Effect of microelements of MS medium in vitro on shoot multiplication of date palm cv Yellow Sukkary.

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Abstract

This study was aimed at standardizing nutrients requirement to increase shoots formation and shoot bud elongation in Date Palm cv.Yellow Sukkary by manipulating microelements levels in MS media and stimulate the production of strong vegetative shoots can be cultured at the stage of rooting easily and effectively. In this work the effect of different microelements of MS medium (Control, H_3BO_3, $FeSO_4.7H_2O$, KI, $NaMoO_4.2H_2O$, $CoCl_2.6H_2O$, $ZnSO_4.4H_2O$, $MnSO_4.4H_2O$ and $CuSO_4.5H_2O$) on shoot proliferation whereas, as the micro salts of MS medium were doubled. $CuSO_4.5H_2O$, $CoCl_2.6H_2O$ and KI concentrations were more effective on number shoot proliferation compared with other treatments and with no significant differences among them. Whereas, $CuSO_4.5H_2O$ was more effective for stimulating the elongation of shoots. The results of leaves SDS-PAGE revealed a total number of eleven bands with molecular weights (MW) ranging from about 15.3 to 82.8 kDa. The analysis of data showed one common band (monomorphic), while the remaining ten bands were polymorphic with 90.9% polymorphisms. Protein band with 78.3 KDa was detected in control and each of Fe_3O_4, EDTA and $MnSO_4$ treatments and absent in another treatments. Lo, protein band with molecular weight 15.1 KDa was present in control and H_3BO_3 treatments and absent in other treatments.

Introduction

Since, the earliest successful demonstrations of date palm micro propagation significant progress has been made to improve plant regeneration through adventitious organogenesis and somatic embryogenesis. Several studies conducted to optimize the regeneration efficiency by examining the components of culture medium including physical status (Fki et al., 2003), sucrose (Vermendi and Navaro 1996; Taha et al., 2001; Fki et al., 2003) ,silver nitrate (Al-Khayri and Al-Bahrany, 2001, 2004) biotin and thiamine (Al-Khayri, 2001) solidifying agents (Taha et al., 2001), salt strength (Taha et al., 2001 ; Al-Khayri, 2003) and partial desiccation (Othmani et al., 2009). Recently, there are eight trace elements considered to be essential for higher plants, Fe, Zn, Mn, Cu, Ni, B, Mo, and Cl. Possibly, other elements will be discovered to be essential because of recent advances in nutrient solution culture techniques and in the commercial availability of highly sensitive analytical instrumentation for elemental analysis. Much remains to be learned about the physiology of micronutrient absorption, translocation and deposition in plants, and about the functions they perform in plant growth and development (Weleh and Shuman, 1995) . Cell growth and morphogenesis of some species may even be promoted by increasing the level of micronutrients above that recommended by Murashige and Skoog medium (1962). The induction and maintenance of callus and growth of cell suspensions of juvenile and mature organs of both Douglas fir and loblolly pine, was said to be improved on LM medium in which Mg, B, Zn, Mo, Co and I were at 5 times the concentration of MS microelements, and Mn and Cu at 1.25 and 20 times respectively (Litvay et al., 1981; Verma et al., 1982). Other authors to have employed high micronutrient levels are Barwale et al., (1986) who found that the induction of adventitious shoots from callus of 54 genotypes of Glycine max was assisted by adding four times the normal concentration of minor salts to MS medium. El Dawayati et al., (2014) conducted that, shoots number increasing can be achieved by duplication in micro elements concentration in MS nutrient salts. This study was aimed at standardizing nutrients requirement to increase

shoots formation and shoot bud elongation in Date Palm cv.Yellow Sukkary by doubling the concentrations of microelements in MS media and stimulate the production of strong vegetative shoots that can be cultured to the stage of rooting easily and effectively Protein analysis is designed to show the status of the cell activity by increasing the micronutrients in culture media.

Materials and Methods

Yellow Sukkary date palm cultivar grown in El-saff- Giza used in this work to study the effect of different microelements of MS medium Control, H_3BO_3, $FeSO_4.7H_2O$, KI, $NaMoO_4.2H_2O$, $CoCl_2.6H_2O$, $ZnSO_4.4H_2O$, $MnSO_4.4H_2O$ and $CuSO_4.5H_2O$,(SIGMA-ALDRICH FLUKA RAIEDL) on shoot proliferation whereas, doubles every element of micronutrients and add to the culture media. The explants used in this experiment were cluster composed of 3 – 5 shoots (0.5 – 1 cm in length) separated from previous proliferating culture at which subculture. The concentrations (mgl-1) in this experiment were as follows:

Control (MS basal medium+0.05 BA+0.1 NAA), 0.0124 H_3BO_3, 0.0557 $FeSO_4.7H_2O$,

0.00166 KI, 0.0745 Na_2EDTA, 0.00005 $CoCl_2.2H_2O$, 0.0172 $ZnSO_4.7H_2O$, 0.0446 $MnSO_4.4H_2O$, 0.00005 $CuSO_4.5H_2O$.

Each treatment included 3 replicates, each replicate = 3 jars and each jar containing one cluster as an explant. All culture jars were incubated in growth room at 27±1 °C under 16 hrs /day exposure to moderate light intensity of 3000 lux illumination(according to Madhuri et al. 1998).

The explants of each multiplication medium were repeatedly subcultured for 2 times (2 subcultures) at 6 week interval into a fresh medium.

Data were calculated in every multiplication culture medium after each subculture. The data were taken as follows:

a) Number of Shoots. b) Shoots length (cm). c) Number of secondary embryos. d) Protein content, the method was as follow:

Protein extraction

Samples of date palm cv.yellow Succary treated with different doses with gamma radiation. total soluble protein were extracted by grounding 0.25 g of each sample in 0.9 ml extraction buffer (10 ml 0.5 MT ris PH6.8, 16 ml 10% SDS, 30 ml D.W) with shaking thoroughly. The extracts were transferred to Eppendorf tubes and centrifuged for 10 min. at 10000 rpm under cooling. Supernatants were transferred fresh tubes and used for SDS-PAGE analysis to as used described by Jonathan and Weeden (1990).

Protein related index

Fractionation electrophoresis was performed under identical conditions on sodium dedocyl sulphate polyacrylamide gel (SDS-PAGE) (12% W/V) vertical slab using BIORAD Tech ware 1.5 mm according to the method of Laemmli (1970). The molecular weights of proteins were estimated relative to marker, a wide range molecular weight protein (Fermentas com.).

At the end of two subcultures, all developed plantlets were transferred to1/2 MS basal medium without any addition of growth regulators and supplemented with activated charcoal (1gl-1) for 4 weeks. And then the plantlets were transferred to rooting medium consisted of 1/2 MS salts and 0.1 NAA and 6gl-l Poly ethylene glycol (PEG) for rooting stage (Sidky et al., 2009)

RESULT AND DISCUSSION

The multiplication stage were carried out to study the effect of doubling micro salts of MS medium on shoot proliferation. Data in Table (1) and Fig. (1) showed the multiplication concentration effect for each element of micronutrients on the number of shoots, length and the number of secondary embryos. Data clearly showed that, the addition of copper sulfate salt, potassium iodide and chloride cobalt with doubled concentrations in the MS medium during the multiplication stage led to increased number of shoots, vegetative by produced with no significant differences among them (16.33, 15.50 and 15.00 shoots/culture respectively). The lowest value of shoot number were recorded with the addition of manganese sulfate doubled to culture medium or control medium. It can be indicated that duplication of micro elements concentration in formula of MS nutrient salts gave the highest significant increase in shoots number/ clusters explants wich agrees with(El-Dawayati, 2014).

Regarding the elongation of shoots produced found that, copper sulfate salt achieved the highest value of shoots length (3.08cm), while, the shoots were short when adding iron sulfate doubled on MS medium. The production of secondary embryos, was formed on the base of developed shoots. It was clearly observed that control medium was more effective for stimulating the production of secondary embryos. While, Na_2EDTA, KI and $MnSO_4.4H_2O$ treatments recorded the lowest values of average number of somatic embryo with no significant differences among them (5.16, 5.33, and 6.16 respectively).

Table 1. Effect of different microelement of MS medium on in vitro shoot proliferation of date palm cv. "Yellow Sukkary" after 2 subcultures.

Treatments mgl-1	Av. No. of shoots	Av. shoots length (cm)	Av. No. of secondary embryos
Control	12.16 ab	2.41 ab	13.50 a
0.0124 H_3BO_3	8.50 b	2.25 ab	8.50 ab
0.0557 $FeSO_4.7H_2O$	12.83 ab	1.66 b	11.50 ab
0.00166 KI	15.00 a	2.25 ab	5.33 b
0.0745 Na_2EDTA	12.83 ab	2.58 ab	5.16 b
0.00005 $CoCl2.2H_2O$	15.50 a	2.25 ab	11.66 ab
0.0172 $ZnSO_4.7H_2O$	13.50 ab	2.16 ab	8.00 ab
0.0446 $MnSO_4.4H_2O$	12.33 ab	2.58 ab	6.16 b
0.00005 $CuSO_4.5H_2O$	16.33 a	3.08 a	8.33 ab
Mean	13.22	2.36	8.68

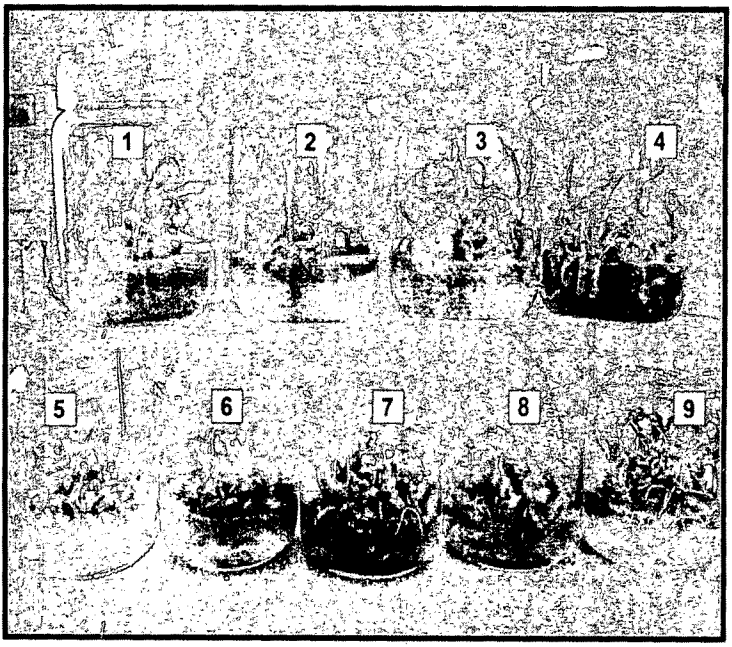

Fig. 1. Effect of double fold of the microelements of MS media on shoots multiplication of date palm "Yellow Sukkary" cv.

Protein content

leaf proteins provides valuable evidence for taxonomic and evolutionary relationships of plant species (Yates et al., 1990).It is worthy to note that, seed protein profiles are often species – specific, highly stable and unlikely to be not influenced by environmental conditions and seasonal fluctuations.

Table2: SDS-protein banding patterns of leaf proteins for date palm cv. "Yellow Sukkary" with different treatments by gamma radiation compared with control.

Band No.	MW KDa	Control	H_3BO_3	$FeSO_4 \cdot 7H_2O$	KI	Na_2 EDTA	$CoCl2 \cdot 2H_2O$	$ZnSO_4 \cdot 7H_2O$	$MnSO_4 \cdot 4H_2O$	$CuSO_4 \cdot 5H_2O$
1	92.8	0	0	1	0	1	0	0	1	0
2	85.6	0	0	0	0	1	0	0	1	0
3	78.3	1	1	1	0	1	0	0	1	0
4	71.3	0	1	1	1	1	1	0	1	0
5	40.2	0	0	0	0	1	0	0	1	0
6	35.7	0	1	0	0	1	0	1	1	0
7	31.9	0	0	0	0	1	0	1	1	0
8	26.6	1	1	1	1	1	0	1	1	0
9	22.3	0	0	1	1	1	1	1	1	0
10	17.3	1	1	1	1	1	1	1	1	1
11	15.1	1	1	0	0	0	0	0	0	0
Total	4	6	6	4	10	3	5	10	1	

SDS-Protein electrophoresis in leaves:

The electrophoretic banding patterns of proteins extracted from leaves of the nine Date Palm treatments are shown in Fig. (2).Their densitometric analyses are illustrated in Table (2). The presence and absence of bands were assessed with (1) and (0), respectively.

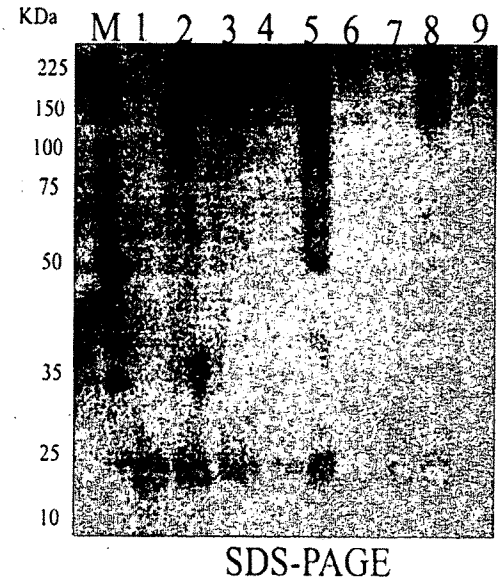

Fig. 2. Effect of different microelement of ms medium on protein content of date palm cv.Yellow Sukkary after 2 subculture.

The results of leaves SDS-PAGE revealed a total number of eleven bands with molecular weights (MW) ranging from 15.3 to 82.8 kDa. The analysis of data showed one common band (monomorphic), while the remaining ten bands were polymorphic with 90.9% polymorphisms. Protein band with 78.3 KDa was detected in control and each of Fe_3O_4, EDTA and $MnSO_4$ treatments and absent in other treatments. Lo, protein band with molecular weight 15.1 KDa was present in control and H_3BO_3 treatments and absent in other treatments. On the other hand, control and some treatments were showed present band at 26.6 KDa except two treatments (CoCl2 and $CuSO_4$) were absent bands, Protein band in control was absent at 92.8, 85.5, 71.3, 40.2, 35.7, 31.9 and 22.3 KDa respectively and was present in other treatments. These results agree with Shankhadhar, et al.,2000

which declared that, the regeneration frequency in salt stressed callus was also lower as compared to control. 15 d and 30 d after inoculation proline content increased several fold whereas total protein content decreased markedly with increase in salt concentration.

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تأثير مضاعفة العناصر الصغرى فى بيئة موراشيجى وسكوج على نباتات نخيل البلح صنف السكرى الأصفر خلال مرحلة التضاعف

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الملخص

الكلمات الدالة: نخيل البلح، بيئة موراشيجى وسكوج، العناصر الصغرى، تضاعف الأفرع، تحليل البروتين

تهدف هذه الدراسة إلى تحديد المتطلبات من المواد المغذية التى تؤدى إلى زيادة تكوين الأفرع الخضرية واستطالتها في نخيل البلح صنف سكرى الأصفر وذلك عن طريق أحداث تغيرات فى مستويات العناصر الصغرى في بيئة MS وتنشيط إنتاج أفرع خضرية قوية يمكن نقلها و زراعتها فى مرحلة التجذير بسهولة وفعالية. وقد قمنا في هذا العمل بدراسة تأثير العناصر الصغرى المختلفة من بيئة MS medium (Control، H_3BO_3، $Fe_3SO_4.7H_2O$، KI، $NaMoO_4.2H_2O$، $CoCl_2.6H_2O$، $ZnSO_4.4H_2O$، $MnSO_4.4H_2O$، $CuSO_4.5H_2O$)

على إنتاج الأفرع الخضرية، حيث تم مضاعفة كل عنصر من العناصر الصغرى فى بيئة الزراعة. وكانت معاملات KI ، $CoCl_2.6H_2O$، $CuSO_4.5H_2O$ أكثر فعالية فى زيادة عدد الأفرع مقارنة مع المعاملات الآخرى وليس هناك أي فروق ذات دلالة إحصائية فيما بينها. في حين كان $CuSO_4.5H_2O$ أكثر فعالية لتحفيز استطالة الأفرع. وكشفت نتائج أوراق تحليل البروتين SDS-PAGE أن إجمالي عدد أحد عشر شريط مع الأوزان الجزيئية (MW) تتراوح بين 82،8-15،3 كيلو دالتون. أظهر تحليل بيانات الموجات مشترك واحد (monomorphic)، في حين كانت الشرائط العشرة المتبقية متعددة الأشكال مع 90.9 ٪. تم الكشف عن البروتين مع الفرقة 78.3 كيلو دالتون، وكل من EDTA، Fe_3O_4 والمعاملة $MnSO_4$ بينما كانت غائبة في المعاملات الأخرى، وكان البروتين مع الوزن الجزيئي 15.1 كيلو دالتون موجودة في H_3BO_3 وغائبة في المعاملات الأخرى.

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