.

Davis, M. J.; Baker, R. and Hanan, J. J. (1977). Clonal multiplication of carnation by micropropagation. J. Amer. Soc. Hort. Sci., 102:48-53.

Duke, J. A. (1985). Handbook of Medicinal Herbs. Boca Raton, FL: CRC Press.

Edenharder, R.; Kurz, P.; John, K.; Burgard, S.; Seeger, K. (1994). *In vitro* effect of vegetable and fruit juices on the mutagenicity of 2-amino-3-methylimidazo[4,5-f]quinoline,2-amino-3,4-dimethylimidazo[4,5-f]quinoline and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline. Food and Chemical Toxicology. 1994; 32(5): 443-459.

El-Shamy, H, A. (2000). *In vitro* culture studies on *Bougainvillea* plant. Ph. D. Thesis. Fac. Agric. Zagazig Univ., Egypt.

Fiola, J. A. and Swartz, H. J. (1986). Somatic embryogenesis, organogenesis and proliferation *in vitro* from *Rubus* embryos.Acta Hort.Sci.,183:91-98.

Fonesbech, M. (1972). Organic nutrients in the media for propagation of *Cymbidium in vitro*. Physiol. Plant., 27 (3): 360-364.

Gebhardt, K. and Friedrich, M. (1987). Micropropagation of *Calluna vulgaris* cv. *Beale*. Plant Cell, Tissue and Organ Culture, 29: 137-145.

George, E. F. (1993). Plant Propagation by Tissue Culture. Exegetics Ltd., Edington,Wilts. BA134Q G, England.

Gudin, C. and Harada, H. (1974). Proliferation *in vitro* of Jerusalem artichoke tissue caused by *Rhodotorula glutinis*. Comptes Rendus Hebdomadaire des Seances de I Academic des Sci., 287: 1853-1854.

Hassan, M. A. (1996). *In vitro* shoot regeneration from strawberry leaf tissues. Zagazig J. Agric. Res., 23: 101-113.

Hegazy, A. E. (2003). Some physiological studies on date palm micropropagation through direct somatic embryogenesis. Ph. D. Thesis. Plant Physiol. Dep. Fac. of Agri. Cairo Univ. Egypt.

Hegazy, A. E; Nesiem, M. R. A.; Ibrahim, I. A. and EL-Ghamrawy, N. K. (2006). Direct somatic embryos of date palm II- Acclimatization and genetic stability. The 3th International Date Palm Conference. Abu Dhabi, United Arab Emirates; February, pp 39.

Hogberg, K. A.; Bozhkov, P. V. and Von-Arnold, S. (2003). Early selection improves clonal performance and reduces intraclonal variation of Norway spruce plants propagated by somatic embryogenesis. Tree Physiol., 23 (3): 211-216.

Maksoud, A. I. (2007). Studies on the production of pharmaceutical compounds through tissue culture techniques. M.Sc. Thesis. GEBRI. Minufiya Univ., Egypt.

Murashige, T. and Skoog, F. A. (1962). A revised medium for rapid growth and bioassays
with tobacco tissue cultures. Physiol. Plant., 15: 433-479.

Nasr, M. I.; Ibrahim,, I. A.; Gergess, A. A.; Hegazy, A.E.and El-Bastawissy, H. H. (2008). Micropropagation of date palm cv. Malakaby through embryogenesis: 1- Effect of plant growth regulators. J. Product & Dev., 13(3): 637- 650.

Ochatt, S. J. (1991). Requirements for plant regeneration from protoplast of the shrubby ornamental honeysuckle, *Lonicera nitida* cv.Maigrum. Plant Cell, Tissue and Organ Culture, 25: 161-167.

Pierik, R. L. M. (1987). *In vitro* Culture of Higher Plants. Martinus Nijhoff Publishers, Dordrecht. Netherlands.

Radojevic, L. and Subotic, A. (1992). Plant regeneration of *Iris setosa* Pall. Through somatic embryogenesis and organogenesis. J. Plant Physiol., 139: 960-966.

SAS (1988). Statistical Analysis System SAS User's Guide: Statistics SAS Institute Inc., Cary, N. S.

Steel, R. G. and Torrie, J. H. (1980). Principles and Procedures of Statistics, a Biomerical Approach. Mc Grow- Hill Book Company, New York, pp 469-517.

Vij, S. P.; Sood, A. and Plaha, K. K. (1984). Propagation of *Rhynchostylis retusa* by direct organogenesis from leaf segment cultures. Botanical Gazette, 145: 210-214.

Zayed, E. M. M. (2000). *In vitro* propagation of *Spathiphyllum.* M. Sc. Thesis, Fac. of Agric., Cairo Univ.

Ziv, M. (1992). The use of growth retardants for the regulation and acclimatization of *in vitro* plants. In: Karssen, C. M.; Van loon, L. C. and vreugdenhil, D. (eds). Progress in Plant Growth Regulation, pp. 809-817.

الإكثار الدقيق لنخيل التمر صنف ملكابى من خلال تكون الاجنة

٣- تاثير المواد الطبيعية مثل التربتون, مستخلص الخميرة, الكازين هيدروليزات ومستخلص الاناناس

عادل أحمد حجازى ، محمود ابراهيم نصر ، ابراهيم عبد المقصود و حامد البسطويسى

معهد الهندسة الوراثية والتكنولوجيا الحيوية ــ جامعة المنوفية

تهدف الدراسة إلى معرفة تاثير اضافة احدى المواد الطبيعية مثل التربتون, مستخلص الخميرة, الكازين هيدروليزات ومستخلص الاناناس الى بيئة الزراعة خلال مرحلة التضاعف العددى للاجنة الجسدية ومرحلة التجزير اثناء إكثار نخيل التمر صنف ملكابى معمليا .

مرحلة التضاعف العددى للاجنة:

- اظهرت النتائج أن الاجنة الجسدية المزروعة على بيئة موراشيجى وسكوج ١٩٦٢ المعدلة والمحتوية على اندول حمض الخليك (٠,١ملجم/لتر) والكاينتين (٠,٥ ملجم/لتر) و الفحم النباتى (١,٥ جرام/لتر) والجرلايت (١,٥ جرام/لتر) بالاضافة الى التربتون (١ جم/لتر) قد سجلت اعلى نسبة فى عدد الاجنة ومعدل تضاعفها والوزن الطازج ومعدل النمو بعد ٣ اسابيع من التحضين. كما سجل اضافة مستخلص الخميرة (١,٥ جم/لتر) الى بيئة التضاعف العددى الى اعلى قيمة معنوية فى عدد الاجنة وأعلى تضاعف عددى للاجنة ووزنها الطازج ومعدل نموها. بينما سجل اضافة الكازين هيدروليزات (١,٥ جم/لتر) الى بيئة التضاعف العددى الى اعلى قيمة معنوية فى عدد الاجنة وأعلى تضاعف عددى للاجنة ووزنها الطازج ومعدل نموها. بينما سجل اضافة مستخلص الاناناس (٩ جم/لتر) الى بيئة التضاعف العددى الى زيادة معنوية فى كل قياسات النمو تحت الدراسة بالمقارنة بالكنترول.

مرحلة تكون النبيتات واقلمتها:

- الافرع الخضرية الناتجة من المرحلة السابقة تم زراعتها على بيئة الاساس لموراشيجى وسكوج مع اندول حمض البيوتريك (٠,٥ ملجم/لتر) واندول حمض الخليك (٠,٥ملجم/لتر) وفيتو اجار (٦ جم/لتر) بالاضافة الى نفس التركيزات من المواد تحت الدراسة معمليا ثم نقلت النبيتات الناتجة للاقلمة على التربة المكونة من خليط من الكمبوست مع البيرليت (١:١ حجم/حجم) لمدة ثلاثة أشهر.
- اشارت النتائج ان البيئة المحتوية على التربتون بتركيز ١ ,١,٥و ٢ جم/لتر) ادت الى تقليل جميع قياسات النمو بعد ٨ اسابيع من الزراعة معمليا وتدهور النسبة المئوية لنجاح النبيتات فى الاقلمة الى ٢٦,٦٧ , ١٣,١٣ , ٦,٦٧ على التوالى بالمقارنة بالكنترول (٤٠) بعد ٣ شهور من الأقلمة.بينما سجل اضافة مستخلص الخميرة (١,٥ جم/لتر) أعلى قيمة فى عدد وطول الاوراق وعدد الجذور وقد ظهر ذلك الأثر بوضوح عندما حقق رفع فى النسبة المئوية لنجاح النبيتات في الاقلمة (٢٦,٦٧) أعلى من الكنترول. وقد سجل اضافة الكازين هيدروليزات (١,٥ جم/لتر) أعلى قيمة معنوية فى عدد الجذور وقد ظهر ذلك الأثر بوضوح عندما حقق رفع فى النسبة المئوية لنجاح النبيتات في الاقلمة (١٣,٣٣) أعلى من الكنترول. كما سجل اضافة مستخلص الاناناس (٩ جم/لتر) الى اعلى قيم فى جميع قياسات النمو وقد ظهر ذلك الأثر بوضوح عندما حقق رفع فى النسبة المئوية لنجاح النبيتات في الاقلمة (٣٣,٣٣) أعلى من الكنترول.