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EFFECT Of SOME AMINO ACIDS, VITAMINS AND PLANT GROWTH REGULATORS ON MAINTENANCE AND LENGTH OF PLANTLETS DERIVED FROM IN VITRO CULTURE OF DATE PALM (Phoenix dactyliferaL.).CV.Barhee

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Abstract

An experiment was conducted at Date Palm tissue culture laboratory-Date Palm Research Center-Basrah University to study the effect of some amino acids, vitamins and plant growth regulators on maintenance and length of plantlets derived from tissue culture of date palm (Phoenix dactylifera L.)cv. Barhee. The obtained results showed that, MS medium that contained a combinations of amino acids (100mg/L glutamine,25mg/l proline,25mg/L asparagine and 50mg/L arginine) and vitamins at (2,2,3,1 and 1,2,2,2mg/L) from biotin, pyridoxine, c-Pantothenate and riboflavin in the presence 0.5 mg/L IBA and 0.4 mg/L BA was the best combinations in the maintenance and Length of date palms plantlets. The 3rd and 4th amino acids treatments whth2nd and3rd combination of vitamins in MS medium contains IBA+BA steadily increased the plantlets Length compared with other treatments. In addition, it was noticed that 1st and 2nd treatments of amino acids, gave the lowest values in plantlets Length in MS medium free of growth regulators regardless the combinations of vitamins. The plantlets were grown up best without circumvolution in MS medium supplemented with growth regulators, and ready to be taken for laboratory acclimatizing process.

الملخص :

أُجريت هذه الدراسة في مختبر زراعة الأنسجة التابع لمركز أبحاث النخيل والتمور–جامعة البصرة-العراق.بهدف دراسة تأثير بعض الأحماض الأمينية والفيتامينات ومنظمات النمو في طول وإدامة النبيتات الناتجة من الزراعة النسيجية.

بينت النتائج المتحصل عليها من هذه الدراسة أن توليفة الأحماض الأمينية (100ملغم/لتر جلوتامين و25 ملغم/لتر برولين و25 ملغم/لتر اسباراجين و50 ملغم/لتر أرجنين) وتوليفة الفيتامينات (2،2،1،3و2،2،2،1 ملغم/لتر) المكونة من البايوتين والبيريدوكسين والكالسيوم-بنتوثينيت والرايبو فلافين المضافة إلي الوسط الغذائي المزود بـ0.5 ملغم/لتر IBA و0.4 ملغم/لتر BA كانت أفضل التوليفات تأثيرا في إطالة إدامة نبيتات نخيل التمر. أدت توليفات الأحماض الأمينية الثالثة والرابعة مع التوليفات الثانية و الثالثة من الفيتامينات في وسطMS المزود بـIBA وBA إلي زيادة مطرده في طول النبيتات مقارنة بالتوليفات الأخرى.وقد لوحظ أن التوليفات الأولى والثانية من الأحماض الأمينية في الوسط الغذائي الخالي من منظمات النمو قد أعطت اقل التأثيرات في طول النبيتات بغض النظرعن توليفات الفيتامينات. كان نمو النبتات جيداً في الوسط الحاوي على منظمات النمو ولم تتأثر بظاهرة الالتفاف وبدت صالحة لعمليات الأقلمة المختبرية

INTRODUCTION :

L-glutamine can serve as the sole source of nitrogen which, can be taken more rapidly than inorganic nitrogen (Thorn *et aI*., 1980) .Abou El-Nil(1989) indicated that amino acids stimulated Date palm callus growth and ranked as follows: glutamine, asparagine, arginine, serine, glycin and alanine. Glutamine caused doubling callus growth compared to control.Saad (2001) reported that the addition of glutamine at(200 and400mg/L) enhanced the somatic embryos germination and the production of plantlets *in vitro*. Hassan(1987) studied five vitamins effects on growth of shoot-tip and proliferation of callus, he found that no significant effect of a vitamin eliminated from a group and he has a different response according to the variety. Al-Khayri(2001)) reported that, the addition of a combination from 0.5mg/L thiamine-HCl and 2mg/L biotin was efficient for embryogenic callus growth, and 0.5mg/L thiamine-HCl and 2 or1mg/L biotin for somatic embryos

protraction.This experiment was conducted to determine the efficacy of combinations from amino acids, vitamins and plant growth regulators on maintenance and Length of plantlets derived from tissue culture of date palm.Cv.Barhee.

MATERIAL AND METHODS :

This experiment was conducted on plantlets of one of the main commercial cultivar Barhee at the Date Palm Research Center – Tissue Culture Laboratory- Basrah University, Iraq.

Culture medium:

The composition and concentration of the medium used in this work were according to Murashige and Skoog (1962).The water used for preparation of the medium was distilled and sterile. The medium was solidified with 0.5% agar; pH was adjusted to 5.8 before autoclaving. The prepared media were poured into test tubes, each contained about 25ml solidified medium. The combinations of studied factors were (biotin +pyridoxine + c-pantothenate + riboflavin) for vitamins,(glutamine +proline +asparagines +arginine) for amino acids and IBA+BA as growth regulators. Media were autoclaved at 121°C and 1.2 Kg/cm^2 for 20min.

Plant materials:

Germinated somatic embryos equalized in age(2 weeks after germination) and Length (2cm) were used in this experiment. Then they transferred to test tubes contained 25 ml fresh MS-medium treated with different combinations

Incubation conditions:

To study the effect of the interactions between the three factors on maintenance and Length of date palm plantlets, the cultures were incubated in growth chamber at 27°C± with 16-hours photoperiod at 1000 Lux. The growth was determined by measuring the length of plantlets at the end of the 4 weeks incubation period. The experiments were repeated twice.

Design and statistical analysis:

Experiments organized as factorial experiments by using the complete randomized design (C.R.D) with five replicates per treatments, each replicate contained five test tubes. The obtained data were analyzed by SPSS statistical Program (version 14.0). The significant differences between the averages were tested with (Revised Least Significant Design) test at $P<0.05$ (Al-Rawi and khalaf Allah, 1980)

RESULTS AND DISCUSSION

Effect of amino acids and vitamins on plantlets maintenance and Length:

Data in table (1) show a significant differences among the treatments, the best interaction between amino acids and vitamins was obtained at the

Table(1):Independent effects of amino acids and vitamins on plantlets maintenance and Length(cm)

Amino acids Glu+Pro+Asp+Arg Mg / L				Vitamins Bio + Pyri + C-Pent + Ribof Mg / L			
				2 2 2 2	2 2 3 1	1 2 2 2	1 2 3 2
200	25	50	50	5.18 h	8.01 kl	8.08 j	8.13 i
200	25	25	50	8.28 g	8.51 f	8.00 l	8.05 jk
100	25	50	50	10.25 e	10.73 c	10.30 d	10.25 e
100	25	25	50	10.98 a	10.85 b	10.85 b	10.85 b

***Diverse letters indicate to the existence of a significant difference at P<0.05**

combination of amino acids100mg/L Glutamine, 25mg/L Proline,25mg/L Asparagine and 50mg/L Arginine with the combination of vitamins 2mg/L Biotin, 2mg/L Pyridoxine, 2mg/L C-Pantothenate and 2mg/L Riboflavin ,the average of effect was 10.98cm.While the first combination of amino acids 200mg/LGlutamin,25mg/L Proline,50mg/L Asparagine and 50mg/L Arginine with the same combination of vitamins exhibited a lower effect on plantlets maintenance and Length, the average decreased to 5.18cm The physiological role of amino acids in Krebs cycle that enhanced different metabolism processes with the aid of vitamins which execute a role of Co-A, may be the cause,the best effect on plantlets maintenance and Length at suitable concentrations. This results was not mentioned in early researches

Effect of amino acids and Plant growth regulators on plantlets maintenance and Length:

MS medium supplemented by auxins and cytokinins in presence of fitting

Table(2): Independent effects of amino acids and Plant growth regulators on plantlets maintenance and Length(cm)

Amino acids Glu+Pro+Asp+Arg Mg / L				PGRs	
				Free of PGRs	IBA+BA
200	25	50	50	7.07 ef	9.11 e
200	25	25	50	7.04 f	9.38 c
100	25	50	50	9.26 d	9.43 b
100	25	25	50	9.30 d	12.46 a

***Diverse letters indicate to the existence of a significant difference at P<0.05**

combination of amino acids, showed the best effect on plantlets maintenance and Length that reached just 12.46cm. The MS medium free of growth regulators exhibited a lower effect on studied characters that recorded 7.07 The fitting treatment was 100mg/L Glutamine, 25mg/L Proline,25mg/L Asparagine and 50mg/L Arginine with IBA and BA may be encouraged plantlets in early stages to achieved plus cellular divisions and develop and cause a protraction of plantlets (Table 2).

Effect of vitamins and Plant growth regulators on plantlets maintenance and Length:

The protraction of plantlets due to the interaction effect amid vitamins and growth regulators was less than the effect of amino acids with the IBA+BA. Best effect was obtained at the treatment (2, 2, 3,1mg/L) of vitamins with IBA+BA that reached to 10.80cm (Table 3). The MS medium devoid from growth regulators with a vitamins combination of(1,2,2,2mg/L) showed less effect on plantlets height that marked 8.09cm.These results give the evidence of the necessity of growth regulators in the maintenance and Length media to obtain the best effects.

Table (3) Independent effects of vitamins and Plant growth regulators on plantlets maintenance and Length(cm)

Vitamins Bio + Pyri + C-Pent + Ribof Mg / L	PGRs	
	Free of PGRs	IBA+BA
2 2 2 2	8.16 e	10.66 b
2 2 3 1	8.34 d	10.80 a
1 2 2 2	8.09 f	10.53 c
1 2 3 2	8.13 ef	10.51 c

***Diverse letters indicate to the existence of a significant difference at P<0.05**

Effect of interactions between amino acids, vitamins and Plant growth regulators on plantlets maintenance and Length:

The best effects of amino acids combinations obtained were (100, 25, 25,50mg/L) and (100,25,50,50mg/L) that attained to 10.88cm and 10.38cm in succession (Table 4,Figure 1) and a lower effect was at(200,25,50,50 mg/L),this results agreed with Abdel-Rahim *et al*.,(1998),they reported that, the addition of amino acids all together was better than individually .In and concern to vitamins, the best combination was(2 ,2, 3, 1mg/L) And the lower effects were at the combinations (1 ,2 ,2, 2 and 1 ,2 ,3 ,2 mg/L) that decreased to 9.31cm and 9.32cm in succession, and this agreed with Al-Khayri(2001),who found that the addition of a combination from 0.5mg/L thiamine-HCl and 2mg/L biotin

Table (4) Effect of amino acids, vitamins and Plant growth regulators on plantlets maintenance and Length(cm)

Amino acids Glu+Pro+Asp+Arg Mg / L	PGRs	Vitamins Bio + Pyri + C-Pent + Ribof Mg / L				Amino acids average	PGRs average
		2 2 2 2	2 2 3 1	1 2 2 2	1 2 3 2		
200 25 50 50	Free of PGRs	7.10	7.02	7.05	7.10	8.09 D	8.17 B
200 25 25 50		7.05	7.00	7.00	7.10		
100 25 50 50		9.00	9.95	9.10	9.00	8.21 C	
100 25 25 50		9.50	9.20	9.20	9.30		
200 25 50 50	IBA+BA	9.20	9.00	9.10	9.15	10.38 B	10.61 *A
200 25 25 50		9.50	10.02	9.00	9.00		
100 25 50 50		11.50	11.50	11.50	11.50	10.88 A	
100 25 25 50		12.45	12.50	12.50	12.40		
Vitamins average		9.41 B	9.55 A	9.31 C	9.32 C		

R.L.S.D OF Interaction=0.08

***Diverse letters indicate to the existence of a significant difference at P<0.05**

was efficient for embryogenic callus growth, and 0.5mg/L thiamine-HCl and 2 or1mg/L biotin for somatic embryos protraction.The MS medium supplemented with growth regulators(IBA+BA) in combination of amino acids was better than MS medium free of growth regulators medium .The high average was 10.61cm. The best interaction amid. combinations of amino acids, vitamins and plant growth regulators was at the treatment (100,25,25,50mg/L)amino acids and (2,2,3,1mg/L) and (1,2,2,2mg/L) vitamins ,in the medium supplemented with IBA+BA, the average attained to 12.50cm while the lower value of average descend to 7.00 cm at the treatment (200,25,25,50 mg/L) amino acids and (2,2,3,1mg/L) and without PGRs, the plantlets suffered from circumvolution. The best effect may be due to the role physiologique of IBA and BA which encourage the division cellular and best growth of plantlets, while resulting the MS medium free of PGRs, cause a dwarf and circumvolution plantlets. (Figure 2).

Figure (2)
Show the effect of 200,25,25,50mg/L amino acids and 1,2,2,2mg/L vitamins in

Figure (1)
show a plantlet after four weeks from culture a somatic embryo on Medium contain IBA+BA with combinations of amino acids and vitamins

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