Enhancing the Growth of Date Palm (*Phoenix Dactylifera) in Vitro* Tissue by Adding Date Syrup to the Culture Medium

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Abstract:

The present experiments were conducted in the Tissue Culture Lab. of the Date Palm Research Center, King Faisal University, Al-Hassa, Saudi Arabia. Different concentrations of date palm syrup locally known as 'Dibs' (5, 10 and 20%) and sucrose (30 g l^{-1}) as control were used for *in vitro* multiplication of date palm cv Khanezi. Preliminary observations have shown that lower concentration of date syrup (5%) have successfully induced buds and shoots formation. Higher concentrations (10% and 20%) of date syrup, however, have resulted in browning and dryness of plant materials. This phenomenon was probably caused by osmotic stress due to the use of higher date syrup concentration . The results have also indicated that most of the parameters like number of shoots, fresh and dry weights of culture have increased when 5% date syrup was used compared to control (sucrose 30 g l^{-1}).

Based on the results of the first experiment another one was carried out using different concentrations of date syrup that were 1, 2, 3, 4, 5 and 6%. The results have shown that date syrup at concentration of 4 to 6% improved production of bud and shoots. In addition, tissues grown on medium containing 1 to 3% date syrup had the lowest number of buds and shoots. The results indicated that date syrup was taken up from the medium, as shown by the increase in total dry weight of culture. Date palm tissues are capable of utilizing date syrup as the sole carbon source for vegetative growth. Furthermore, date syrup at concentration of 4 to 6% can be used totally as a replacement of 30 g l^{-1} sucrose which was the normal sugar used in most of plant tissue culture. This is the first report on the use of date syrup on the multiplication of date palm cv Khanezi.

Key words:

date palm, *Phoenix dactylifera*, *in vitro*, sucrose, date syrup

Introduction:

Date palm (*Phoenix dactylifera* L.) is the major fruit crop in the Kingdom of Saudi Arabia. Its ability to tolerate arid environmental conditions, made Saudi Arabia quite unique for its cultivation (Ibrahim and Khalif, 1998 and Alkhateeb and Ali-Dinar, 2002). The estimated annual production of the kingdom is 830000 tons from an area of 140000 hectares planted with date palms (FAO, 2004). It is well known that date palm is propagated sexually through seeds and vegetatively by offshoot (Alkhateeb *et al.*, 2006). Tissue culture is a technique mainly used for rapid propagation of several perennial fruit trees including date palm. Date palm is propagated *in vitro* by two methods; the first is by embryogenesis in which vegetative embryos are formed from embryogenic callus. The second is through organogenesis which produces date palm buds that eventually give plantlets without passing through the callus stage (Alkhateeb and Ali-Dinar, 2002).

It is well known in plant tissue cultures a continuous supply of carbohydrates is essential, because the photosynthetic activity of *in vitro* plant tissues is reduced due to low light intensity, high relative humidity and limited gas exchange (Kozai, 1991). Sucrose is the most widely used carbohydrate and carbon source in plant tissue culture as indicated by numerous studies (Hildebrandt and Riker, 1949; Vuke and Mott, 1987; Alkhateeb, 2001, 2007).

It has also been reported that in many plant species adding of plant extracts juice of coconut, tomato, potato, onion, banana, orange, apple, pineapple and yeast to the culture medium enhanced the growth of tissues (Chen *et al*., 2005; Lo *et al*., 2004; He *et al*., 2003; Hong *et al*., 2003; Amo-Marco and Picazo, 1994). Kinnersley and Henderson (1988) found that addition of corn syrup to the basic culture media improved embryogenesis of wild carrot. El-Assar *et al*. (2004) studied the effects of natural extracts of coconut water, date palm merstimatic tissues extract and casein hydrolysate on the growth of date palm cv. Sewi tissue culture grown *in vitro*. They found that date extract was superior to other natural extracts in producing growing tissues which were longer, larger in diameter and more highly coloured.

Although there are data on the effect of different plant extracts on *in vitro* plant culture, no data are currently available for the use of date syrup on date palm tissue culture or other plants. The objective of the present study was to investigate the the possibility of using date syrup locally known as 'Dibs' in tissue culture and to determine the optimum level of date syrup concentration that enhance the *in vitro* growth of date palm cv. Khanezi.

Materials and Methods:

These experiments were conducted in the Tissue Culture Lab. of the Date Palm Research Center, King Faisal University, Al-Hassa, Saudi Arabia. Date palm of cv. Khanezi approximately 3-years-old off-shoots and weighing 5-7 kg were separated from healthy mother palm. Offshoots were thoroughly cleaned and the outer leaves were carefully removed to expose the shoot tips and lateral bud. The exposed regions were excised and immediately placed in antioxidant solution containing 150 mgl^{-1} ascorbic acid and 100 mgl^{-1} citric acid. The shoot tip and lateral buds were sterilized in 20% sodium hypochlorite (domestic bleach) containing 2-3 drops of Tween-20 for 15 min, followed by rinsing 3 times with distilled water.

The shoot tips and lateral buds were sectioned into approximately 1 cm explants which were used for organogenesis culture as described by Alkhateeb and Ali-Dinar (2002). Two buds that resulted from direct organogenesis were transferred to 100 ml flasks filled with 50 ml of modified MS salts media (Murashige and Skoog, 1962) supplemented with 170 mg l^{-1} $NaH_2PO_4.2H_2O$; 125 mg l^{-1} inositol; 200 mg l^{-1} glutamine; 1 mg l^{-1} thiamine HCl; 1 mg l^{-1} pyridoxine HCl; 1 mg l^{-1} nicotinic acid; 1 mg^{-1} calcium pantothenate; 1 mg/l biotin; 7 g^{-1} purified agar; 0.2 mg l^{-1} kinitin; 0.1 mg l^{-1} 2ip; 0.1 mg l^{-1} NAA; 0.1 mg l^{-1} NOA and 0.1 mg l^{-1} IAA. The modified MS media was further supplemented with sucrose at a concentration of 30 g l^{-1} as control and different concentrations of date syrup (5, 10 and 20 %) in the first experiment.

Based on the results of the first experiment another one was carried out using different concentrations of date syrup that were 1, 2, 3, 4, 5 and 6%. Cultures were incubated at 25±2°C in 16 h of light daily supplied by 65/80

Warm White Weisse 3500 fluorescent tubes for both experiments. Each treatment was represented by 10 replicates and 2 buds per replicate in a completely randomized design. After 6 weeks from the onset of the experiment, number of buds and shoots per culture were determined. Also, shoot length was determined by measuring the longest shoot. After obtaining fresh weights of the cultures, it was placed in a forced air oven at 75°C for 72 hours to determine dry weights.

The data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the completely randomized design (Gomez and Gomez, 1984). The treatment means were compared using the least significant difference (LSD) at 5 % level of probability (Waller and Duncan, 1969).

Results and discussion:

The results shown in Table 2 indicates that date syrup at 5% concentration enhances number of buds, fresh and dry weight of culture as compared to control (sucrose 30 g l^{-1}). However, higher concentrations of 10 and 20% date syrup are detrimental for tissue growth (Table 2 and Fig 1 and). Tissues grown on medium containing 4 to 6 % date syrup had the highest number of buds and shoots, whereas tissues grown on medium containing 1 to 3% date syrup had the lowest. In addition, tissues grown on sucrose medium had vegetative growth rates similar to those grown on 4 to 6 % date syrup. Similar results were obtained in other studies related to adding of plant extracts juice of coconut, tomato, potato, onion, banana, orange, apple, pineapple and yeast to the culture medium (Chen *et al*., 2005; Lo *et al*., 2004; He *et al*., 2003; Hong *et al*., 2003; Amo-Marco and Picazo, 1994).

Most of the vegetative growth characteristics measured were enhanced with increasing date syrup in the medium up to 6%. However, with further increase of date syrup up to 20%, the growth of tissues was suppressed (Fig 2). This reduction of growth could be attributed to a supra-optimal effect caused by the more negative osmotic potential generated by the increase of date syrup. It is noted that the sever symptom of toxicity (Table 2 and Fig 1 and 2) caused by high syrup concentration is similar to that caused by high sugar concentration (Alkhateeb, 2007) except dryness

of tissue in the case of toxicity caused by high date syrup concentration. This dryness looks like as if the tissues have lost water.

In the present study the importance of sugars for bud formation was clearly shown as indicated by the reduction of bud formation in the medium supplemented with low date syrup concentrations 1 to 3% (Fig 3, 6 and 7). It seems that there was apparently insufficient sugar available in such concentrations of date syrup, which led to its reduction. In the future, adding 1 to 3% date syrup to the 30 g l^{-1} sucrose may be benefitial and enhance the shoot and bud formation of date palm.

The reason for the superior effect of the media with date syrup at 4 to 6 % was presumably due to the expend no energy to break down sucrose into monosaccharide. Al-Hooti *et al*. (2002) reported that glucose and fructose are the major sugars presented in date syrup and total sugar contents were reaching 88%. This is in accordance with findings that sucrose is degraded in to smaller units before uptake into cells in culture (Fowler, 1982). Alternatively date syrup contains in addition to sugar, macro and micro elements (Table 1) which may contribute to the enhancement effect of date syrup in micropropagation of date palm.

In this study, it is recorded that rooting percentage and the number of roots were enhanced (Fig 4) with increasing date syrup concentration. This raise the possibility that root formation required more energy than bud or shoot formation since shoot formation was enhanced under low date syrup concentration.

In conclusion**,** the results indicated that date syrup was taken up from the medium, as shown by the increase in total dry weight of culture (Fig 5). Date palm tissues are capable of utilizing date syrup as the sole carbon source for vegetative growth. Furthermore, date syrup at concentration of 4 to 6% can be used totally as a replacement of 30 g l^{-1} sucrose which was the normal sugar used in most of plant tissue culture**.** It is clear that the substitution of date syrup at 4 to 6% for 30 g l^{-1} sucrose clearly promoted the bud and shoot formation of date palm cultures and to a large degree, better than sucrose. It is well known that date syrup contains minerals and vitamins in addition to sugars (Table 1). Therefore, further experiments that look into the effect of date syrup and agar only on the

tissue culture of plants are needed. Such experiments would be beneficial to those who are interested in the production of plant tissue culture. If such experiments succeed, this will reduced the cost of the production of plants tissue culture.

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Table (1)

The constitution of date palm syrup

Components	Value
Moisture content (%)	**16**
Ash content (%)	**6.8**
Total solids on dry weight (%)	**84.0**
Total sugar (%)	**79.45**
Reduced sugar (%)	**4.87**
Invert sugar (%)	**74.83**
Total proteins (as N) (%)	**0.83**
Total lipids (fats) (%)	**1.98**
Pectin content (as calcium pectate) (%)	**1.46**
Vit. C. content (mg/100 g)	**0.185**
Minerals (mg/100 g)	
Sodium	**13**
Potassium	**202.8**
Iron	**7.8**
Magnesium	**143**
Calcium	**338**

Table (2)

Effect of date syrup in enhancing the growth of date palm (*Phoenix dactylifera*) cv. Khanezi *in vitro* tissue culture

Parameters	% Date Syrup*		
	Control (30 g l^{-1} sucrose)	5	10
Number of buds /culture	3.888a**	4.444a	0.888b
Number of shoots/culture	1.222a	1.000a	0.000b
Length of longest shoot/culture (cm)	2.211a	1.644a	0.000b
Percentage of rooting	0.444a	0.333ba	0.000b
Number of roots/culture	0.555a	0.666a	0.000b
Length of longest root/culture (cm)	0.411a	0.466a	0.000b
Fresh weight of culture (g)	2.122ba	2.652a	1.544b
Dry weight of culture (g)	0.228a	0.377a	0.318a

- *Since all plants were killed at 20% date syrup concentration, therefore this treatment was omitted from the table.
- ** Means in each column followed by the same letter(s) did not differ at < 0.50 according to Duncan's multiple-range test.

0 5% 10% 20%
(30 gl^{-1} sucrose)

Fig. (1): Effect of syrup (Dibs) of date palm on bud and shoot formation in flasks of cv. Khanezi date palm cultivar (note color media degree) in the first experiment.

0 5% 10% 20%
(30 gl^{-1} sucrose)

Fig. (2): Effect of syrup (Dibs) of date palm on bud and shoot formation in Khanezi date palm cultivar in the first experiment.

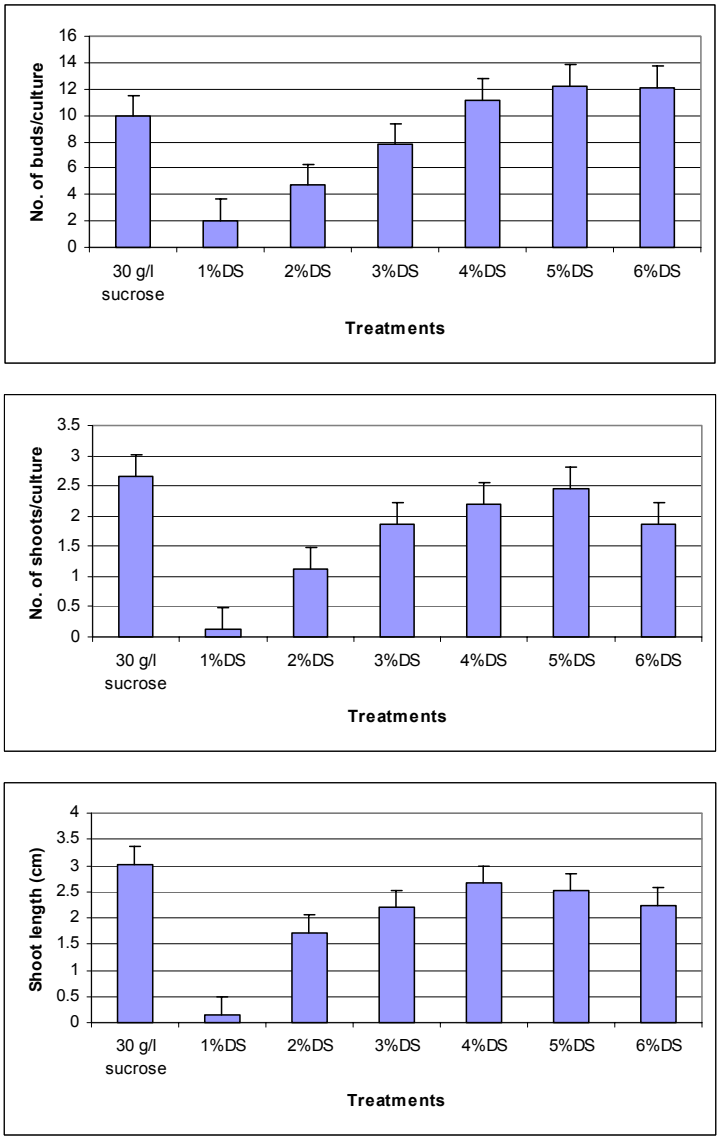

Fig (3): Number of buds and shoots/culture as well as length of longest shoot of date palm (*Phoenix dactylifera)* cv. Khanezi *in vitro* tissue culture as affected by different concentration of date syrup. Lines over bars represent LSD at 5%.

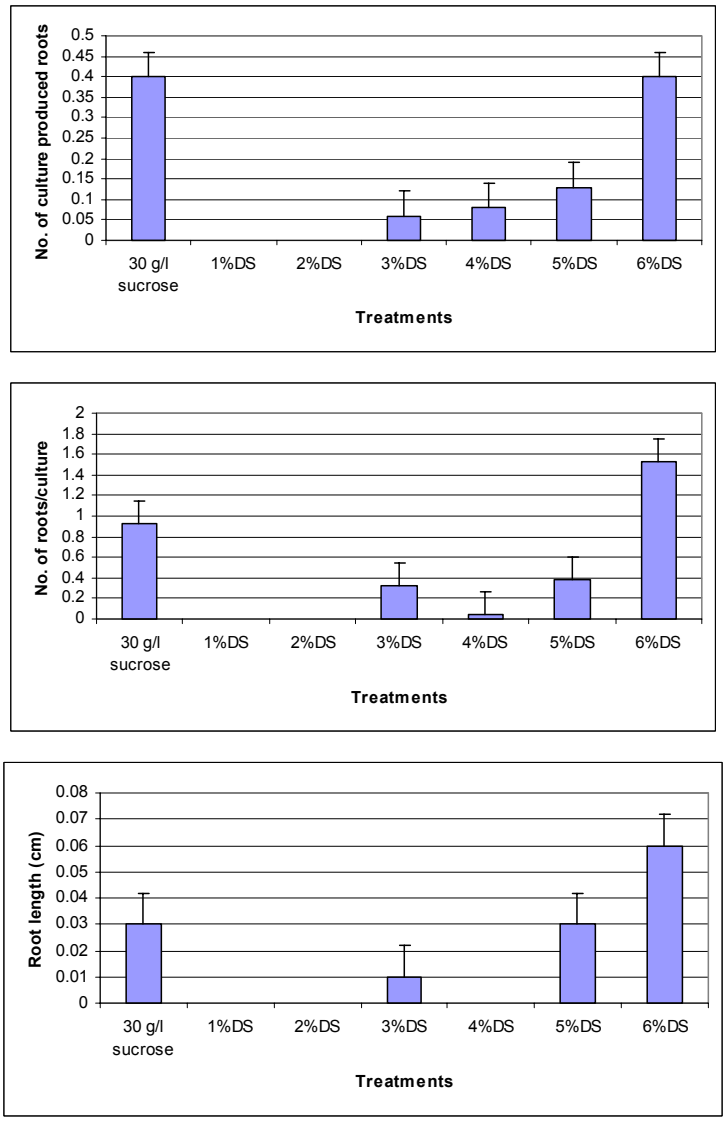

Fig (4) : Number of culture produced root, no. of roots/culture and longest of root length of date palm (*Phoenix dactylifera)* cv. Khanezi *in vitro* tissue culture as affected by different concentration of date syrup. Lines over bars represent LSD at 5%.

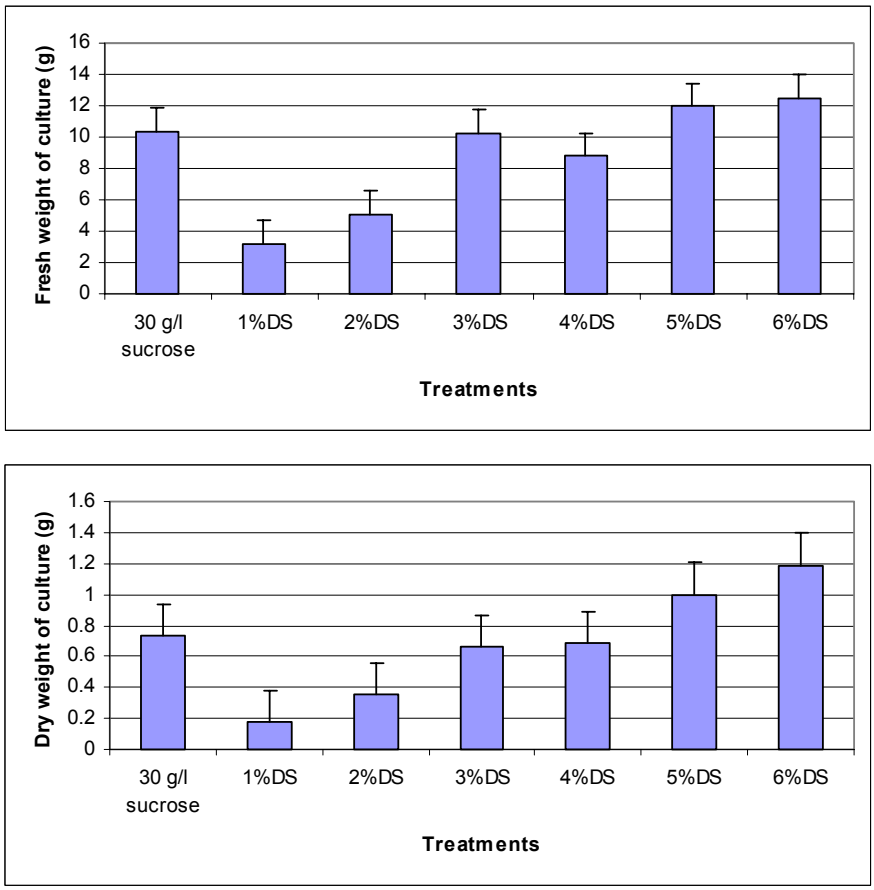

Fig (5): Fresh and dry weights of culture of date palm (*Phoenix dactylifera)* cv. Khanezi *in vitro* tissue culture as affected by different concentration of date syrup. Lines over bars represent LSD at 5%.

0 1% 2% 3% 4% 5% 6%
(30 gl^{-1} sucrose)

Fig. (6): Effect of date syrup (Dibs) on bud and shoot formation in flasks of Khanezi date palm cultivar (note color media degree) in the second experiment.

0 1% 2% 3%
(30 gl^{-1} sucrose)

4% 5% 6%

Fig. (7): Effect of date syrup (Dibs) on bud and shoot formation of Khanezi date palm cultivar in the second experiment.

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تحسين نمو نخيل التمر صنف خنيزي بإضافة دبس التمر لبيئة الإكثار النسيجي

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الملخص:

أجرى البحث بمعمل زراعة الأنسجة بمركز أبحاث النخيل والتمور، جامعة الملك فيصل وذلك بغرض تحسين نمو الأنسجة في نخيل التمر صنف خنيزي عن طريق إضافة عدة تركيزات من دبس التمر وهي كالآتي ٠,٥ ، ١٠ ، ٢٠٪ بالإضافة إلى سكروز بمعدل ٣٠ جم/لتر معاملة (الكنترول). وأظهرت النتائج أن التركيزات المنخفضة من دبس التمر (٥٪) نشط تكوين البراعم بنجاح بينما التركيز الأعلى (١٠ و ٢٠ %) أدّى إلى التلون باللون البنى وجفاف الأجزاء النباتية. هذه الظاهرة محتمل أن سببها التأثيرات الأسموزية لهذين التركيزين العاليين من الدبس لذلك لا ينصح باستخدامهما. كما بينت هذه الدراسه أن تركيز ٥٪ دبس التمر قد حسن معظم الصفات الخضرية تحت الدراسة مثل النموات القابلة للتجذير والوزن الرطب والجاف للنموات الخضرية.

على ضوء هذه النتائج أجريت تجربه أخرى أستخدم فيها التركيزات التالية من دبس التمر ١, ٢, ٣, ٤, ٥ و ٦٪. بينت هذه الدراسة أن التراكيز ٤ و ٥ و ٦٪ أدت إلى تكوين البراعم و بدرجه كبيره مقارنه لتركيز ٣٠جم/ل سكروز إن لم تكن متفوقة عليه, وصلت الى درجة المعنويه وخاصة تركيزي ٥ و ٦٪ دبس التمر, بينما التراكيز ١ و ٢ و ٣٪ أدت إلى قلة تكوين البراعم والنموات القابلة للتجذير بسبب قلة تركيز السكريات في هذه التراكيز. كما أوضحت النتائج مقدرة نموات النخيل على إستخدام دبس التمر كمصدر وحيد للكربوهيدرات بدلا من السكروز. يعتبر هذا هو التقرير الأول في إستخدام دبس التمر في عملية الإكثار النسيجي للنخيل. وأخيرا يمكن القول أن التراكيز ٤, ٥ , وكذلك ٦٪ من دبس التمر يمكن أن تحل كاملا مكان ٣٠جم/ل سكروز وهو التركيز المستخدم عادة في الإكثار النسيجي لنخيل التمر.

الكلمات الدالة: النخيل, الإكثار النسيجي, سكروز, دبس التمر