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*Journal*

*J. Biol. Chem.  
Environ. Sci., 2013,  
Vol. 8(3):1-10  
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## USING SOME MICROELEMENTS TO IMPROVE SHOOT AND ROOT INDUCTION OF DATE PALM (*PHOENIX DACTYLIFERA L.*) CV. SUKARRI.

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### ABSTRACT

This study was developed to improve shoot and root induction in *in vitro* propagation of date palm cv. Sukarri using various micronutrient elements salts ( $MnSO_4 \cdot 4H_2O$ ), ( $ZnSO_4 \cdot 4H_2O$ ) and ( $CuSO_4 \cdot 7H_2O$ ) of different concentrations which added to three media formulation (M1, M2 and M3). Shoots derived from somatic embryos germination were used as explants in the multiplication stage for a period of 30 days of culture. Shoots length, shoot number and leaves number were evaluated and measured. The shoots were separated and sub-cultured to the rooting medium and for 45 days. Same evaluations and measurements were repeated. At the multiplication stage the highest shoots number were formed with the lowest concentration of ( $MnSO_4 \cdot 4H_2O$ ), ( $ZnSO_4 \cdot 4H_2O$ ) and ( $CuSO_4 \cdot 7H_2O$ ) supplemented in M1 medium. However, the rooting stages the highest concentrations of the microelements supplemented in M3 medium were found the best to develop the highest numbers of roots.

**Key Words:** *In vitro* propagation, Micronutrient, Multiplication stage, Rooting Induction.

### INTRODUCTION

Date palm is a monocotyledonous and dioecious species belonging to the Arecaceae family, is widely cultivated in arid regions of the Middle East and North Africa (Al-Khayri, 2001). It is considered as one of the most important cash crops in the Middle East as about 70% of the total production of dates is from Arab world. Worldwide about 3000 named date palm cultivars exist, though some

names are probably synonyms, the result of a local or national name given to one cultivar which also exists in another location under another name. It is propagated sexually through seeds and vegetatively by offshoot (Bonga, 1982).

When plants are grown from seeds, about half of the palms turn out to be males while the other half would be female. However, it can be identified only at the time of flowering. Moreover, the plants obtained through seeds are genetically heterogeneous. Consequently, for uniformity of the orchards date palms cultivars should be propagated through offshoots only. The use of plant tissue culture to supplement propagation by offshoots became necessary since the first attempts at date palm propagation by tissue culture (Reuveni et al., 1972).

Somatic embryogenesis is a very important method for plant propagation, but it requires further genetic investigations to be employed with safety for true to type progeny in some monocotyledons as oil palm *Elaeis guineensis*. This technique also provides a rapid system for production of large number of genetically uniform and disease free plantlets for agriculture and forestry. Regeneration of date palm by somatic embryogenesis has been reviewed by Tisserat (1984), and Branton and Blake (1989) Somatic embryo should closely resemble their zygotic counterparts with appropriate root, shoot and cotyledonary organs and there should not be any vascular connection with the mother plant and should be able to grow into an independent plant and the protocols of somatic embryogenesis of date palm needs to be improved.

For long time research scientists had evidence that, both laboratory and field testing, to show that micronutrients are beneficial to plant growth and reproduction. Micronutrients are as important as the primary and secondary nutrients in plant nutrition. However, the amounts of micronutrients required for optimum nutrition is much lower. Soil and foliar applications are the most prevalent methods of micronutrient addition but the cost involved and difficulty in obtaining high quality micronutrient fertilizers are major concerns with these in developing countries. Variation exists within crops and varieties/genotypes/hybrids in their response to various treatments (Abo-Rekab, 2010, farooq et al 2012).

Recent studies have been focusing on the use of various microelements such as Manganese (Mn), Zinc (Zn) and copper (Cu) to

improve plant tissue culture. Mn is necessary for the maintenance of chloroplast ultra-structure and plays an important role in redox reactions, although it can be toxic at high concentration (Sarkar et al., 2004). Zn deficient plants suffer from reduced enzyme activities and a consequent diminution in protein, nucleic acid and chlorophyll synthesis. Plants deprived of zinc often have short internodes and small leaves (Eichhorn, 1980). High concentration of Cu can be toxic, although various authors report strong increases of growth when Cu is added (Dahleen, 1995).

The objective of this research was to develop reliable method for multiplication and rooting of the date palm by regulating the addition of some micro-elements.

## MATERIALS AND METHODS

The present study was carried out through 2012 and 2013 at the Central Laboratory of Date Palm Research and Development, Agric. Res. Center, Giza, Egypt.

**Plant materials:** the explants were germinated shoots from induced embryogenesis of date palm cv. Sukarri.

### Preparation of basic nutrient medium

The media were prepared by adding 50 ml of modified MS salts medium (Murashige and Skoog, 1962) to 100 ml flasks. Media were supplemented with 170 mg/L  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ; 100 mg/L inositol; 200 mg/L glutamine; 0.1 mg/L thiamine HCl; 0.5 mg/L pyridoxine; 0.5 mg/L nicotinic acid; 0.1 mg/L biotin; 7 g-L purified agar; 30 mg/L sugar; This media was autoclaved at 120 C° for 20 minutes at 15 psi.

### Shoot multiplication induction

Germinated shoots of 0.5 cm in length derived from somatic embryos germination were transferred to the basic nutrient medium supplemented by 0.5 mg/l. BA, 0.1mg/L NAA and 3 different sets of concentrations of microelements as shown in table (1). Each set of media were placed in three jars. After 30 days of culture, the shoot number and leave number were counted and the shoot length was measured by centimeter. All culture treatments were incubated at 27±2C° under 2000 lux illumination by cool white fluorescent light for 16 hours photoperiod.

### Root induction

Multiplied shoots were transferred to the basic nutrient medium supplemented by 1.0 mg/L NAA and 3 different sets of concentration of the micronutrient elements as shown in table (1). The different treatments were incubated in a growth chamber at  $27 \pm 2^\circ\text{C}$  under 4000 lux illumination by cool white fluorescent light for 16 hours photoperiod. After 45 days of the transfer, the roots and leaves numbers were counted and the root length and shoot length were measured.

**Table (1): Different concentrations of the micronutrient elements that were used within the three cultured media.**

Micronutrient elements	Concentration in media (mg/L)			
	Control	M1	M2	M3
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	0	0.1	0.25	0.5
$\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$	0	1	2.5	5
$\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$	0	0.05	0.15	0.3

### Statistical analysis

Both experiments were arranged in a complete randomized design and data were analyzed according to methods described by Snedecor and Cochran (1980). The averages were compared using L.S.D test.

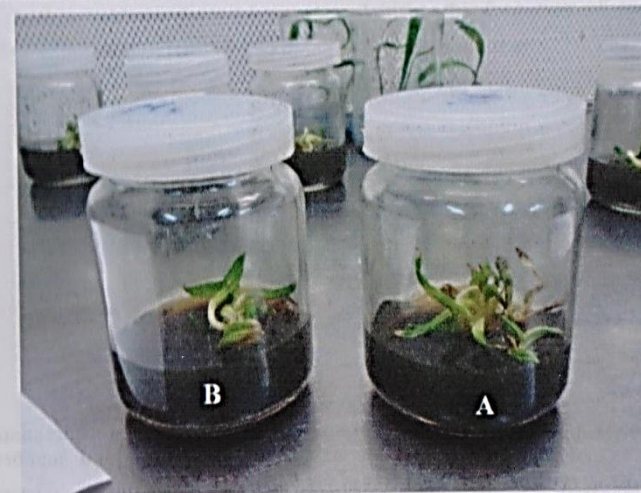
## RESULTS AND DISCUSSION

Date palm needs optimum amounts of minerals for their best growth. Optimization of the supplying of macro and micro nutrient supplements is necessary to increase quantitative, qualitative and economical output of date production (Saleh 2008).

### • Multiplication culture

After 30 days, the explants were found to form shoots with good leaves. Only shoots with average from 3.0 to 5.0 were counted in this study. The increasing in the shoots length varied from 0.43 to 0.73 cm per explants and the leave number approximately from 3.7 to 7.0.

The results shown in figure (1 and 2) indicated that the microelements ( $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ ,  $\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$  and  $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ ) of different concentrations enhancing of the shoot number, shoot length and leaves number of cultured explants compared to the control (Table 2).



**Figure (1): Effect of different concentrations of microelements on the shoot number, shoot length and leaves number at the multiplication stage after 30 days of culture in which A represent the best results (M1) and B represents control.**

The shoot numbers were formed with the lowest concentrations of ( $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ ), ( $\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$ ) and ( $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ ) added to M1 medium gave the highest significant number of shoots among all other treatments. In addition, shoots grown on M2 and M3 culture medium gave similar results that recorded 4.007, 3.667 respectively. Shoots grown on the control medium recorded the lowest result (3 shoots).

As presented in the same figure (2), the measured parameters of all treatment indicated that the micronutrient elements had no significant improvement of shoot length and leaves number. These results are in accordance with these obtained by Bekheet, S.A and Saker, M.M. (1998), El-Hammady, A.A (1999) and Al-Khaliffah, N.S. (2000).

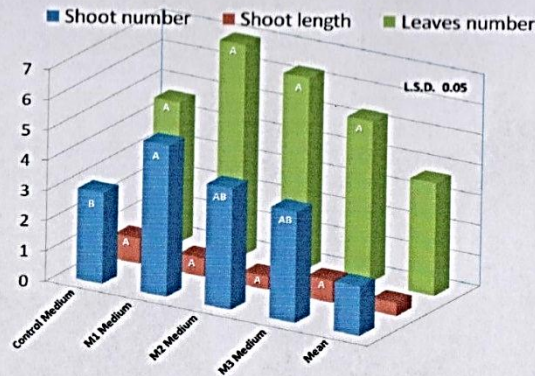


Figure (2): The effect of different concentration of some micronutrient elements on shoot number, shoot length and number in multiplication stage of date palm cv. Sukarri.

Table (2): Effect of different concentration of some micronutrient elements on shoot number, shoot length and number in multiplication stage of date palm cv. Sukarri.

Concentration (mg/L)	Shoot number	Shoot length	Leave number
Control	3.0 B	0.8333 A	4.667 A
M1	5.00 A	0.6000 A	7.00 A
M2	4.007 AB	0.4333 A	6.333 A
M3	3.667 AB	0.7333 A	5.333 A
Mean	1.632	0.4285	3.723

L.S.D 0.05

• Rooting culture

Shoots formed in the shooting stage medium were separated and transferred to the rooting medium in this study. Results of rooting stage after 45 days of culture are shown in figure (3) which indicated that the addition of micronutrient elements of Mn SO<sub>4</sub>. 4H<sub>2</sub>O, Zn SO<sub>4</sub>.4H<sub>2</sub>O and CuSO<sub>4</sub>.7 H<sub>2</sub>O leads to increase the root quality in this study specially the root numbers.

Data in figure (4) show significant differences in the root number, the M3 medium was the most effective by forming the highest amount of root numbers which presented 3.0 numbers. In addition, it is noted that at

low level or without the micronutrient element, the formation of root number was lower than M3 medium.



Figure (3): Effect of different concentrations of micronutrient elements on the root number, root and shoot length and leaves number at the rooting stage after 45 days of culture in which A represent M3, B represent M1 and C represent control medium.

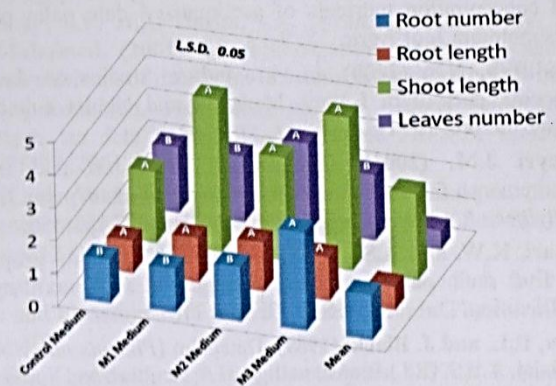


Figure (4): Effect of the different concentration of some micronutrient elements on the root number, root length, shoot length and leave number in the rooting stage of date palm cv. Sukarri.

These data clearly indicated that root and shoot length did not exhibit any significant difference between all treatments. According to these results, the rooting stage was practically successful as a protocol for micropropagation. ( Tisserat 1984, Al-Maari and Al-Ghamdi 1997, El-Sharabasy et al (2001), El-Sharabasy et al, 2009).

**Table (3): Effect of the different concentration of some micronutrient elements on the root number, root length, shoot length and leave number in the rooting stage of date palm cv. Sukarri.**

Concentration(mg/L)	Root number	Root length	Shoot length	Leave number
Control	1.333 B	1.00 A	2.167 A	2.00 B
M1	1.333 B	1.35 A	4.667 A	2.00 B
M2	1.667 B	1.500 A	3.167 A	2.667 A
M3	3.000 A	1.33 A	4.667 A	2.00 B
Mean	1.373	0.7259	2.627	0.5756
L.S.D 0.05				

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### استخدام بعض العناصر الصغرى لتحسين النبيتات والجذور لنخيل البلح في المختبر

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طورت هذه الدراسة لتحسين جودة الجذور و النبيتات لنخيل التمر السكري في الإكثار الدقيق باستخدام بعض أملاح العناصر الصغرى ( $ZnSO_4, 4H_2O$ ), ( $MnSO_4, 4H_2O$ ) و ( $CuSO_4, 5H_2O$ ) حيث تتكون التركيزات في ثلاث بيئات غذائية (M1,M2,M3) وتم الحصول على الأجزاء النباتية من الأجنه الجسمية حيث تم عزلها اثناء مرحلة النمو وبعد 30 يوماً من زراعتها تم قياس كل من طول النبيتات وعددها وأوراقها وبعد ذلك تم فصل النبيتات ونقلها الى بيئة غذائية لتكوين الجذور وبعد 45 يوماً من زراعتها تم قياس طول الجذور واعداد الجذور والاوراق وقد اوضحت النتائج أن في مرحلة النمو للنبيتات أن البيئة الغذائية M1 كانت الأكثر عدداً للنبيتات حيث إنها تحتوي على اقل تركيز من الزنك و المنجنيز و النحاس بينما في مرحلة التجذير وجد ان العدد الاعلى من الجذور تكونت في البيئة الغذائية M3 التي تحتوي على اعلى تركيز من العناصر الصغرى.