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IN VITRO PROPAGATION OF MONO AND POLYEMBRYONIC MANGO CULTIVARS

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ABSTRACT

In order to propagate mango cvs (*Mangifera indica* L.) vegetatively by means of in vitro-culture using shoot tip and stem node explants; the effect of culture medium, explant type and phytohormones, on the morphogenetic capability was investigated. Stem node gave the best results compared with shoot tip culture during the establishment stage. High proliferated shoots number was obtained with modified WPM medium (20gl^{-1} sucrose) containing a combination of 30 mgl^{-1} adenine, 2 mgl^{-1} 2ip and 0.5 mgl^{-1} IBA plus L- glutamine and casein hydrolysate or 30 mgl^{-1} adenine combined with 0.2 or 0.5 mgl^{-1} IBA. Also, 40 mgl^{-1} adenine alone increased the proliferation percentage of shoot cultures (100%) while high average shoot length of the proliferated shoots occurred with 30 mgl^{-1} adenine. High rooting percentage and average root number were obtained with modified WPM medium (20 gl^{-1} sucrose) containing a combination of 30 mgl^{-1} adenine, 1 mgl^{-1} BAP and 4 mgl^{-1} IAA.

Key words: Mango, *In vitro*-culture, Cytokinins, Auxin, Proliferation, Rooting

INTRODUCTION

Mango is a tropical fruit crop of major significance. Propagation by cuttings is not very successful due to poor rooting. So cultivars are grafted on rootstocks. Nevertheless seedling-rootstocks often lack in uniformity or defined origin, respectively. Propagating mango vegetatively by means of in vitro-culture has not been realised so far (**Yang *et al* 1991**). Mango micropropagation is hindered by the rapid activation of oxidative enzymes during the excision of explants leading to the eventual death of excised cultured tissues.In vitro techniques used for the regeneration of mango include somatic embryogenesis (**Litz *et al* 1982 and 1984; Litz, 1984; DeWald *et al* 1989a and 1989b; Litz *et al* 1998 and Patena *et al* 2002**). However, shoot-tip and stem node culture of mango cultivars has not yet been described widely so far few attempts were performed with success by **Yang and Ludders (1993) and Thomas and Ravindra (1997)**. The purpose of this study was to investigate the effect of culture medium, cultivar, explant factors

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and phytohormones on regeneration capability of two mango cultivars; Zebda which is known of being polembryonic and Fagri-Kalan the monoembryonic one.

MATERIAL AND METHODS

This study was achieved through the period from year 1999 till 2001 in the Fruit Trees Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Preparation of the explants

Intact shoots (20 weeks old) were defoliated 20 days before the excision of explants. The explants collected in April were soaked afterwards in a cold antioxidant solution (150 mgl^{-1} ascorbic acid and 100 mgl^{-1} citric acid) and kept in a refrigerator at 5°C for about 2 hours until being surface disinfected. Two types of explants (terminal shoot tips and stem nodes which were 6cm in length) of two cultivars; Zebda polyembryonic (6-24month old) and monoembryonic Fagri kalan (17 years old) were prepared and subjected to continuous flow of tap water for at least half an hour. The explants were then sterilized using mercuric chloride ($HgCl_2$) at 0.05% for 10 mins. then rinsed three times in sterile distilled water. After sterilization, shoot tip and stem nodes of monoembryonic cv. were air-dried under laminar flow hood to getting rid of the water drops before being placed on culture medium which was supplemented with 100 mgl^{-1} ascorbic acid + 150 mgl^{-1} citric acid as antioxidant. Glass test tubes (150 x 15 mm) capped with aluminum foil filled with 10 ml of (MS) or (WPM) medium were used with young cv. while test tubes (150 x 25 mm) capped with bellco kaptus filled with 25 ml medium were used with adult cv. during establishment, proliferation and rooting stages. These explants were used in the following experiments:

1. Effect of explant type, media type and physical status of the media on survival and color retention percentages of mango cvs. during establishment stage

Two types of medium i.e., MS (**Murashige and Skoog, 1962**) and WPM (**Lloyd and McCown, 1980**) were used. Each medium used in two physical status, solid (solidified with purified agar at $7gl^{-1}$) and liquid (using filter paper bridges as a support). Sucrose used in two concenterations; $30gl^{-1}$ for the basic form and 20 gl^{-1} for the modified form of the medium. The effect of various treatments was assessed 4 weeks after culture based on explant survival and color retention percentages .

2. Effect of adenine, BAP and 2ip concentrations on culture establishment of mango cvs

This experiment aimed to determine best concentrations of cytokinin which allowed the presence of high number of uniform, and normal shoots to be used through micropropagation experiments. The explants were cultured onto modified WPM ($20gl^{-1}$ sucrose). The explants were placed in test tubes containing solid modiefied WPM medium supplemented with either benzyl amino purine (BAP) or isopentyl adenine (2ip) each in the concentrations of 0, 0.5, 1 and 2 mgl^{-1},

While adenine was used in concentrations of 0, 20, 30 and 40 mgl^{-1}. Survival and bud burst percentages, average shoot length (shoots were counted when its length was 1cm), average number of shoots and leaves per explant were measured after four weeks from culture.

3. Effect of auxin type and concentrations on the establishment of mango cvs. culture

Explants were placed in test tubes containing modified WPM medium supplemented with adenine 40 mgl^{-1} and either IAA (indole-3- acetic acid) or IBA(indole-3-butyric acid) or NAA (α-Naphthalen acetic acid) each in the concentrations of 0 , 0.5 and 1 mgl^{-1}. Survival and bud burst percentages, average shoot length ,average number of shoots and leaves per explant were measured after four weeks from culture.

4. Effect of adenine, BAP, 2ip and IBA concentrations on the proliferation of mango shoots cv. Zebda

This experiment was performed to study the influence of some cytokinin / auxin combinations on the proliferation rate (No. of proliferated shoots/explant) in shoot cultures. Benzyl amino purine (BAP) and isopentyl adenine (2ip) at 0, 0.5, 1.0, or 2.0 mgl^{-1} were incorporated into modified WPM medium , also, adenine at 0 , 20,30 or 40 mgl^{-1} was added. While indole-3-butyric acid (IBA) was added at 0.0 , 0.2 and 0.5 mgl^{-1} to the medium. The shoots that were produced from the terminal shoot tip and stem node cultures during the initiation stage were used as explants in this experiment. Survival ,bud burst percentages, average number and length of new proliferated shoots and leaves (shoots were counted when its length was 1cm long at least) were measured after four weeks from culture.

5. Effect of auxin /cytokinin combination levels on the rooting of shoot culture

The effect of indole butyric acid (IBA) or α-Naphthalen acetic acid (NAA) in five concentrations; (0, 1, 2, 3 and 4 mgl^{-1}) and indole acetic acid IAA (0, 2, 4 and 8 mgl^{-1})and benzyl amino purine (BAP) as a cytokinin at 0.0, 0.5, 1 and 2 mgl^{-1} on rooting and adventitious root initiation. Also, the main objective of this experiment was to determine the best combination of auxin / cytokinin concentration, physical form of the medium (solid or liquid) on the rooting of proliferated shoots of *Mangefira indica* cv. Zebda.

Shoots (3-4 cm in length with 3-4 leaves) after one passage *in vitro* were used in this experiment while modified WPM supplemented with 30 mgl^{-1} adenin was used as a rooting medium . After 4 weeks of subculturing, percentage of rooted shoots, number and length of roots per shoot were recorded.

In all of the experiments, each treatment consisted of there replicates and three explants per each in a completely randomized design. The data were statistically analyzed as a factorial experiment in a completely randomized design. For all previously mentioned experiments, the Duncan's multiple range test 5% was used to differentiate means (**Duncan, 1955**).

RESULTS AND DISCUSSION

Effect of explant type, medium type and physical status of the medium on survival and color retention percentages of mango cvs. during establishment stage.

Table (1) illustrated the effect of explant type, medium type and physical form on survival percentage during the establishment stage for adult monoembryonic mango cv. Fagri Kalan. As for the effect of physical phase of medium despite of medium type, it has a significant effect on survival and color retention percentages with all medium types for terminal shoot tip and stem node explants of monoembryonic mango cv. Fagri Kalan . Solid phase showed higher significant survival and color retention percentage with all tested medium and explants compared to liquid one.

Table 1. Effect of explant type, medium type and physical phase of the medium on survival and color retention percentages of mango explants cv. Fagri Kalan.

		Survival %		Mean	Color retention %		Mean
		ST	SN		ST	SN	
MS	Solid	22.2 d	33.3 c	27.7 D	0.00 d	22.2 b	11.1B
	Liquid	0.00 f	0.00 f	0.00 G	0.00 d	0.00 d	0.00 D
Mean		11.1 F*	16.6 E*		0.00 E°	11.1 C°	
modified MS	Solid	22.2 d	44.4 b	33.3 C	0.00 d	22.2 b	11.1 B
	Liquid	11.1 e	22.2 d	16.6 F	0.00 d	11.1 c	5.50 C
Mean		16.6 E*	33.3 C*		0.00 E°	16.6 B°	
WPM	Solid	33.3 c	44.4 b	38.8 B	11.1 c	33.3 a	22.2 A
	Liquid	11.1 e	33.3 c	22.2 E	0.00 d	22.2 b	11.1 B
Mean		22.2 D*	38.8 B*		5.50 D°	27.7 A°	
Modified	Solid	33.3 c	55.5 a	44.4 A	11.1 c	33.3 a	22.2 A
WPM	Liquid	11.1 e	33.3 c	22.2 E	0.00 d	22.2 b	11.1 B
Mean		22.2 D*	44.4 A*		5.50 D°	27.7 A°	
Mean		18.0 B`	33.3 A`		2.70 B`	20.8 A`	

* Means followed by the same letter are not significantly different from each other at 5% level
* ST = shoot tip * SN = stem node

Regarding medium type, survival percentages was significantly higher with modified woody plant medium (WPM) compared with other media when used for stem node cultures. While, insignificant difference was observed between WPM and modified WPM on survival and color retention percentages when used for terminal shoot tip culture.

As for the explant type, significant difference in survival and color retention was clear in all cases.

The interaction between type of explants, medium type and physical phase showed that modified WPM and WPM induced high significant survival and color retention percentage with stem node culture when cultured in solid form of the medium rather than liquid one. The highest survival and color retention percentages were recorded for stem node explants cultured on solid modified WPM medium.

Table (2) illustrated the effect of explant type, medium type and physical form on the different criteria during the establishment stage for young polyembryonic mango cv. Zebda.

Table 2. Effect of explant type, medium type and physical phase on survival and color retention percentages of mango explants cv.Zebda.

		Survival %		Mean	Color retention %		Mean
		ST	SN		ST	SN	
MS	Solid	33.3 h	44.4 g	38.8 G	11.1 g	22.2 f	16.6 E
	Liquid	11.1 I	33.3 h	22.2 H	0.00 h	0.00 h	0.00 F
Mean		22.2 H*	38.8 G*		5.5 G°	11.1 F°	
modified MS	Solid	55.5 f	66.6 d	61.1 D	33.3 e	44.4.d	38.8 D
	Liquid	44.4 I	55.5 f	49.9 F	33.3 e	44.4 d	38.8 D
Mean		49.9 F*	61.1 E*		33.3 E°	44.4 D°	
WPM	Solid	77.7 c	77.7 c	76.6 B	66.6 c	66.6 c	66.6 B
	Liquid	55.5 f	55.5 e	56.0 E	44.4 d	44.4 d	44.4 C
Mean		66.1 D*	66.6 C*		55.5 B*	55.5 B*	
WPM modified	Solid	100 a	88.8 b	94.4 A	88.8 a	77.7 b	82.7 A
	Liquid	66.6 d	66.6 d	66.6 C	44.4 d	33.3 e	38.8 D
Mean		83.3 A*	77.7 B*		66.6 A°	54.9 C°	
mean		55.4 B`	61.0 A`		40.3 B`	41.5 A`	

* Means followed by the same letter are not significantly different from each other at 5% level
* ST = shoot tip * SN = stem node

The mean of medium type showed that survival and color retention percentages of either, shoot tip or stem node of young polyembryonic mango explants cv. Zebda cultured in modified WPM was significantly higher compared to the other medium types. Also, the mean of physical phase showed that survival percentage of young polyembryonic terminal shoot tip and stem node cultured in solid modified WPM was significantly higher compared to liquid medium phase. Also, difference

in survival percentages between the terminal shoot tip and stem node was significant under all types of medium irrespective physical phase of the medium. Whereas the color retention percentages were significant for the terminal shoot tip explants when modified WPM was used .The difference between stem node and the terminal shoot tip was significant.

The interaction between medium type and physical phase showed that the highest significant survival and color retention percentage were visible in shoot tip cultured in solid form of modified WPM medium compared with liquid phase and other medium types.

The conclusion go in parallel with that found by **Yang and Ludders (1993)** who reported that solid WPM resulted in a highr survival rate and shoot forming. Also, **Thomas and Ravindra (1997)** indicated that maximum phenolics was found in full strength MS medium whilas semisolid and liquid medium showed more black exudation in full than half strength of MS medium.

On the other hand, **Raghuvanshi and Srivastava (1995)** indicated that MS medium was the best for plant regeneration of *Mangifera indica* from leaf explant compared with **Nitsch (1969)** and modified white medium **(Rangaswamy, 1961).**

Effect of adenine, BAP and 2ip concentrations on culture establishment of mango cvs.

Tables (3 and 4) cleared the effect of cytokinin type and concentrations on the ability of young polyembryonic (Zebda) and adult monoembryonic (Fagri Kalan) mango cultures to proliferate new shoots.

Table 3. Effect of adenine, BAP and 2iP concetrations on average shoot length , average number of shoots and leaves per stem node cv. Fagri Kalan.

		Avg. shoot no.	Avg. shoots length	Avg. leaf no./shoot
Adenine	0.0 mg	1.00 a	1.75 b	3.25 ab
	20 mg	1.00 a	1.75 b	2.75 cd
	30 mg	1.00 a	2.10 a	3.40 ab
	40 mg	1.00 a	1.83 b	3.33 ab
Mean		1.00 A°	1.85 A*	3.18 A“
BAP	0.0 mg	1.00 a	1.75 b	3.25 ab
	0.5 mg	1.00 a	1.33 c	3.00 bc
	2.0 mg	1.00 a	1.16 cd	2.33 d
	5.0 mg	1.00 a	1.00 d	1.00 e
Mean		1.00 A°	1.31 C*	2.39 B“
2ip	0.0 mg	1.0 a	1.75 b	3.25 ab
	0.5 mg	1.00 a	1.33 c	2.66 cd
	2.0 mg	1.00 a	1.83 b	3.50 a
	5.0 mg	1.00 a	1.80 b	3.40 ab
Mean		1.00 A°	1.67 B*	3.20 A“

*Means followed by the same letter are not significantly different from each other at 5% level.

Table (3) cleared the effect of cytokinin type and concentrations on the ability of adult mango stem node explants cv. Fagri Kalan (polyembryonic) to proliferate new shoots. The three types of cytokinins have insignificant effect on average shoots number. The data on cytokinin type indicated that using adenine at 30 mgl^{-1} had resulted in a significant increase in the average shoots length BAP concentrations from 0.5 - 5.0 mgl^{-1} gave shoot length significantly lower than similar concentration of 2ip and the same trend was obvious for the avg. leaf no / shoot. While average leaf number per shoot with adenine or 2ip was significantly greater than BAP treatment. On the other hand, when terminal shoot tip was used as explants, it was found that no result obtained.

Table (4) showed the effect of cytokinin type and concentrations on the ability of young mango explants cv. (Zebda polyembryonic) to proliferate new shoots.

The mean of cytokinin and explant type indicated significant increase in average shoot number with stem node explant and significant increase in average shoot length and leaf number achieved with terminal shoot tip explant cultured with adenine as a cytokinin. The mean of cytokinin and its concentrations showed that 2 mgl^{-1}2ip was significant in average shoot number as will as 1 mgl^{-1} that showed significant increase in average shoot length. Also 2ip treatments at 0.0, 0.5 and 1.0 mgl^{-1} led to higher rate of increase in average leaf number per shoot.The interaction between the three variables indicated that highest significant shoot number was achieved in stem node explants cultured in 2mgl^{-1} 2ip. However, 2mgl^{-1} BAP significantly reduced shoot number concentrations for both types of explants. Although, there was a significant increase in average shoot number with all adenine levels used for stem node cultures compared to the control. With regard to average shoot length, 30 mgl^{-1} adenine led to the highest significant increase with terminal shoot tip explants was used. Concerning the average leaf number per explant data in Table (4) revealed that 30 mgl^{-1} adenine or 1.0 mgl^{-1} 2ip produced the highest significant number of leaves for terminal shoot tip explant.

The obtaind results are in agreement with those of **Yang and Ludders (1993)**. The authers reported that 2ip at 2.0 mgl^{-1} stimulate the production of shoots. On the other hand, they revealad that BAP at 5.0 mgl^{-1} stimulate the production of shoots and there are no apparent differences in survival rate between both cytokinins nor concentration. While **Yang *et al* (1991)**, stated that shoot growth was optimal with combination of 1 mgl^{-1} BAP, 1 mgl^{-1} zeatin and 2 mgl^{-1} 2ip.

Effect of auxin type and concentrations on the establishment of mango cvs. culture

Data shown in Table (5) presented the effect of auxin types and concentrations on proliferation of shoots of adult mango stem node culture cv. Fagri Kalan (mpnpembryonic). The data declared that auxin type did not significantly affect average shoot number. Whereas IBA and IAA exhibited the highest average shoots length and leaf number per shoot. It was also clear that increasing NAA concentrations (0.5 and 1.0 mgl^{-1}) led to a significant decrease in shoot length and insignificant increase in leaf no. No data

Table 4. Effect of adenine,BAP and 2iP concentrations on average shoot length , average number of shoots and leaves per mango explants cv. Zebda.

		Avg. shoot no.		Mean	Avg. shoot length		Mean	Avg. leaf no.		Mean
		ST	SN		ST	SN		ST	SN	
Adenine	0.0 mg	1.00 e	1.50 d	1.25 CD	1.83 bcd	1.37 cde	1.60 CD	4.66 abcd	4.50 abcd	4.58 A
	20 mg	1.00 e	1.00 e	1.00 D	2.16 ab	1.16 de	1.66 BCD	5.00 abc	2.66 f	3.83 AB
	30 mg	1.00 e	2.00 bc	1.50ABC	2.62 a	1.33 cde	1.97 ABC	5.25 a	4.00 bcde	4.75 A
	40 mg	1.00 e	2.25 ab	1.62 AB	2.08 ab	1.20 de	1.64 BCD	3.66 cdef	2.41 f	3.04 BC
Mean		1.00 C°	1.68 A°		2.17 A•	1.27 C•		4.70 A“	3.39 C“	
BAP	0.0 mg	1.00 e	1.50 d	1.25CD	1.83 bcd	1.37 cde	1.60 CD	4.66 abcd	4.50 abcd	4.58 A
	0.5 mg	1.00 e	1.66 cd	1.33 BCD	1.90 bc	1.50 bcde	1.70 ABC	3.60 cdef	4.16 abcd	3.83 AB
	1.0mg	1.00 e	2.25 ab	1.62 AB	1.50 bcde	0.95 e	1.22 D	2.75 ef	2.58 f	2.66 C
	2.0mg	0 00 f	0.0 f	0.00 E	0.00 f	0.00 f	0.00 E	0.00 g	0.00 g	0.00 D
Mean		0.75 D°	1.35 B°		1.30 C•	0.95 D•		2.75 D“	2.81 CD“	
2ip	0.0 mg	1.00 e	1.5 d	1.25 CD	1.83 bcd	1.37 cde	1.60 CD	4.66 abcd	4.50 abcd	4.58 A
	0.5 mg	1.00 e	1.00 e	1.00 D	2.16 ab	2.00 abc	2.08 AB	4.33 abcd	5.00 abc	4.66 A
	1.0mg	1.00 e	2.00 bc	1.50ABC	2.12 ab	2.12 ab	2.12 A	5.25 ab	4.24 abcd	4.75 A
	2.0mg	1.00 e	2.50 a	1.75 A	1.75 bcd	1.35 cde	1.55 CD	3.50 def	2.24 f	2.87 BC
	Mean	1.00 C°	1.75 A°		1.96 A•	1.62 B•		4.40 AB“	4.07 B“	
Mean		0.91 B`	1.59 A`		1.81 A`	1.28 B`		3.96 A`	3.42 B`	

*Means followed by the same letter are not significantly different from each other at 5% level.
* ST = shoot tip * SN= stem node

Table 5. Effect of NAA, IBA and IAA concnetrations on average shoot length , average number of shoots and leaves per stem node cv. Fagri Kalan.

		Avg. shoot no.	Avg. shoots length	Avg. leaf no./shoot
	0.0 mg	1.00 a	2.10 a	3.40 c
NAA	0.5 mg	1.00 a	1.83 bc	3.40 c
	1.0 mg	1.00 a	1.75 c	3.50 c
Mean		1.00 A°	1.89 B*	3.46 B"
	0.0 mg	1.00 a	2.10 a	3.40 c
IBA	0.5 mg	1.00 a	2.10 a	4.20 ab
	1.0 mg	1.00 a	2.25 a	4.70 a
Mean		1.00 A°	2.15 A*	4.11 A"
	0.0 mg	1.00 a	2.10 a	3.40 c
IAA	0.5 mg	1.00 a	2.00 ab	4.00 bc
	1.0 mg	1.00 a	2.00 ab	4.00 bc
Mean		1.00 A°	2.03 A*	3.80 A"

*Means followed by the same letter are not significantly different from each other at 5% level.

regarding terminal shoot tip, was obtained in this concern.

Data in Table (6) illustrated the effect of auxin types and concentrations on proliferation of shoots of young mango explants cv. Zebda (polyembryonic).The highest mean number of shoots resulted from auxin-free medium, also, 0.5 and1.0 mgl^{-1} IBA produced significant mean number of shoots per explant compared to NAA and IAA. However the highest mean of shoot length resulted from 0.5 and 1 mgl^{-1} IBA and the highest leaf no. from 1 mgl^{-1} IBA and 0.5 mgl^{-1} IAA. Also insignificant increase in shoot length was recorded for auxin types. The interaction between the three variables showed that the highest significant shoot number was produced on stem node explant at zero concentration for all auxin types.With regard to average shoot length NAA at 1.0 mgl^{-1} led to the highest significant increase in the shoot length produced by stem node compared with terminal shoot tip explant. Whereas IBA at 1.0 mgl^{-1} produced the highest significant number of leaves with shoot tip explant.

In view of the current results a similar trend was found through the working of **Yang and Ludders (1993)**, as 0.5 mgl^{-1} of IAA achieved the highest percentage of shoot formation and IBA was the best

Table 6. Effect of NAA, IBA and IAA concentrations on average shoot length , average number of shoots and leaves per mango explants cv. Zebda.

		Avg. shoot no.		Mean	Avg. shoot length		Mean	Avg. leaf no.		Mean
		ST	SN		ST	SN		ST	SN	
NAA	0.0 mg	1.00 d	2.25 a	1.62 A	2.08 cd	1.20 e	1.64 C	3.66 bcd	2.41 e	3.04 C
	0.5 mg	1.00 d	1.60 bc	1.30 AB	2.00 d	2.35 abcd	2.17 B	4.00 abc	3.60 cd	3.80 A
	1.0 mg	1.00 d	1.00 d	1.00 B	2.25 bcd	3.16 a	2.70 AB	3.75 bcd	4.00 abc	3.87 A
Mean		1.0 C^{+}	1.61 B^{+}		2.11 A°	2.24 A°		3.80 $A^{\bullet}$	3.33 $B^{\bullet}$	
IBA	0.0 mg	1.00 d	2.25 a	1.62 A	2.08 cd	1.20 e	1.64 C	3.66 bcd	2.41 e	3.04 C
	0.5 mg	1.00 d	1.83 ab	1.40 A	2.60 abcd	3.04 ab	2.82 A	4.00 abc	33.3 d	3.66 AB
	1.0mg	1.00 d	1.80 ab	1.40 A	2.75 abcd	2.90 abc	2.82 A	4.50 a	3.30 d	3.90 A
Mean		1.0 C^{+}	1.96 A^{+}		2.47 A°	2.38 A°		4.05 $A^{\bullet}$	3.01 $C^{\bullet}$	
IAA	0.0 mg	1.00 d	2.25 a	1.62 A	2.08 cd	1.20 e	1.64 C	3.66 bcd	1.41 e	3.04 C
	0.5 mg	1.00 d	1.25 cd	1.12 B	2.00 d	2.87 abcd	2.43 AB	4.20 ab	3.62 cd	3.91 A
	1.0 mg	1.00 d	1.66 bc	1.33 AB	2.37 abcd	2.75 abcd	2.56 AB	3.50 cd	33.3 d	3.41 B
	Mean	1.00 C^{+}	1.72 AB^{+}		2.15 A°	2.27 A°		3.78 $A^{\bullet}$	3.12 $BC^{\bullet}$	
Mean		1.00 B`	1.76 A`		2.24 A`	2.30 A`		3.88 A`	3.16 B`	

*Means followed by the same letter are not significantly different from each other at 5% level.

* ST=shoot tip * SN=stem node

for shoot elongation.On contrary, the authers stated that NAA was the best auxin for sprouting.

Effect of cytokinin/auxin combination levels on the proliferation of mango shoots cv. Zebda.

The mean of cytokinins in Table (7) showed that proliferating shoots percentage significantly higher with adenine combined with 0.2 or 0.5 mgl^{-1} IBA had similar effect on average proliferated shoot number. Regardless of average shoot length, IBA at 0.5 mgl^{-1} with BAP (0.5 mgl^{-1}), 2ip (0.4, 1.0 and 2.0 mgl^{-1}) and adenine (30 and 40 mgl^{-1}) recorded the highest significant length.

The interaction between cytokinin types and levels with IBA concentrations cleared that the highest percentage of proliferated shoots was significantly achieved by BAP at 1.0 mgl^{-1} only or combined with 0.2 mgl^{-1} IBA. Also, 2 mgl^{-1} 2ip with 0.5 mgl^{-1} IBA or adenine at 40 mgl^{-1} with 0.2 and 0.5 mgl^{-1} IBA had similar effect. With regard to average proliferated shoot number, the highest significant number recorded with 2ip at 2.0 mgl^{-1} combined with 0.5 mgl^{-1} IBA. The highest significant proliferated shoot length (1.37 and 1.32 cm) was achieved with adenine, at 20 and 30 mgl^{-1}, respectively.

The results is in agreement with **Yang and Ludders (1993)** who reported that combination of cytokinin BAP, ZT, 2ip and auxins IAA, IBA stimulated shoot production of mango rootstocks and had less effect on axillary bud growth.

While **Raghuvanshi and Srivastava (1995)** stated that multiple shoots of mango were produced in several combinations with the largest number being in medium supplemented with 1.1 μM IAA and 13.0μM kinetine, also, stated that only one or two of the multiple shoots attained maximum height (5.2cm), the rest remained comparatively small (8mm-2cm).

Effect of auxin / cytokinin combination levels on the rooting of shoot culture

The data in Table (8) cleared the effect of adenine, BAP and IAA on the rooting of shoot culture. As shown in Table (8) data showed that rooting percentages increased significantly by IAA/BAP combination compared to either the auxin or the cytokinin alone and other auxin / cytokinin combinations. The most effective IAA level in the induction of rooting was 4.0 mgl^{-1} combined with 1.0 mgl^{-1} BAP which led to 66.6% rooting. Also similar effect on average number of roots and average root length was recorded. The significance was clear between 2.0, 4.0 and 8.0 mgl^{-1} IAA. Furthermore, no significancy in the results, could be detected between 0.0 or 0.2 mgl^{-1} IAA combined with 1 mgl^{-1} BAP. With regard to average root number it was clear that this character was significantly increased with 4.0 mgl^{-1} IAA combined with 1 mgl^{-1} BAP. Conversely, the root number was significantly decreased when higher concentration (8.0 mgl^{-1}) was used.

From Table (8) it was clear that average root length was increased with increasing IAA concentration from 4.0 to 8.0 mgl^{-1} combined with 1 mgl^{-1} BAP. Also, it was noticeable that insignificant differences was found between 0.0 and 0.2 mgl^{-1} IAA combined with 1 mgl^{-1} BAP. It is worthy to mention that when

Table 7. Effect of cytokinin / auxin combinations levels on proliferating shoots percentage, avg. shoots no. and length of proliferating shoot during multiplication stage.

		Proliferating shoots %			Mean	Avg. shoot no. proliferatd			Mean	Avg. shoot length of proliferatd shoots			Mean
IBA mg/L		0.0	0.2	0.5		0.0	0.2	0.5		0.0	0.2	0.5	
BAP	0.0 mg	0.00 i	0.001	0.00 i	0.00 G	0.00 f	0.00 f	0.00 f	0.00 D	0.00 e	0.00 e	0.00 e	0.00 E
	0.5 mg	0.00 i	0.00 i	0.00 i	0.00 G	0.00 f	0.00 f	1.50 bcde	0.50 D	0.00 e	0.00 e	1.25 a	0.41 D
	1.0 mg	100 a	100 a	80.0 d	93.3 B	1.16 de	1.33 cde	2.50 ab	1.66 BC	0.66 cd	0.72 cd	0.93 bc	0.77 BC
	2.0 mg	0.00 i	0.00i	0.00i	0.00 G	0.00 f	0.00 f	0.00 f	0.00 D	0.00 e	0.00 e	0.00 e	0.00 E
Mean		25.0 G^{+}	25.0 G^{+}	19.9 H^{+}		0.29 C°	0.33 C°	1.00 B°		0.16 B^{*}	0.18 B^{*}	0.54 A^{*}	
2ip	0.0	0.00 i	0.00 i	0.00 I	0.00 G	0.00 f	0.00 f	0.00 f	0.00 D	0.00 e	0.00 e	0.00 e	0.00 E
	0.5 mg	0.00 i	0.00 i	0.00 I	0.00 G	0.00 f	0.00 f	0.00 f	0.00 D	0.79 cd	0.75 cd	1.20 ab	0.91 AB
	1.0 mg	37.4 g	37.4 g	75.0 e	49.9 E	1.66 abcde	1.66 abcde	1.66 abcde	1.66 BC	0.79 cd	0.75 cd	1.20 ab	0.91 AB
	2.0 mg	85.7 e	75.0 e	100 a	86.9 C	2.33 abc	2.33 abc	2.57 a	2.40 A	0.61 cd	0.62 cd	1.21 ab	0.81 BC
Mean		30.8 E^{+}	28.1 F^{+}	43.7 C^{+}		1.0 B°	1.00 B°	1.06 B°		0.35 B^{*}	0.34 B^{*}	0.60 A^{*}	
adenine	0.0 mg	0.00 i	0.00 I	0.0 i	0.0 F	0.00 f	0.00 f	0.00 f	0.00 D	0.00 e	0.00 e	0.00 e	0.00 E
	20 mg	12.4 h	37.4 g	62.5 f	37.4 D	1.00 e	1.00 e	1.5 bcde	1.16 C	0.50 d	0.91 bc	1.37 a	0.92 AB
	30 mg	62.5 f	87.5 b	87.5 b	79.1 A	1.40 cde	1.42 bce	1.71 abcde	1.51 BC	0.87 c	0.83 cd	1.32 a	1.01 A
	40 mg	87.5 b	100 a	100 a	95.8 A	1.85 abcde	1.87 abcde	2.12 abcd	1.95 AB	0.62 cd	0.62 cd	0.94 bc	0.73 C
Mean		40.6D^{+}	56.2B^{+}	62.5A^{+}		1.06B°	1.07A°	1.33A°		0.50C°	0.59B°	0.91A°	
Mean		32.1 C`	36.4 B`	42.0 A`		0.78 B`	0.80 B`	1.13 A`		0.34 B`	0.37 B`	0.68 A`	

* Means followed by the same letter are not significantly different from each other at 5% level.

Table 8. Effect of IAA levels on rooting percentage , average root number and length per shoots during rooting stage.

IAA	Rooting %	Avg. root no.	Avg. root length
0.0 mg	0.00 c	0.00 c	0.00 c
2.0 mg	0.00 c	0.00 c	0.00 c
4.0 mg	66.6 a	4.16 a	1.09 b
8.0 mg	11.1 b	3.00 b	1.33 a

*Means followed by the same letter are not significantly different from each other at 5% level.

liquid medium was used for rooting no result was obtained.

On the contrary, **Raghuvanshi and Srivastava (1995)** reported that maximum rooting percentage obtained was 20% when shoots cultured on solid MS medium supplemented with 9.8 µM IBA only without cytokinin.

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ، ١١(١) ، ٣٤٣ -٣٥٧ ، ٢٠٠٣

الاكثار المعملى لبعض أصناف المانجو وحيدة ومتعددة الأجنة

[٢٦]

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من أجل اكثار المانجو خضريا بواسطة تقنية زراعة الانسجة أستخدمت القمة النامية والعقد الساقية كمنفصلات من صنفين زبدة (وحيد الجنين) والذى نتج من شتلات بذرية عمر ٦ - ٢٤ شهر أو من صنف فجرى (عديد الأجنة) كلان أشجار عمر ١٧ سنة و تم دراسة تأثير كل من بيئة الزراعة ، نوع المنفصل ومنظمات النمو على القدرة التجديدية للمزارع.

العقد الساقية أعطت أفضل النتائج بالمقارنة بالقمة النامية خلال مرحلة التأسيس كذلك أعلى نسبة لتكوين المرستيمات الخضرية تم الحصول عليها باستخدام بيئة WPM المعدلة المحتوية على ٣٠ملجم / لتر أدينين , ٢ ملجم/ لتر 2ip (٢ ايسوبنتيل أدنين) و ٠,٥ ملجم / لتر اندول حمض البيوتريك و ٢٠ جم / لتر سكروز.

أيضا ٤٠ ملجم / لتر أدينين منفردا فى البيئة يزيد من نسبة تكون المرستيمات الخضرية (١٠٠%) فى حين أن أعلى متوسط لطول الأفرع المكثرة أمكن الحصول علية من البيئة المحتوية على ٣٠ ملجم / لتر أدينين.

أعلى نسبة تجذير و متوسط عدد جذور أمكن الحصول علية مع بيئة WPM المعدلة المحتوية على مزج من ٣٠ ملجم / لتر أدينين , ١ ملجم / لتر بنزيل أمينو بيورين و ٤ ملجم / لتر اندول حمض الخليك و ٢٠ جم / لتر سكروز.

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