Plant Archives Vol. 20 No. 1, 2020 pp. 517-528 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ENHANCED *IN VITRO* MULTIPLICATION AND ROOTING OF DATE PALM CV. YELLOW MAKTOUM BY ZINC AND COPPER IONS

Zeinab E. Zayed*, Maiada M. EL-Dawayati, Fadia A Hussien and Tahani Y. Saber

The Central Laboratory of Date Palm Research and Development, Agriculture Research Center, Giza, Egypt.

Abstract

The present work was carried out to study the effect of $ZnSO_4$ and $CuSO_4$ on the *in vitro* multiplication stage during two types of subculture intervals (4 and 6 weeks) and rooting stage of date palm *cv.* Yellow Maktoum. The pigment content have been estimated (chlorophyll a, b, total chlorophyll and carotenoids), protein and proline contents during multiplication stage from this study. Blending have been microelements compounds $ZnSO_4$ or $CuSO_4$ separately at different concentrations (0.0, 10.0, 25.0. 50.0. 75.0. 100.0 µM) to a Murashige and Skoog (MS) medium as a basal nutrient medium with recommendation of auxins and cytokinins during both multiplication and rooting stages. After three subculture from culturing on the same concentration of $ZnSO_4$ and $CuSO_4$ data showed that, $ZnSO_4$ at 50 µM or $CuSO_4$ at 25 µM induced a maximum morphogenic responses (number of shoots, number of secondary embryos, shoots length (cm) growth vigor/expaln) and physiological responses (Chl a, b, total Chl, Car content and total protein content) during the two subculture intervals under investigation. Further, proline content increased by increasing $ZnSO_4$ or $CuSO_4$ concentrations in MS medium. Regarding the subculture interval, usually 6 weeks was better than 4 weeks of all measurements. Otherwise, The best treatments for the *in vitro* rooting were the strength MS basal nutrient medium supplemented with $ZnSO_4$ at (75 µM or 100 µM) or $CuSO_4$ at (50 µM or 75 µM) whereas, number of roots, roots length, plantles length(cm) and growth vigor/explant achieved a maximum values with that treatments. All rooted plantlets were transferred to green house for acclimatization. These plantlets achieved 90% survival rate after 6 months from culturing in greenhouse. The present study was aimed at standardizing the nutrient requirements for improved micropropagation of date palm cv. Maktoum during multiplication and rooting stages by manipulating zinc and copper levels in MS medium for obtaining full plantlets with good root system are able to resume their development successfully at acclimatization.

Key words: Date palm, Carotenoids, $CuSO_4$, *In vitro*, Multiplication, proline content, Rooting, Subculture interval, total chlorophyll, total protein and, $ZnSO_4$

Introduction

Date palm, *Phoenix dactylifera* L., is a perennial, dioecious, and monocotyledonous tree well adapted to arid environments. Typically, date palm propagation is sexually by seed or vegetatively by offshoots. However, both techniques are economically inefficient and fail to meet the demand for large quantities of planting material and the clonal propagation of selected superior genotypes (Al Khayri 2007 and Aahmane 2017). There have been previous reports on date palm micropropagation through the organogenesis and somatic embryogenesis (Fki *et al.*, 2011; Zayed 2017). Although the great achievement in date palm *in vitro* propagation there are still serious problems during its reproduction cycle inside lab which may be defused or stop the successfully transferring to green house. This work presents noticeable problem during multiplication stage as the converted plantlets (resulted from somatic embryos differentiation) did not pass to rooting stage in good manner of growth due to their shortness, weakness and low number of multiplied shoots, so that may decrease their opportunity for successful transfer to acclimatization stage. Inorganic macronutrient and micronutrient levels used in most plant tissue culture media are based on levels established by Murashige and Skoog (1962) for tobacco tissue culture. However, many plant species and varieties do not respond to classical approach, demonstrating that alterations in hormonal ratios cannot be the sole mechanism controlling *in vitro* developmental processes (Ramage and Williams 2002).

Zn and Cu are micronutrients of growth medium that

***Author for correspondence :** E-mail : zemmz2005@yahoo.com

are needed by plants for growth and various biochemical and physiological pathways **(**Narula and Srivastava 2005).

Zn plays a vital role in the cell division, cell expansion, proteins synthesis, and also in carbohydrate, nucleic acid and lipid metabolism. Zn is required for the synthesis of tryptophan (Tsonev and Lidon 2012), which is a precursor of IAA this metal also has an active role in the production of auxin, an essential growth hormone **(**Brennan 2005**)**.zinc increases the biosynthesis of chlorophyll and carotenoids (Broadley *et al.,* 2007).

Copper (Cu) is an important part of enzymes and protein involved in plant metabolic processes such as photosynthesis and mitochondrial electron transport. It is an essential micronutrient required for proper plant growth and development (Shahid, *et al.,* 2015). Copper is a micronutrient important for normal plant growth and development. It takes part in processes of photosynthesis, respiration, transport and other physiological and biochemical functions (Yruela, 2005). In many plant species increasing of copper level in culture medium has a positive effect on *in vitro* regeneration, elongation and micropropagation (Bardar *et al.,* 2014). Therefore, optimum Cu and Zn concentrations in the medium positively affect development of the membrane system of chloroplasts and chlorophyll content. Proline accumulation is reported to occur in response to heavy metal toxicity (Sharma and Dietz 2009 and Ahmed *et al.,* 2015).

The present study was aimed at standardizing the nutrient requirements for improved micropropagation of date palm *cv.* Maktoum during multiplication and rooting stages by manipulating zinc and copper levels in MS medium for obtaining full plantlets with good root system are able to resume their development successfully at acclimatization.

Materials and methods

This experimental work was performed at the Central Laboratory of Date Palm Researches and Development Giza, Egypt. Date palm off shoots cv. Yellow Maktoum have been received from Iraq country under suppression of Dr. Zeinab Zayed to propagate this cultivar under Egyptian condition. Sterilization protocol for meristematic shoots tip were performed according to (Zayed, 2017**)**.

Effect of zinc sulphate ($ZnSO_4 . 7H_2O$) and copper sulphate ($CuSO_4 . 5H_2O$) on multiplication stage.

Plant material: Explants material in this stage were shoots cluster multiplied consist of (4-5 shoots at 0.5-0.7 cm length) which received by indirect somatic embryogenesis protocols according to (El-Dawayati *et al.,* 2014**)**.

Medium component: MS **(**Murashige and Skoog 1962) salt strength medium and vitamins were used as a basal nutrient medium, supplemented with 40 gl^{-1}sucrose, 0.54 µM NAA and 0.222 BA µM (control treatment). Studied levels of zinc sulphate ($ZnSO_4 . 7H_2O$) and copper sulphate ($CuSO_4 . 5H_2O$) were added separately to nutrient medium at different concentrations 0.0 (control treatment, having normal zinc or copper concentration in MS basal medium), 10.0, 25.0, 50.0, 75.0 and 100.0 µM. All the salts used were of analytical laboratory.

The pH of the medium was adjusted to 5.7 ± 0.1 before adding bacteriological grade agar (*Qualigens*) at $8gl^{-1}$ and medium was dispensed into small jars 150 ml (40ml/jar) before autoclaving at 121ºC and 1.1 kg/cm^2 for 20 min. Culture jars of each treatment were divided to three replicates. Each replicate consists of three culture jars. Each jar contained one of shoot cluster explant.

All culture jars of each treatment were incubated under 100μmol/ m^2/s provided by florescent lamps for 16 and 8 hrs. dark at 25 ± 2ºC.

Data were recorded after three subcultures during the two subcultures intervals 4 weeks and 6 weeks about morphogenic responses (shoots number, number of secondary embryos formation, shoots length (cm) and growth vigor/ explant) and physiological responses (chlorophyll a, b, total chlorophyll, carotenoids content and total protein content). Proline content was determined to indicate the effect of using high concentrations of $ZnSO_4$ and $CuSO_4$ as a sign of stress.

The data of growth vigor/ explant were scored visually according to Pottino 1981; Mujib *et al.,* 2005 ; Zayed, 2014 as follows:-

1 - Negative result (-)

2 - Below average result (+)

3 - Average result (++)

4 - Good result (+++)

5 - Very good result (++++)

The pigments were determined (mg g^{-1} FW) using the method described by Arnon (1949). Determination of protein content mg g^{-1} FW of leaves was assessed by the method described by Bradford (1976). The proline content mg g^{-1} FW of leaves was determined by Bates *et al.,* (1973).

Effect of zinc sulphate ($ZnSO_4$) and copper sulphate ($CuSO_4$) on rooting stage

The same concentrations of $ZnSO_4$ and $CuSO_4$ were studied during rooting stage, subculture interval in this

testing stage will detriment according to the results obtain from multiplication stage.

Plant material: Explant material in this stage were elongated shoots (shootlet) 7 cm about with 2 leaves were excised from elongated shoots received from microprpagation protocols according to (Zayed 2017).

Medium component: medium components in the rooting studying of date palm *cv.* Yellow Maktoum are the same components above mentioned during multiplication studying except sucrose concentration is 50 gl^{-1} and growth regulators are 1.36 µM paclobutrazol (PBZ), 5.37 µM NAA and 4.92 µM IBA (Zayed 2017). Culture tube (2.5 × 25 cm) containing 20 ml rooting medium adding to different concentrations of zinc sulphate ($ZnSO_4$. 7 H_2O) and copper sulphate ($CuSO_4$. 5 H_2O) separately as mentioned above. Culture tubes of each treatment were divided to three replicates. Each replicate consists of three culture tube. Each tube contained one shootlet explant.

All culture tubes of each treatment were incubated under 200μmol $m/^2$ / s provided by florescent lamps for 16 and 8 hrs. dark at 27 ± 2°C.

Data were recorded after three subcultures about shoot length/shootlet, root number/shootlet, root length/ shootlet (cm) and growth vigor/shootlet which described above mentioned.

Rooting and acclimatization

Elongated shootlets which were received from all previous treatments of multiplication experiment were collected and cultured on rooting medium consisting of 1/ 2 MS basal nutrient medium supplemented with 5.37 µM NAA, 4.92 µM IBA and 1.36µM paclobutrazol (PBZ) and then, the shootlets were separated to individual shoots and cultured on preacclimatization medium (Zayed 2017).

All rooted plantlets from either multiplication or rooting experiments were transferred to liquid preacclimatization medium composed of 1/4 MS medium containing 10.0g^{-1} sucrose and 6g^{-1} polyethylene glycol 8000 (PEG). Plantlets with well developed shoot and root system were carefully transferred to pots containing peatmos : vermiculate: sand 1 : 1 : 1 after it washed with tap water. Humidity was maintained initially by covering the pots with transparent polythene bags.

Statistical analysis

The factorial design in completely randomized arrangement was used and data were subjected to analysis of variance. Separation of means among treatments was determined using L.S.D test at 5% probability level according to Snedecor and Cochran (1972).

Result

In the present study, date palm cv. Yellow Maktoum were grown *in vitro* and the effect of different levels of $ZnSO_4$ and $CuSO_4$ were assessed. Analysis of Variance (ANOVA) showed significant effect ($P \leq 0.05$) for $ZnSO_4$, $CuSO_4$ treatments and subcultures intervals for

Table 1: Effect of different concentrations $ZnSO_4$ and the subculture interval (4 and 6 weeks) on shoots number of date palm *cv.* Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$µM(A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	14.75	18.25	16.5
10	20.75	26.50	23.62
25	25.50	32.25	28.87
50	32.25	40.00	36.12
75	28.00	34.50	31.25
100	24.57	28.75	26.66
Mean(B)	24.30	25.70	
$LSD_{0.05}$	(A)3.82	(B)1.04	(AB)2.54

Table 2: Effect of different concentrations $ZnSO_4$ and the subculture interval (4 and 6 weeks) on Secondary embryos number of cluster explants of date palm *cv.* Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$µM(A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	5.25	7.32	6.28
10	6.75	7.75	7.25
25	8.25	9.25	8.75
50	10.25	11.75	11.00
75	8.75	8.00	8.37
100	3.20	4.25	3.72
Mean(B)	7.07	8.05	
$LSD_{0.05}$	(A)2.41	(B)1.23	(AB)1.56

Table 3: Effect of different concentrations $ZnSO_4$ and the subculture interval (4 and 6 weeks) on shoots length (cm) of date palm *cv.* Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$µM(A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	4.25	5.00	4.62
10	4.37	5.37	4.87
25	6.12	5.50	5.81
50	6.25	7.50	6.87
75	6.00	6.25	6.12
100	5.00	5.25	5.12
Mean(B)	5.33	5.81	
$LSD_{0.05}$	(A)0.82	(B)0.35	(AB)0.78

all measurements during multiplication and rooting stages

Effect of zinc sulphate ($ZnSO_4$) on multiplication stage

After three subculture from culturing shoots cluster explants of date palm *cv.* Yellow Maktoum on different levels of $ZnSO_4$ under two subculture intervals (4 weeks and 6 weeks). The control MS medium supplemented with 0.222 µM BA and 0.54 µM NAA (recommended medium during multiplication stage) reproduction 14.75 shoots/explant after 4 weeks and 18.25 shoots/explant after 6 weeks. The addition of different levels of $ZnSO_4$ from 10.0 to 100.0 µM was optimization of shoots number healthy compared with control medium. $ZnSO_4$ at 50.0 µM was good result of number of shoots which recorded 36.12 shoots/explant. There are significant differences

Table 4: Effect of different concentrations $ZnSO_4$ and the subculture interval (4 and 6 weeks) on growth vigor of clusters explants of date palm cv.Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$ µM(A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	2.50	3.00	2.75
10	2.50	3.50	3.0
25	2.75	3.75	3.25
50	3.75	4.00	3.87
75	3.50	3.75	3.62
100	3.00	2.50	2.75
Mean(B)	3.00	3.41	
$LSD_{0.05}$	(A)0.25	(B)0.23	(AB)0.45

Table 5: Effect of different concentrations $CuSO_4$ and the subculture interval (4 and 6 weeks) on shoots number of date palm cv. Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$ µM(A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	14.75	18.25	16.5
10	26.25	22.00	24.12
25	34.25	38.00	36.12
50	29.00	40.50	34.75
75	22.25	34.75	28.50
100	18.25	28.00	23.12
Mean(B)	24.12	30.25	
$LSD_{0.05}$	(A)4.25	(B)4.02	(AB)3.45

Table 6: Effect of different concentrations$CuSO_4$ and the subculture interval (4 and 6 weeks) on Secondary embryos number of cluster explants of date palm cv.Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$ µM(A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	5.25	7.32	6.28
10	6.25	12.50	9.37
25	7.00	11.25	9.12
50	6.20	10.50	8.35
75	5.25	9.00	7.12
100	4.75	6.75	5.76
Mean(B)	5.78	9.55	
$LSD_{0.05}$	(A)1.06	(B)1.89	(AB)1.37

Table 7: Effect of different concentrations $CuSO_4$ and the subcultures interval (4 and 6 weeks) on shoots length (cm) of date palm cv.Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$ µM(A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	4.25	5.00	4.62
10	5.50	5.75	5.62
25	5.75	6.50	6.12
50	6.45	7.50	6.97
75	5.00	6.25	5.62
100	3.75	4.25	4.00
Mean(B)	5.11	5.87	
$LSD_{0.05}$	(A)0.44	(B)0.33	(AB)0.73

Table 8: Effect of different concentrations $CuSO_4$ and the subculture interval (4 and 6 weeks) on growth vigor of clusters explants of date palm cv.Yellow Maktoum after three subculture of culture during multiplication stage.

$ZnSO_4$ µM(A)	Subculture interval(B)		Mean(A)
	4 weeks	6 weeks	
0(Control)	2.50	3.00	2.75
10	2.75	3.75	3.25
25	3.75	4.00	3.87
50	3.75	4.00	3.87
75	3.25	3.50	3.37
100	2.25	2.50	2.37
Mean(B)	3.04	3.45	
$LSD_{0.05}$	(A)0.43	(B)0.22	(AB)0.47

Table 9: Effect of different concentrations zinc sulphate ($ZnSO_4$) on roots formation of date palm cv. Yellow Maktoum after three subculture from culturing.

$ZnSO_4$ µM	Shoot lengt(cm)	No.of root	Root length(cm)	Growth vigor
Control	12.4	3.4	4.7	2.4
10	15.7	7.2	6.0	2.8
25	15.7	7.2	7.0	3.2
50	16.2	7.6	7.5	3.6
75	16.0	8.2	7.5	3.8
100	16.5	8.1	8.0	4.0
$LSD_{0.05}$	0.47	0.33	0.36	0.32

Table 10: Effect of different concentrations copper sulphate ($CuSO_4$) on roots formation of date palm cv. Yellow Maktoum after three subculture.

$ZnSO_4$ µM	Shoot lengt(cm)	No.of root	Root length(cm)	Growth vigor
Control	12.4	3.4	4.7	2.4
10	13.7	4.0	6.3	3.2
25	14.4	6.6	8.3	3.8
50	16.9	7.2	8.6	3.8
75	17.6	9.2	7.6	4.0
100	15.4	8.8	6.5	4.0
$LSD_{0.05}$	0.89	0.52	0.45	0.25

between subcultures intervals (4 and 6 weeks) of the shoots numbers produced where 6 weeks were the perfect than 4 weeks (25.70 shoots /explant and 24.30 shoots /explant respectively) Table 1. On the other hand interaction between different levels of $ZnSO_4$ and subculture intervals had significant effect of shoots number/explant. Cluster explants when cultured on $ZnSO_4$ at 50.0 µM for three subcultures and subculture intervals were 6 weeks produced the superior of shoots number (40.0 shoot/explant) during multiplication stage.

The number of secondary embryos formed on the bases of the cluster explants was also evaluated during two subculture intervals under investigation Table 2. It was found that 50.0 µM $ZnSO_4$ promoted secondary embryos formation. The higher concentrations of $ZnSO_4$ (75.0 and 100.0 µM) reduced secondary embryos formed (8.37 and 3.72 secondary embryos/explant respectively).

Concerning subculture intervals, there wasn't significant differences between secondary embryos number formed after 4 weeks or 6 weeks during multiplication stage (7.07 and 8.05 secondary embryo/ explant respectively). Interaction effect between treatments studied and subculture intervals clearly affected significantly of the secondary embryos numbers formed on the bases of the cluster explants during multiplication stage, cluster explants cultured on 50.0 µM $ZnSO_4$ showed increasing in the secondary embryos number formed with 6 weeks as a subculture interval.

The effect of different levels of $ZnSO_4$ and subculture intervals on shoots length (cm) was studied during multiplication stage in Table 3. The results showed that $ZnSO_4$ at 50.0 and 75.0 µm gave the longest shoots without significant differences in between (6.87 and 6.12 cm/ explant respectively). The subculture interval every 6 weeks was better than 4 weeks of shoots length. Regarding interaction effect between different levels of $ZnSO_4$ and subculture intervals of shoots length (cm) data indicated that $ZnSO_4$ at 50.0 or 75.0 µM and 6 weeks as a subculture interval was optimum of shoots length (7.5 cm/explant) during multiplication stage.

From observation in Table 4 cluster explants cultured on $ZnSO_4$ at 50.0 or 75.0 µM showed the highest significant results in growth vigor signs during multiplication stage (3.87 and 3.62 /explant respectively) without significant differences in between, best signs of growth vigor appeared in strong shoots and well green color without marks of browning or welting. Where cluster explants cultured on high concentration of $ZnSO_4$ at 100.0 µM showed bad marks of growth as browning and weak shoots. The interaction effect between different levels of $ZnSO_4$ and subculture intervals on growth vigor clusters explant, data observed that $ZnSO_4$ at 50.0 µM after 6 weeks was the best of growth vigor (4.0) during multiplication stage. Fig. 1.

The Chl *a*, Chl *b* and total Chl content in the multiplication *in vitro* of date palm *cv.* Yellow Maktoum increased with the increasing zinc concentrations up to the level 50.0 µM $ZnSO_4$. Higher concentrations of $ZnSO_4$ caused a decline in the photosynthetic pigments. While the Car content increased up to level of $ZnSO_4$ 25.0 μM

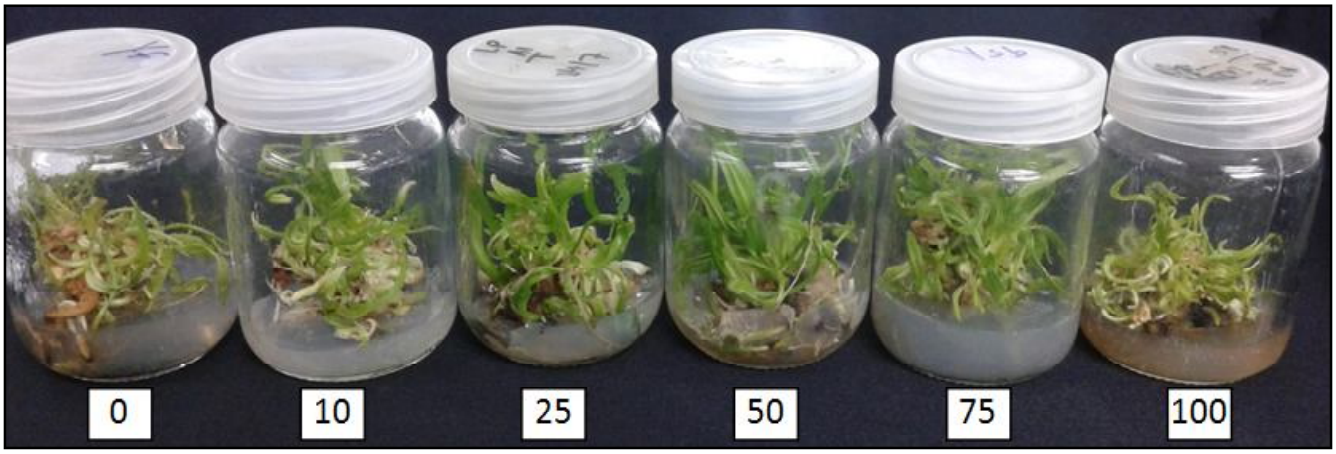

Fig. 1: Effect of different concentrations of $ZnSO_4$ on shoot growth.

and declined thereafter. Regarding the subculture interval, 6 weeks was better than 4 weeks of all pigments content (Fig. 2).

Zinc is an essential micronutrient and plays an important role in protein synthesis, enzyme activation and growth regulation, so data presented in Fig 3. indicated that addition $ZnSO_4$ at 50.0 µM to date palm multiplication medium increased significant in total protein content after 6 weeks as a subculture interval (1.6 mg/g FW) whereas that, $ZnSO_4$ at 75.0 µM achieved the same value of total protein content (1.6 mg/g FW) after 4 weeks. The level of ZnSO4 above 100.0 µM reduced total protein content of date palm shoots during multiplication stage.

Results showed that proline accumulation increased significantly by increasing $ZuSO_4$ concentrations from 10.0 to 100.0 µM and do not significant differences of proline accumulation between two type of subculture interval (4 and 6 weeks) Fig. 4.

Effect of copper sulphate ($CuSO_4$) on multiplication stage

Moreover, studies were conducted with modified levels of copper in the multiplication medium of date palm *cv.* Yellow Maktoum for three subculture under two type of subculture intervals (4 and 6 weeks). The addition of $CuSO_4$ at the concentration from 10.0 to 100.0 µM was beneficial for production of shoots and their growth as compared to the control treatment (having normal copper concentration in MS basal medium). There was concomitant increase in number of shoots with increasing concentration of copper till the level for mineral is optimized at 50.0 µM which achieved 40.50 shoot / explant after 6 weeks of culture. In addition to that increase in copper concentrations had adverse effect on number of shoots formed /explant. The subculture intervals 6 weeks was the perfect than 4 weeks of shoot number formed /explant (30.25 and 24.12 / explant respectively) with high significant differences in between (Table 5).

The results in Table 6 showed that the addition of $CuSO_4$ at 100.0 µM for three subcultures to multiplication medium of date palm cv. Maktoum depressed the formation of secondary embryos to give the lowest significant results both of subculture interval under investigation (4.45 and 6.75 secondary embryo/explant respectively). Where the highest induction of secondary embryos/explant was observed with $CuSO_4$ at 10.0 and 25.0 µM in formula of MS nutrient salts (12.50 and 11.25 secondary embryo/explant respectively) after 6 weeks without significant differences in between. The high efficiency of secondary embryo production ensures the production of large numbers of explants for shoot regeneration.

As regards shoots length (cm), the addition of $CuSO_4$ at 50.0 µM to culture multiplication medium achieved the highest significant value in increasing shoots length/ cluster explants (6.97cm) during multiplication stage (Table 7). On the other hand cluster explants cultured on the high level of $CuSO_4$ (100.0 µM) of two types subculture interval under investigation gave the lowest significant results in increasing shoots length (3.75 and 5.50 cm /explant). There was significant effect of subcultures intervals on shoots length cluster explants during multiplication, increasing of subculture interval of cluster explant cultured on different levels $CuSO_4$ from 4 weeks to 6 weeks achieved the longest shoots. Data showed that increasing in shoot length for all cultured explants during multiplication stage had affected significantly with interaction between studied levels of $CuSO_4$ and the subculture intervals (4 and 6 weeks) .This was improved clearly with cluster explants cultured on $CuSO_4$ at 50.0 µM by means of 6 weeks (7.50 cm /explant).

Clearly from data in Table 8 clusters explants culture on different levels of $CuSO_4$ in formula of MS from 10.0 to 75.0 µM nutrient salts showed the highest significant

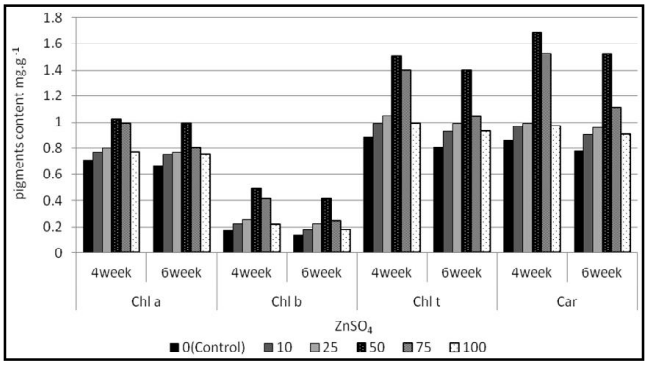

Fig. 2: Effect of different concentrations $ZnSO_4$ and two types of subculture interval (4 and 6 weeks) on the pigments content (Chl a, Chl b, Chl t and Car mg.g^{-1} FW) of date palm cv. Yellow Maktoum.

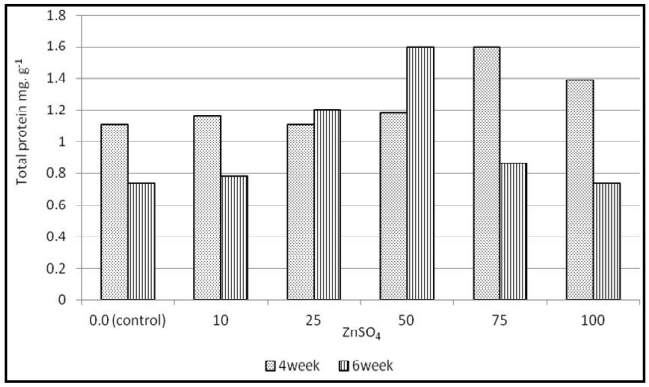

Fig. 3: Effect of different concentrations $ZnSO_4$ and two types of subculture interval (4 and 6 weeks) on total protein content (mg.g^{-1} FW) of date palm cv. Yellow Maktoum.

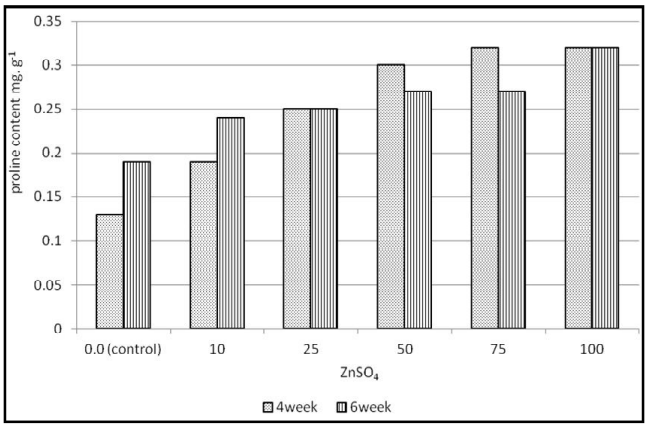

Fig. 4: Effect of different concentrations $ZnSO_4$ and two types of subculture interval (4 and 6 weeks) on proline content (mg.g^{-1} FW) of date palm cv. Yellow Maktoum.

results in growth vigor signs during multiplication stage (3.25, 3.87, 3.78 and 3.37 /explant respectively). The best signs of growth vigor appeared in strong shoots and well green color without marks of browning or welting. Where cluster explants cultured on high concentration of $CuSO_4$ seemed bad marks of growth and visible symptoms of toxicity were observed especially at the 100 µm Fig. 5. The interaction effect between all different levels of $CuSO_4$ and intervals subcultures showed big impact on growth vigor/cluster explants. Where the highest significant value of growth vigor/cluster explants was recorded the same value after 6 weeks on $CuSO_4$ at 25 and 50 µM (4.0).

The Chl *a*, Chl *b*, total Chl and car content in the multiplication *in vitro* of date palm *cv.* Yellow Maktoum increased with the increasing copper concentrations up to the level 50.0 µM $CuSO_4$. Higher concentrations of $CuSO_4$ caused a decline in the photosynthetic pigments. Concerning the subculture interval effect, there wasn't significant differences between 4 and 6 weeks of all pigments content (Fig. 6).

Concerning the effect of different levels $CuSO_4$ and two types of subculture interval on total protein content where cu is an important part of protein involved in plant metabolic processes such as photosynthesis and it is an essential micronutrient required for suitable plant growth. Clearly from data in Fig. 7 showed that low concentrations of $CuSO_4$ (10.0 and 25.0 µM) encouraged increasing significant of total protein content compared with the higher concentrations (50.0, 75.0, 100.0 µM) both of the subculture interval under investigation (4 and 6 weeks). With regard to the effect of subculture interval on total protein content during multiplication stage of date palm, 4 weeks was the perfect than 6 weeks of total protein content.

The effect of different concentrations of $CuSO_4$ and subculture interval on proline content was recorded in Fig. 8, the proline accumulation increased significantly by increasing $CuSO_4$ concentrations and the maximum proline content of 0.77 or 0.78 mg g^{-1} (fw) was on MS medium containing either 75.0 or 100.0 µM $CuSO_4$ without significant differences in between. On the other hand, there aren't significant differences in between 4 weeks and 6 weeks as a subculture interval on proline content.

Effect of zinc sulphate ($ZnSO_4$) on roots growth

Shootlet derived from somatic embryos (5-7 cm shoot length and 2 leaves/plantlet) were cultured on rooting medium of date palm cv. Maktoum supplemented with different concentration of $ZnSO_4$ (0.0, 10.0, 25.0, 50.0, 75.0 and 100.0 µM) in formula of MS nutrient salts in order to enhance and encourage root formation Fig. 9.

Data in Table 9. showed clearly effect different concentrations $ZnSO_4$ on root growth of shootlets date palm cv. Maktoum for three subcultures. The addition of $ZnSO_4$ with different concentrations from 10.0 to 100.0 µM to rooting medium was beneficial to form good root system compared to the control treatment (having normal zinc concentration in MS basal medium).

The high concentrations of $ZnSO_4$ promoted roots

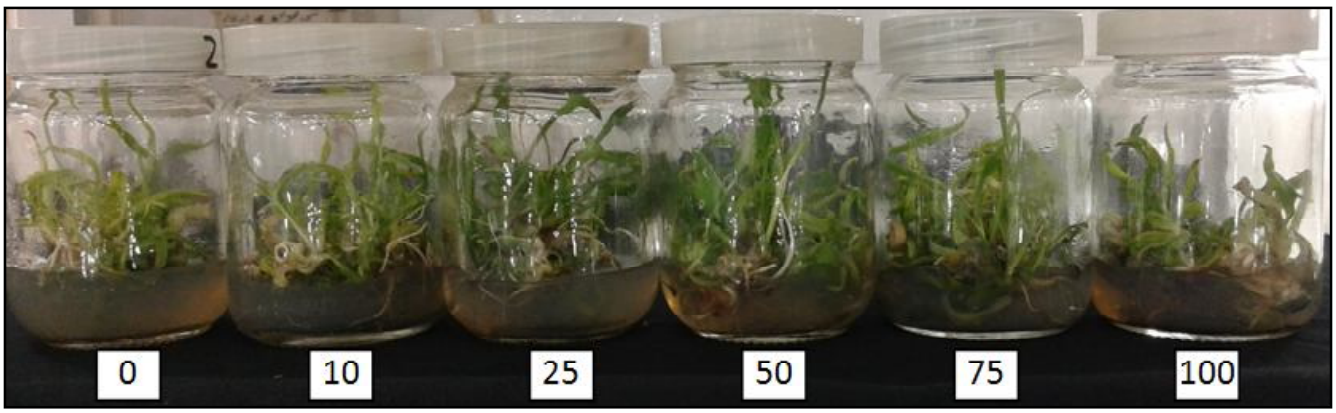

Fig. 5: Effect of different concentrations $CuSO_4$ on shoot growth.

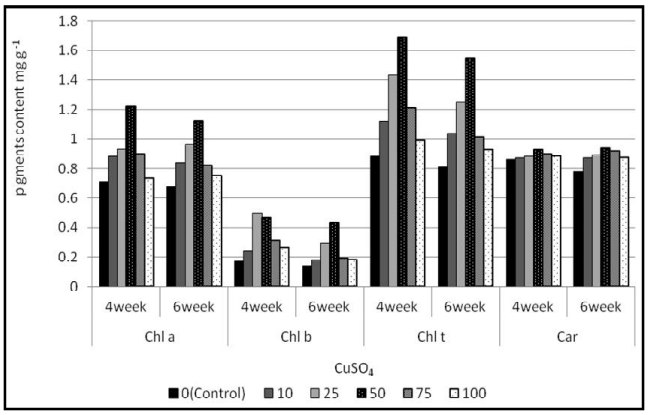

Fig. 6: Effect of different concentrations $CuSO_4$ and two types of subculture interval (4 and 6 weeks) on the pigments content (Chl a, Chl b, Chl t and Car mg.g^{-1} FW) of date palm cv. Yellow Maktoum.

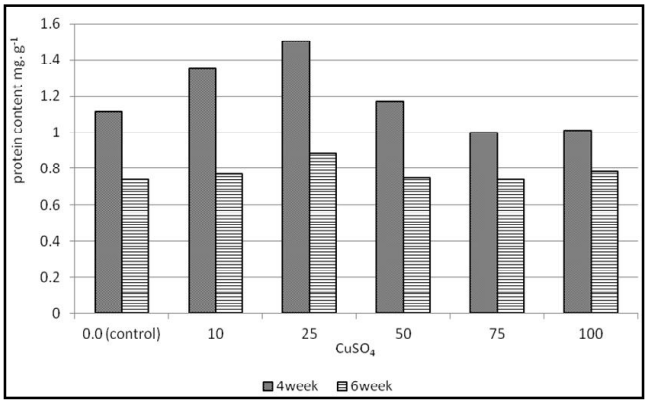

Fig. 7: Effect of different concentrations $CuSO_4$ and two types of subculture interval (4 and 6 weeks) on protein content (mg.g^{-1} FW) of date palm cv. Yellow.

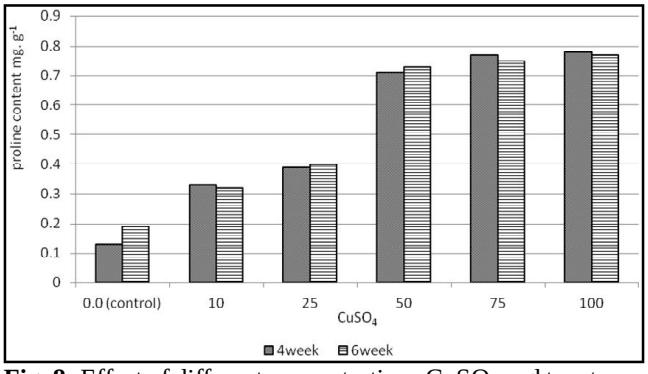

Fig. 8: Effect of different concentrations $CuSO_4$ and two types of subculture interval (4 and 6 weeks) on proline content (mg.g^{-1} FW) of date palm cv. Yellow Maktoum.

growth, where $ZnSO_4$ at 100.0 µM was the superior concentration of shoots length (12.5 cm/explant), number of roots (8.1/explant) and root length (8.0 cm/explant) comparative with control medium and other treatments. Further the shootlets which cultured on rooting medium containing $ZnSO_4$ at 100.0 µM appeared stronger shootlets and well green color without marks of browning or welting as the best sign of growth vigor of shootlets explants (4.0 /shootlet explant).

Effect of copper sulphate ($CuSO_4$) on roots growth

Data in Table 10 showed the effect of copper sulphate ($CuSO_4$) on root growth during rooting stage of date palm cv. Yellow Maktoum for three subcultures. The addition of $CuSO_4$ at 75.0 µM in formula of MS nutrient salts achieved the highest significant value of shoots length (13.4 cm/explant) and number of roots (9.2 root/explant) after three subcultures from culturing. While either concentrations 25.0 and 50.0 µM of $CuSO_4$ were the optimum concentration of root length without significant differences in between (8.3 and 8.6 cm /explant respectively). As regard growth vigor of shootlets date palm cv. Yellow Maktoum during rooting stage, data clearly observed that growth vigor as a sign of stronger shootlets increased with increasing $CuSO_4$ level in formula of MS nutrient salts from 10.0 to 100.0 µM (2.4, 3.2, 3.8, 3.8, 4.0 and 4.0/shootlets explant respectively) (Fig. 10).

Generally, the highest concentrations of $ZnSO_4$ (100.0 µM) and $CuSO_4$ (75.0 µM) were the best concentrations of all growth parameters during rooting stage *in vitro* (shoots length, number of roots, roots length and growth vigor) to produce optimize plantlets able to transfer acclimatization stage successfully.

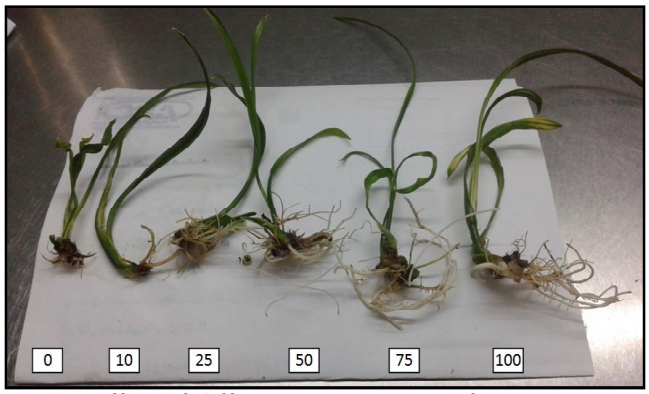

Fig. 9: Effect of different concentration of $ZnSO_4$ on root growth.

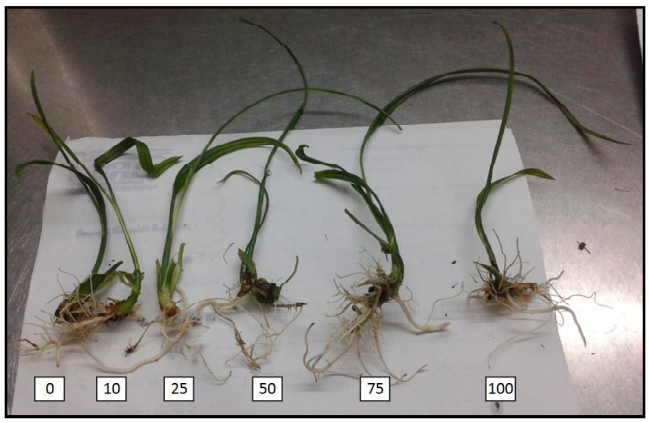

Fig. 10: Effect of different concentration $CuSO_4$ on root growth.

Eventually, all plantlets have been received from using $ZnSO_4$ or $CuSO_4$ at the certain best treatments during multiplication stage were accelerated in growth during rooting stage as well as the all plantlets have been received from using $ZnSO_4$ or $CuSO_4$ at the certain best treatment during rooting stage were collected and transferred to preacclimatization liquid medium. All rooted plantlets were transferred to green house for acclimatization with maintaining high humidity by covering transparent polyethylene bags. These plantlets achieved 90% survival rate after 6 months from culturing in the greenhouse. Thus our results promote the process of date palm micropropagation.

Discussion

In the present study, date palm *cv.* Yellow Maktoum was grown *in vitro* and the effect of different levels of $ZnSO_4$ and $CuSO_4$ were assessed during multiplication and rooting stages after three subcultures. We observe here that manipulating the salt strength might also modify the growth of plantlets since a suitable salt strength may work as important elicitor of *in vitro* morphogenesis. Furthermore, cell growth and morphogenesis of some species may be enhanced by increasing levels of mineral salts above those recommended by Murashige and Skoog (1962).

Zinc is an essential micronutrient of growth medium that is needed by plants for growth and various biochemical and physiological process such as protein synthesis, enzyme activation and growth regulation and the maintenance of membrane structure. It has been confirmed by our results whereas, there was concomitant increase in all growth parameters (number of shoots, number of secondary embryos, shoots length (cm), growth vigor) with increasing concentration of zinc till the level for mineral is optimized at 75.0 µm and thereafter decreased. The positive role of $ZnSO_4$ has been documented earlier by Ali *et al.,* 1999 in *Bacopa monniera*, Sharma *et al.,* 2010 in *Pisum sativum* L. *Cicer arietinum*, Fatima *et al.,* 2011 in *Withania somnifera* and El-Jassani 2013 in *Phoenix dactylifera*. Also Shahid *et al.,* 2015 declared that, optimized MS medium supplemented with different concentration of $ZnSO_4$ was better than control with respect to multiple shoot induction and also length of shoots. Medium containing $ZnSO_4$ (100 µM) induced maximum number (5.20 ± 0.37) of shoots with shoot length (0.80 ± 0.027 cm). Similar stimulatory effect of zinc in MS basal nutrient medium has been reported by Ahmed *et al.,* 2015 who said, incorporation of 25 µM $ZnSO_4$ to a Murashige and Skoog (MS) medium with optimized concentrations of auxins and cytokinins induced a maximum number of shoots per explant (45.47 ± 0.24) in *Rauvolfia serpentina*. The level of $ZnSO_4$ above the optimum prove to be inhibitory for shoots formation. In this study, the Chl *a* Chl *b* and Car increase up to the optimal Zn concentration (50.0 µM) and thereafter decreased. High concentration of $ZnSO_4$ (100.0 µM) inhibited Chl total and Car content this result is in accordance with the earlier reports like Khudsar *et al.,* 2004 in *Artemisia annou*, Sharma *et al.,* 2010 in *Pisum sativum* and Ahmed *et al.,* 2015 in *Rauvolfia serpentine*.

Our results proved that, the optimum level of $ZnSO_4$ (50.0 Mµ) increase total protein content but above 100.0 Mµ decrease total protein content of date palm shoots during multiplication stage, these results are line with Ali *et al.,* 2000; Hansch and Mendel 2009; Tsonev and Lidon 2012 who reported that, Zn is implicated in protein synthesis and energy production. It is also involved in nucleic acid synthesis, carbohydrate and lipid metabolisms. Proline accumulation increased with increasing $ZnSO_4$ concentrations in the current study, Parlak and Yilmaz 2012 said that proline content increased under Zn in three tested plants, Lemna gibba, Lemna minor, and *Spirodela polyrrhiza* L. Same results were in agreement with Al Khateeb and Al-Qwsemeh 2014 who reported that proline content of both S. nigrum and S. lycopersicum increased with high concentrations of Zn as a result of stress. Proline prevents membrane damage and had a protective role in lipid proxidation induced by metals (Thounaojam *et al.,* 2012). On the other hand current results declared that, the subculture interval every 6 weeks are the better than 4 weeks and these results agree with (Haris and Mantell 1991) and (Grant and Hammatt 1999).

Copper is a micronutrient important for normal plant growth and development. It takes part in processes of photosynthesis, respiration, transport and other physiological and biochemical functions (Yruela 2005). Our results from Tables 5, 6, 7 and 8 indicated that, addition different concentrations of $CuSO_4$ in the MS medium exhibited good growth and healthy of number of shoots, number of embryos, shoots length (cm) and growth vigor/ cluster explant of two types of subculture intervals (4 and 6 weeks) compared with control medium except for the higher concentration of $CuSO_4$ (100.0 Mµ) exhibited weak shoots and symptoms of toxicity. In accordance to an earlier report by Joshi and Kothari 2007 on *Capsicum annuum*, the increasing of Cu concentration in the medium significantly favored the induction of shoot-buds and their elongation in the second stage subculture. Similar findings were also reported by *Elucine coracana* (Kothari *et*

al., 2004) *Stevia ebaudiana* (Jain *et al.,* 2009), *Withania somnifera* (Fatima *et al.,* 2011), *Phoenix dactylifera* (Madi and Al Mayahi 2014) and *Capsicum annuum* (Grozeva 2015). Also Shahied *et al.,* 2015 tested effect different concentrations of copper sulphate (in increasing order) for nodal explant culture of *Rauvolfia tetraphylla*. The cultures maintained on different concentrations of copper sulphate exhibited better growth and multiplication in comparison to control after 4 weeks of culture.

Cu is an important constituent of several enzymes like cytochrome oxidase, ascorbic oxidase, phenolase, diamine oxidase, super-oxide dismutase, as well as plastocyanin, a pigment participating in electron transfer. The optimum Cu concentration in medium positively affects development of membrane system of chloroplasts and Chl content. In our experiments the Chl *a*, Chl *b*, total Chl and car content in the multiplication *in vitro* of date palm *cv.* Yellow Maktoum increased with the increasing copper concentrations up to the level 50.0 µM $CuSO_4$, which is in accordance with earlier reports such *Lupinus luteus* (Mourato *et al.,* 2009), *Jatropha curcas* (Khurana-Kaul *et al.,* 2010) and *Rauvolfia serpentine* (Ahmed *et al.,* 2015). Higher concentrations of $CuSO_4$ caused a decline in the photosynthetic pigments this result agree with (Romeo-Puertas *et al.,* 2004 and Vuksanoviæ *et al.,* 2017).

The stimulating effect of Cu can be ascribed to its role in several metabolic activities like protein and carbohydrate metabolism. The various Cu containing enzymes involved in electron transport, protein and carbohydrate biosynthesis might play a role in plant regeneration. From our results in Fig. 7, the low concentrations of $CuSO_4$ (10.0 and 25.0 µM) encouraged increasing significant of total protein content compared with the higher concentrations (50.0, 75.0, 100.0 µM) these results were supported by Kothari *et al.,* 2008 and Shahied *et al.,* 2015.

In the present study, proline accumulation increased by increasing $CuSO_4$ concentrations in MS medium and the maximum proline content of 0.78 mg g^{-1} FW was on the MS medium containing 100.0 µM $CuSO_4$ (Fig. 8). These results agree with Fatima *et al.,* 2011 of *Withania somnifera* and Al-Khateeb and Al-Qwasemeh 2014 of Solanum nigrum**.** The production of proline at higher Cu concentrations correlated with a lower regeneration frequency and a decrease in biomass and pigment content. Such toxic responses of Cu have recently been reported (Jain *et al.,* 2009 and Ahmed 2015). On the other said current results indicated that, the subculture interval every 6 weeks are the better than 4 weeks for all parameters and these results agree with (Haris and Mantell 1991**)** and **(**Grant and Hammatt 1999).

Furthermore, zn influences cell division and cell expansion besides having a role in chlorophyll formation and our study in Table 9 showed that during rooting stage, the addition of $ZnSO_4$ with different concentrations from 10.0 to 100.0 µM to rooting medium was beneficial for good growth of shoots and roots compared to the control treatment (having normal zinc concentration in MS basal medium). Additional supply of zinc in rooting medium stimulated rooting percentage and root length (Ali *et al.,* 2000). Tsui 1948 reported that, zinc is required directly for the synthesis of tryptophane and indirectly for the synthesis of auxins.

Also, our results in Table 10 showed that $CuSO_4$ at 75.0 µM was the best concentrations of all growth parameters during rooting stage *in vitro* (shoots length, number of roots, roots length and growth vigor/explan). These results were line with Jiang *et al.,* 2000 of *Zea mays* and Joshi and Kothari 2007 of *Capsicum annuum* who noticed that 10^{-5} M Cu stimulated root growth. The various Cu containing enzymes involved in electron transport, protein and carbohydrate biosynthesis might play a role in plant regeneration (Purnhauser and Gyulai 1993) that might explain our good results during the rooting stage.

In conclusion, the addition of $ZnSO_4$ and $CuSO_4$ in their different concentrations to the growth medium of date palm cv. Yellow Maktoum during the multiplication stage and also the rooting stage had the greatest effect on the improvement of growth and strong plantlets were able to transfer to the adaptation stage and achieve the highest success rate in agriculture within the acclimatization greenhouse.

Reference

Abahmane, L. (2017). Cultivar-Dependent Direct Organogenesis of Date Palm from Shoot Tip Explants. In: J.M. Al-Khayri S.M. Jain and V.J. Dinnes (eds.), Date palm protocols. Springer, Berlin, 3-16.

Ahmad, N., A.A. ALatar, M.M. Faisal, I. Khan, N. Fatima, M. Anis and A.K. Hegazy (2015). Effect of copper and zinc on the *in vitro* regeneration of *Rauvolfia serpentine*. *Biol. Plant,* **59:** 11-17.

Ali, G., P.S. Srivastava and M. Iqbal (1999). Morphogenic and biochemical responses of Bacopa monniera cultures to zinc toxicity. *Plant Sci.*, **143:** 187–193.

Ali, G., P.S. Srivastava and M. Iqbal (2000). Influence of Cadmium and Zinc on Growth and Photosynthesis of Bacopa monniera Cultivated *in vitro*. *Biol. Plant,* **43:** 599–601.

Al-Khayri, J.M. (2007). Date palm *Phoenix dactylifera* L.

micropropagation. In: S.M. Jain and H. Haggman (eds.), Protocols for micropropagation of woody trees and fruits. Springer, Berlin, 509–526.

Al Khateeb, W. and H. Al-Qwasemeh (2014). Cadmium, copper and zinc toxicity effects on growth, proline content and genetic stability of *Solanum nigrum* L., a crop wild relative for tomato; comparative study. *Physiol. Mol. Biol. Plants,* **20:** 31–39.

Aron, D. (1994). Copper Enzymes In Isolated Chloroplasts. Polyphenoloxidase In *Beta Vulgaris*. *Plant Physiol.,* **24:** 1-15.

Bardar, S., V. Khurana Kaul, S. Kachhwaha and S.L. Kothari (2014). Nutrient optimization for improved *in vitro* plant regeneration in *Eclipta alba* L. Hassk. and assessment of genetic fidelity using RAPD analysis. *Plant Tissue. Cult. & Biotech.,* **24:** 223–234.

Bates, L.S., R.P. Waldren and I.D. Teare (1973). Rapid determination of free proline for water-stress studied. *Plant and Soil,* **39:** 205-207.

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of micrograms quantities of protein utilization the principle of protein – dye binding. *Analytical Biochemistry,* **72:** 248-254.

Brennan, R.F. (2005). Zinc Application and Its Availability to Plants. Ph. D. dissertation, School of Environmental Science, Division of Science and Engineering, Murdoch University.

Broadley, M.R., P.J. White, J.P. Hammond, I. Zelko and A. Lux (2007). Zinc in plants. *New Phytologist,* **173:** 677-702.

El-Dawayati, M.M., Z.E. Zayed and R.A. Sidky (2014). An Efficient protocol for the *in vitro* multiplication of date palm (*Phoenix dactylifra* L.) *cv.* Gondela to optimize shooting stage. *Egypt. J. of Appl. Sic.,* **29:** 318-332.

EL-Jassani, I.F. (2013). Studies on micro propagation of Eucalyptus sp. and *Phoenix dactylifra* L. M.Sc. Thesis, Department of Ornamental, Faculty of Agriculture, Cairo University, Egypt.

Fatima, N., N. Ahmad and M. Anis (2011). Enhanced *in vitro* regeneration and change in photosynthetic pigments, biomass and proline content in *Withania somnifera* L. (Dunal) induced by copper and zinc ions. *Plant Physiol Biochem.,* **49:** 1465–1471.

Fki, L., R. Masmoudi, W. Kriaa, A. Mahjoup, B. Sghaier, R. Masid, A. Mliki, A. Rival and N. Drira (2011). Date palm micropropagation via somatic embryogenesis. In: S. Jain, J. Al-Khairy and D. Johonson (eds.), .p. Date Palm Biotechnology. Springer, Dordrecht, 47-68.

Grant, N.J. and N. Hammatt (1999). Increased root and shoot production during micropropagation of cherry and apple rootstocks: effect of subculture frequency *Tree Physiology*, **19:** 899–903.

Grozeva, S. (2015). Effect of copper levels in the culture medium on shoot regeneration in pepper. *Banat's J. of Biotech.,* **2:** 86-91.

Hänsch, R. and R.R. Mendel (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl), Curr. Opin. *Plant Biol.,* **12:** 259–266.

Harris, R.A. and S.H. Mantell (2015). Effects of Stage II subculture durations on the multiplication rate and rooting capacity of micropropagated shoots of tree paeony (*Paeonia suffruticosa* Andr.), *J. of Horti. Sci.,* **66:** 95-102.

Jain, P.K., S. achhwaha and S.L. Kothari (2009). Improved micropropagation protocol and enhancement in biomass and chlorophyll content in *Stevia rebaudiana* (Bert.) Bertoni by using high copper levels in the culture medium. *Sci. Hort.,* **119:** 315-319.

Jiang, W., D. Liu and X. Liu (2000). Effect of copper on root growth, cell division, and nucleolus of *Zea mays*. *Biol. Plant.,* **44:** 105-109.

Joshi, A. and S.L. Kothari (2007). High copper levels in the medium improves shoot bud differentiation and elongation from the cultured cotyledons of *Capsicum annuum* L. *Plant Cell Tiss Organ Cult.* **88:** 127–133.

Khudsar, T., Mahmooduzzafar, M. Iqbal and R.K. Sairam (2004). Zinc induced changes in morpho-physiological and biochemical parameters in *Artemisia annua*. *Biol. Plant.,* **48:** 255-260.

Khurana-Kaul, V., S. Kachhwaha and S.L. Kothari (2010). Direct shoot regeneration from leaf explants of *Jatropha curcas* in response to thidiazuron and high copper contents in the medium. *Biol. Plant,* **54:** 369–372.

Kothari, S.L., K. Agarwal and S. Kumar (2004). Inorganic nutrient manipulation for highly improved *in vitro* plant regeneration in finger millet-*Eleusine coracana* L. Gaertn. *In vitro Cell Dev Biol. Plant,* **40:** 515–519.

Kothari, C.A., M. Sharma, S. Kachhwaha and S.L. Kothari (2008). Micronutrient optimization results into highly improved *in vitro* plant regeneration in kodo (*Paspalum scrobiculatum* L.) and finger (*Eleusine coracana* L. Gaertn.) millets. *Plant Cell Tiss and Organ Cult.,* **94:** 105 112.

Madi, A. and W. AL-Mayahi (2014). Effect of copper sulphate and cobalt chloride on growth of the *in vitro* culture tissues for date palm (*phoenix dactylifera* L.) *cv.* Ashgar. *Amer. J. of Agric. and Biol. Sci.,* **9:** 6-18.

Mourato, M.P., L.L. Martins and M.P. Campos-Andrada (2009). Physiological responses of *Lupinus luteus* to different copper concentrations. *Biol. Plant.,* **53:** 105-111.

Mujib, A.S., P. Banrjee and D. Ghosh (2005). Origin, development and structure of somatic embryos in selected Bulbous ornamentals: BAP as inducer In: A. Mujib and J. Samaj (eds.), Somatic Embryogenesis, Springer-Verlag, Berlin, Heidelberg, 15-24.

Murashige, T. and F.A. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.,* **15:** 473-479.

Narula, A., S. Kumar and P.S. Srivastava (2005). Abiotic metal stress enhances diosgenin yield in *Dioscorea bulbifera* L. cultures. *Plant Cell Rep.*, **24:** 250–254.

Parlak, K.U. and D.D. Yilmaz (2012). Response of antioxidant defences to Zn stress in three duckweed species. *Ecotoxicol Environ Saf.*, **85:** 52–58.

Pottino, B.G. (1981). Methods in plant tissue culture. Dept. of Hot. Agric, College. Maryland University. 8–29.

Purnhauser, L. and G. Gyulai (1993). Effect of copper on shoot and root regeneration in wheat, triticale, rape and tobacco tissue cultures. *Plant Cell Tiss Org Cult.*, **35:** 131–139.

Ramage, C.M. and R.R. Williams (2002). Mineral Nutrition and Plant Morphogenesis. *In vitro* Cellu. and Develop. *Biol. Plant*, **38:** 116-124.

Romeo-Puertas, M.C., M. Rodriguez-Serrano, F.J. Corpas, M. Gomez, L.A. Del Rio and L.M. Sandalio (2004). Cadmium induced subcellular accumulation of O_2 and H_2O_2 in pea leaves. *Plant Cell Environ.*, **27:** 1122-1134.

Shahid, A., N. Ahmad, M. Anis, A. Alatar and A. Faisal (2015). Morphogenic responses of *Rauvolfia tetraphylla* L. cultures to Cu, Zn and Cd ions. Rend. Fis. Acc. Lincei. DOI 10.1007/s12210-015-0491-5.

Sharma, S.S. and K.J. Dietz (2009). The relationship between metal toxicity and cellular redox imbalance. *Trends in Plant Sci.*, **14:** 43-50.

Sharma, S., P. Sharma, S.P. Datta and V. Gupta (2010). Morphological and Biochemical Response of *Cicer arietinum* L. *var.* pusa-256 towards an Excess of Zinc Concentration. *Life Sci. J.*, **7:** 1-110.

Snedecor, G.W. and W.G. Cochran (1972). Statistical Method 6^{th}. The Iowa State University Press, Ames., Iowa U.S.A., p59.

Thounaojam, T.C., P. Panda, P. Mazumdar, D. Kumar, G.D. Sharma, L. Sahoo and S.K. Panda (2012). Excess copper induced oxidative stress and response of antioxidants in rice. *Plant Physiol Biochem.*, **53:** 33–39.

Tsonev, T. and F.J.C. Lidon (2012). Zinc in plants - An overview. *Emir. J. Food Agric.*, **24:** 322-333.

Tsui, C. (1948). The Role of Zinc in Auxin Synthesis in the Tomato Plan. *Americ. J. of Bot.*, **35:** 172-179.

Vuksanoviæ, V., B. Kovaèeviæ, M. Kataniæ, S. Orloviæ and D. Miladinoviæ (2017). *In vitro* Evaluation of Copper Tolerance and Accumulation in *Populus nigra Arch Biol Sci.*, **69:** 679-687.

Yruela, I. (2005). Toxic metal in plants. Copper in plants. *Brazil. J. of Plant Physiol.*, **17:** 145–156.

Zayed, Z.E. (2014). Effect of different types of cytokinins on the regeneration ability of direct somatic embryos and adventitious shoots induced from immature inflorescences of date palm. *Egypt. J. of Appl. Sci.*, **29:** 142-153.

Zayed, Z.E. (2017). Enhanced Indirect Somatic Embryogenesis from Shoot-Tip Explants of Date Palm by Gradual Reductions of 2, 4-D Concentration. In: J.M. Al-Khayri, S.M. Jain and V.J. Dinnes (eds.), Date Palm Biotechnology protocols. Springer, Berlin, 77-88.