

Full Length Research Paper

Identification of the effect of different levels of activated charcoal and sucrose on multiplication shoots of date palm *phenixdactylifera* L.C.v. *sufedy in vitro*

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Accepted 22 July, 2013

This study was conducted at the Laboratory of Plant Tissue Culture of the Palm Research Center / University of Basra from March 2010 to September 2011. The aim is to determine the effect of the use of activated charcoal and sucrose on the multiplication of shoots, rate of elongation, average number of roots and length of plantlets of date palms C.v *Elsafada in vitro*. Activated charcoal concentrations were zero, 0.5, 0.75, 1, and 1.25 g/l. Those of sucrose were 20 (control), 35, 50, 65, and 80 (g/l). Nutritional media were prepared with some other chemical substances as well as 3 mg/l Naphthalene acetic and 10 mg/l 2-Isopentenyl adenine (2iP). Transplants were incubated under illumination intensity lighting of 1000 Lux for 16 h a day and a temperature of $27 \pm 1^\circ\text{C}$. A re-culture was done every four weeks. The use of 0.75 g/l of activated charcoal caused a significant improvement in the percentages of shoots multiplication (9.6%), rate of elongation (7.3 cm), number of roots (4.6 root/plantlets) and their length. However, the 0.5 concentration exceeded others in root length (5.3 cm). Use of 65 g/l sucrose increased the multiplication ratio (5.3%), elongation (7 cm) and number of roots (4.6 root/plantlet). While use of 80 g/l led to higher length of roots (5.8 cm) compared to the other concentrations. Results also showed that the use of 0.5 g/l activated charcoal and 65 g/l sucrose improved the multiplication ratio (9.2%), elongation (8.1 cm) and average number of roots (5.3 root/plantlet). Furthermore, the treatment of 0.75 g/l activated charcoal and 65 g/l sucrose significantly increased the average of root length (6.9 cm) compared to other treatments.

Key words: Date palm, elongation, activated charcoal.

INTRODUCTION

Tissue culture is a very important modern technology, which concentrates on planting various tissues of plants' parts. To obtain many plants genetically identical to the mother plant (AL-Maarri and Al-Ghamdi, 1998), propagation of date palm offshoots was done either by

organogenesis from the shoot tip and auxiliary buds, or by configuring somatic embryogenesis during callus phase which makes embryos through the cultivation of plant tissues on industrial sterile nutritional medium (Abhman et al., 2001).

Table 1. Concentrations of additives to the Nutritional media for the emergence of lateral shoots.

Quality (g/L)	Substrate
0.17	Sodium hydrogen ortho phosphates
0.1	Meso inositol
0.04	Adenine sulphates
0	Thiamine-Hcl
0	Biotin
0	Nicotine amide
6	Agar
0	NAA
0.01	2iP

The presence of activated charcoal in the nutritional medium balances plants' growth regulators and other materials inside the nutritional medium; it also helps to stimulate tissue cultivated, differentiated and modified (Zaid, 1993). The use of sucrose as an energy source has been indicated by many researchers who have obtained good response from plant parts cultivated from date palm *in vitro* (Rhiss et al., 1979; Omar et al., 1992).

The addition of sucrose is essential to the nutritional media as a source of carbon to all plant tissue cultivated including leaves of plantlets (Badr, 1982). Al-Maarri and Ghamdi (1997) stated that an increase in sucrose in the media of five cultivars of date palm from 30 to 70 g/L led to an increase in the proportion of plantlets' root to 90%. Al-Maarri and Alghamdi (1998) concluded that raising the proportion of sucrose led to an increase in the proportion of the side shoots from the tip developing on MS medium. They found that 60 g/L of sucrose increased the rate of the lateral buds and developed multiple buds from the tip compared to 30 g/L. However, Hamid (2001) found that using 45 g/L of sucrose has significantly affected the stimulation of elongation of buds from shoot tip of Maktoum cultivar. Taha et al. (2001) found in their study of the date palm, that addition of 40 g/L sucrose increased lengths of plantlets significantly like that of 30 g/l and also improved the number of leaves per plantlet.

The aim of this study is to investigate the effect of adding different levels of activated charcoal and sucrose on shoot multiplication, rate of elongation, number of roots and length of date palm plantlet *in vitro*.

MATERIALS AND METHODS

Preparation of nutritional medium

The nutritional medium consists of a group of inorganic salts described by Murashige and Skoog (1962) and known as MS salts. It was prepared in the form of salt stock solution, which consists of five groups. Stock solution was prepared by weighting element of each group separately and dissolved in volumetric flask capacity of 150 cm³. Each volumetric flask contains 50 cm³ of distilled water. For the purpose of preparing ten liters, elements of each group

were multiplied by 10, weighed and dissolved in distilled water; the volume was completed to 100 cm³ and kept in the refrigerator.

Stimulating multiplication, elongation and root of shoots

For the purpose of stimulating multiplication, elongation and root of shoots resulting from lateral shoots, the following experiments were made:

1. Activated charcoal was added to the nutritional medium and led to different concentrations: 0, 0.5, 0.75, 1 and 1.25 g/L. Nutritional media contain materials listed in Table 1 plus sucrose concentration of 30 g/L,
2. After selecting the appropriate concentration of the activated charcoal, the different concentrations of sucrose (20, 35, 50, 65 and 80 g/l) was added to the media in Table 1,
3. After determining the best concentrations of each activated charcoal and sucrose, their interaction effect was studied.

Test tube size of 2.5 x 18 cm was used; it contained 20 ml of nutritional media and set at PH of 5.7. Experiment included planting lateral bud (ten replicates per treatment). Transplant was incubated at a temperature of 27 ± 1°C under the intensity of 1000 lux illumination for 16 h a day. Replanting was done every four weeks.

Statistical analysis

Each experiment was carried out separately in a simple experiment by completely randomized design (CRD) either in one or two way experiment. Significant difference among treatment means was done by using Revised Least Significant Differences Test (RLSD), at a significant level of 5% (Alraway and Khalaf Allah, 1980).

RESULTS AND DISCUSSION

Percentage of multiplication, the rate of elongation and root of shoots

Results shown in Table 2 revealed that the addition of activated charcoal to the nutritional media helped to raise the percentage of multiplication shoots consisting of lateral buds of date palms cultivar Elsofydi. Adding 0.75 g/L concentration gave the highest proportion of

Table 2. Effect of different concentrations of activated charcoal on the percentage of multiplication and the rate of elongation and rooting shoots resulting from lateral shoots.

% vegetative shoots	Root length (cm)	No. of root/plantlets	Rate of elongation (cm)	Concentration of activated charcoal (g/l)
2.3 ^d	1.6 ^c	2.6 ^b	2.9 ^c	Zero
7.6 ^b	5.3 ^a	4.3 ^a	6.9 ^a	0.5
9.6 ^a	4.3 ^b	4.6 ^a	7.3 ^a	0.75
3.6 ^c	2.6 ^d	0.7 ^c	5.3 ^b	1
2.3 ^d	2.3 ^d	0.4 ^c	3.3 ^c	1.25

Different letters denote significant differences at the 0.05 level of probability.

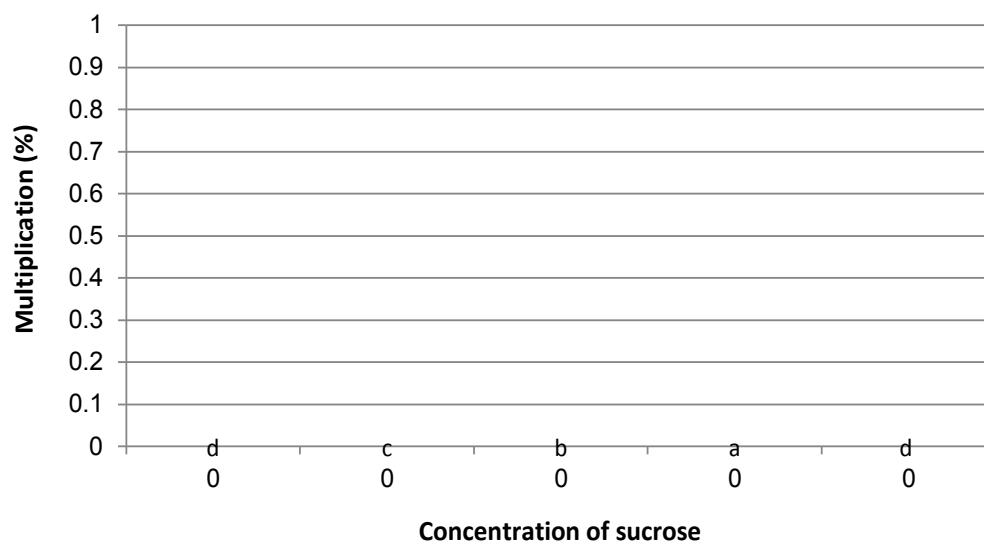


Figure 1. The effect of different concentrations of sucrose on the percent of shoots multiplication. *Different letters denote significant differences at the 0.05 level of probability.

multiplication (9.6%), which was significantly different from the other concentrations. Percentage of multiplication got to its lowest level (2.3%) when 1.25 g/l concentration was used. However, this result was not significantly different from the control group. Table 2 also showed that the rate of elongation of the branches of vegetative reached the highest rate at concentrations of 0.75 g/l (7.3 cm) and 0.5 g/l (6.9 cm); while the rate of elongation decreased at zero concentration (control treatment, 2.9 cm), with no significant difference from that of 1.25 g/l treatment (3.3 cm). The average number of roots in concentrations of 0.75 g/l and 0.50 g/l was the highest (4.6 and 4.3 root/plantlets, respectively). 0.5 g/l concentration gave the highest ($p < 0.05$) mean of roots length (5.3 cm). The lowest length of roots was shown by 1.25 g/l concentration (2.3 cm). Superiority of 0.75 and 0.5 g/l of activated charcoal in both percentage of multiplication shoots arising from lateral bud as well rate of elongation and number of roots may be due to inhibition act of toxic phenolic compounds and lack of

adsorption material useful for growth regulators; also other nutritional components of media which help plant parts to absorb these materials, leading to a good response (El-Shafey et al., 1999). The presence of activated charcoal in the nutritional media balanced plant growth regulators and other materials in the nutritional media and helped stimulate and supply the transplanted tissue; it also helped in differentiation and modification (Zaid, 1993). Rhiss et al. (1979) pointed out that the use of activated charcoal helped to reduce secretions of phenols and simulate budding and multiplication.

The effect of different concentrations of sucrose on the percentage of shoots multiplication

Results shown in Figure (1) indicated that there was significant difference ($P < 0.05$) in the percentage of shoots multiplication arising from lateral buds of date palm Elsofydi cultivar *in vitro*. It also showed a highest

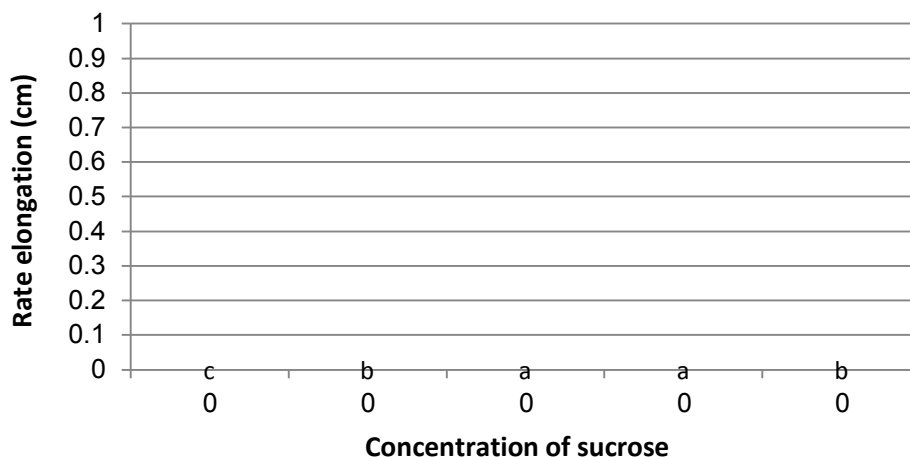


Figure 2. The effect of different concentrations of sucrose in the rate of elongation shoots. Different letters denote significant differences at the 0.05 level of probability.

Table 3. Effect of different concentrations of sucrose in the average number of roots / plantlet and lengths.

Average of root number/plantlets	Average of root length (cm)	Sucrose concentration g/l
2.3 ^b	1.6 ^c	20
2.6 ^b	2.1 ^c	35
4.3 ^a	5.6 ^b	50
4.6 ^a	5.9 ^b	65
2.6 ^b	6.8 ^a	80

Different letters denote significant differences at the 0.05 level of probability.

($P < 0.05$) multiplication percent due to the addition of 65 g/l of sucrose (5.3%) in comparison with other concentrations. There was a decline in the ratio (1.2%) of 20 g/l concentration (control), which was not significantly different from that of 80 g/L (2.5%).

The effect of different concentrations of sucrose on the rate of elongation shoots

There were significant ($P < 0.05$) differences in the rate of elongation of shoots arising from lateral bud of Elsofydi cultivar (Figure 2). Superior concentration (65) g/L gave the highest elongation (7 cm) and the difference was not significant from that of 50 g/L concentration, which was 6.8 cm. While, low rate was noticed at concentrations of 20, 35 and 80 g/L (2.2, 4 and 4.3 cm, respectively).

The effect of different concentrations of sucrose on the average number of roots and length

Table 3 shows that the addition of sucrose concentration of 65 g/l resulted in the highest number of roots/plantlets

(4.6 roots). Difference was not significant from that of 50 g/L concentration (4.3 roots). The number of roots reached the lowest level at a concentration of 20 g/L (2.3). Root difference was not significant than those of 40 and 70 g/L concentrations.

The concentration of 80 g/l gave the highest root length (6.8 cm) and was significantly different from those of other concentrations. 20 g/L concentration gave the lowest rate of root length (1.6 cm) and the difference was not significant from that of 35 g/l concentration.

The presence of sucrose in the nutritional media is one of the most important factors in plant tissue culture and its importance lies on its carbon content, which is a source of energy for tissue division and different growth stages (Hennigar, 1990; Trigian and Gray, 1999).

Taha et al. (2001) concluded that the concentration of sucrose in the nutritional media of date palm tissue culture had great effect on plantlet growth when it was added at a level of 50 g/L. It caused an increase in plantlet height and leaves number compared to 30 g/L where plantlets roots were weak (verifications). The plantlets tissue response differs due to the difference of variety, sucrose concentration and genetic factor of the plant (Singh and Shymal, 2001).

Table 4. Effect of interaction between the activated charcoal and sucrose on the ratio of multiplication and elongation rate and the number and length of roots.

Multiplication (%)	Rate of root length (cm)	Number root/plantlets	Rate of elongation (cm)	Activated charcoal (g/l)	Sucrose (g/l)
6.3 ^c	4.1 ^c	3.9 ^b	5.8 ^b	0.5	50
7.8 ^b	4.3 ^c	4.9 ^a	7.9 ^a	0.75	50
9.2 ^a	5.1 ^b	5.3 ^a	8.1 ^a	0.5	65
5.1 ^d	6.9 ^a	5.1 ^a	6.1 ^b	0.75	65

Different letters denote significant differences at the 0.05 level of probability.



Image 1. Multiplication shoots on the medium is equipped with 65 g/L sucrose and 0.5 activated charcoal.



Image 2. Multiplication shoots on the medium is equipped with 50 g/L sucrose and 0.75 activated charcoal.

The effect of interaction between the activated charcoal and sucrose

It was noted from the results shown in Table 4 that there was a significant effect of the interaction between the concentration of activated charcoal and sucrose. The use of activated charcoal concentration of 0.5 g/l and sucrose concentration of 65 g/L led to the highest rate of shoot multiplication (9.2%, Image 1) followed by the impact of the use of 0.75 g/L of activated charcoal and 50 g

sucrose/l (7.8%, Image 2). These were more significant than the effect of other treatments. The use of the same treatment also showed the highest shoot elongation (8.1 cm, Image 3). in the average number of roots and length, the result indicated a significant effect 0.5 g concentration of activated charcoal and 65 g/L sucrose. However, treatment of 0.75 g/L activated charcoal and 65 g/L sucrose resulted in highest rate of root length (6.9 cm), which is significantly different from other treatments (Images 4, 5 and 6). It can be concluded that adding 0.5 g/l of activated charcoal and 65 g/L sucrose to nutrient media gave the best results in cultivated Elsofydi date palm cultivar *in vitro*.



Image 3. Shoots elongation on the medium is equipped with 65 g/L sucrose and 0.5 activated charcoal.



Image 5. Rooting shoots on the medium is equipped with 65 g/L sucrose and 0.5 activated charcoal.



Image 4. Shoots elongation on the medium is equipped with 50 g/L sucrose and 0.75 activated charcoal.



Image 6. Rooting shoots on the medium is equipped with 65 g/L sucrose and 0.75 activated.

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