Research Journal of Agriculture and Biological Sciences, 5(6): 1169-1175, 2009

In vtro Propagation of Manfalouty and Nab El-gamal Pomegranate Cultivars

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Abstract:Shoot tips of 2-3 cm long of two pomegranate cultivars (Manfalouty and Nab El-Gamal) were cultured on three different media at full strength, namely Murashige and Skoog; (MS), Nitsch & Nitsch; (NN) and Woody Plant Medium; (WPM). Media were prepared as a basal medium supplemented with GA_3 at 5.0 mg/l, BA at 0.1 mg/l, IBA at 0.02 mg/l and activated charcoal at 3.0 g/l for establishment stage. For proliferation stage, BA and kinetin were tested at 0.0, 1.0 and 2.0 mg/l. For rooting stage, two different auxins; IBA and NAA were tested at 0.0, 0.25 and 0.5 mg/l on WPM at full (FSW) and half (HSW) strength. The plantlets grown on WPM were found to be significantly better in average survival (100 and 60%), plantlet height (5.10 and 4.58 cm), and average leaves number per shoot (11.3 and 10.0) for Manfalouty and Nab El-Gamal pomegranate cvs., respectively compared to other media. The two cultivars grown on WPM containing 1.0 mg/l BA had significantly the highest proliferation rate (6.8 and 5.8 shoot/explant) compared to 1.0 mg/l kinetin which produced the least value of proliferation rate (2.2 and 2.8 shoot/explant) for both investigated cultivars, respectively. The same trend was found concerning the average leaves number in response to BA and kinetin treatments. NAA at 0.25 mg/l significantly produced the highest rooting response (100% as an average for HSW and FSW) for Nab El-Gamal pomegranate cv., while 0.25 mg/l IBA induced the highest value of rooting (85% as an average) for Manfalouty pomegranate cv. IBA significantly increased the average number of roots compared to NAA treatments on both strengths of woody plant medium. The average roots length of the plantlet grown on HSW medium was longer (5.82 and 2.46 cm) than those grown on FSW medium (5.32 and 2.03 cm) in Manfalouty and Nab El-Gamal cvs., respectively.

Key words: micropropagation, proliferation, rooting, *Punica granatum*

INTRODUCTION

Pomegranate (*Punica granatum* L.) is generally known in a distinct family (Punicaceae), which comprises only one genus (*Punica*) and only two species, *P. granatum* and *P. proptopunica*,[1]. Pomegranate fruit is high in vitamin C, potassium and antioxidant polyphenols, a good source of fiber and low in calories[2]. Pomegranate is propagated vegetatively, by the rooting of hard wood cuttings, but the establishment of new plants requires one year[3].

Micropropagation in fruit tree would help in overcoming difficulties of vegetative propagation, producing true to-type plants and rapid & mass production of planting materials.

The aseptic culture was established when shoot tips of some pomegranate cultivars were individually cultured on the MS basal medium[4] supplemented with activated charcoal (2.5 g/l) and (per leter; 1.0 mg BA and 2ip; 5.0 mg GA_3 and 0.2 mg IBA). The highest survival percentage was obtained from the shoot tips cultured at the beginning of the growing season (March)[5]. The proliferation rate ranged from 5.3 to 8.6 shoot/explant when the MS medium was supplemented with 0.5-1.0 mg/l BA and 0.1-0.5 mg/l NAA[6,7,8,9,10]. Moreover, rooting was induced in 86% of the regenerated shoots in a half-strength MS medium supplemented with 1.0 mg/l^{-1} indol-3-butyric acid (IBA)[6,8,9,10,11].

Consequently, this work has been designed to study the effect of medium type (Murashige & Skoog, Nitsch & Nitsch and Woody plant medium) and growth regulators on establishment, multiplication and rooting on two main pomegranate cultivars (Manfalouty and Nab El-Gamal) as a step for further studies of improvement and breeding.

MATERIALS AND METHODS

This work was conducted at Fruit Crops Orchard and Tissue Culture Laboratory, Faculty of Agriculture, Assiut University during the period from 2005 to 2007 to establish a micropropagation protocol of two main pomegranate cultivars (Manfalouty and Nab El-Gamal) grown in Egypt.

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Material for Propagation: Shoot tips of about 2-3 cm long of Manfalouty and Nab El-Gamal pomegranate cultivars were collected from mature trees, cleaned under running tap water for about[1-2] hours, soaked in antioxidant solution (150 mg/l ascorbic acid and 100 mg/l citric acid) for 15 minutes under the laminar flow hood and sterilized using 0.1% mercuric chloride for 2 minutes, followed by three times rinses in sterile bidistilled water[12].

Culture Media: Three different media; MS[4], NN[13] and WPM[14] were tested to micropropagat the pomegranate cultivars. Media were prepared as a basal medium supplemented with organic acids and vitamins. Sucrose was added at 30.0 g/l and Myo-instol at 0.1 g/l. pH of the prepared media was then adjusted to 5.6-5.8. Gelrite (Phytogel, SIGMA) was added as 2.5 g/l for media solidification. For establishment stage, GA_3 at 5.0 mg/l, BA at 0.1 mg/l and IBA at 0.02 mg/l were used. For proliferation stage, BA and kinetin were tested at 0.0, 1.0 and 2.0 mg/l. For rooting stage, two different auxins; IBA and NAA were tested at 0.0, 0.25 and 0.5 mg/l on WPM at full (FSW) and half (HSW) strength. Media were then poured in 200 ml jars; media volume for each jar was 25 ml. Media were autoclaved at 121°C and 1.5 Kg/cm^2 pressure for 20 minutes. The jars containing media were left to be air-cooled for solidification.

Measurements:

Aseptic culture establishment:

- Survival percentage.
- Vegetative growth characteristics (plantlet height, leaves number, nodes number and internode length).

Proliferation:

- Average number (per explant) of proliferated shoots, leaves and nodes.
- Average length (in cm) of proliferated shoot and internode.

Rooting:

- Rooting percentage.
- Average number of roots/explant.
- Average length of roots (cm).

Statistical Analysis: The experiment of effect of IBA and NAA was set up as a split-split plot design, while the other experiments were designed as a randamized complet blocks experiment and means separation were made according to the Least Significant Differences (L.S.D.) at 5% level[15].

RESULTS AND DISCUSSION

Effect of Medium Type on Survival Percentage: Data in Table (1) cleared that the highest average survival was recorded on WPM medium (80%) followed by MS medium (50%) while NN medium produced the least value (40%) as an average for the two investigated cultivars. Moreover, Manfalouty cv. gave significantly higher average of survival (73.3%) compared to Nab El-Gamal cv. (40%) for the three tested media.

Effect of Medium Type on Vegetative Growth Characteristics: Data presented in Table (2) showed that WPM produced the tallest plantlets (5.10 and 4.58 cm average) for Manfalouty and Nab El-Gamal cvs., respectively, while NN medium gave the shortest plantlets (3.44 and 3.62 cm). The average plantlet height was 4.84 cm on WPM followed by 4.02 cm on MS medium and 3.51 cm on NN medium for both studied cultivars. Moreover, the average height of Manfalouty plantlets grown on the three used media was higher (4.27 cm) than those of Nab EL-Gamal plantlets (3.99 cm).

The highest average number of leaves per explant (10.67 average for two cultivars) was produced on WPM followed by MS medium (9.67), while NN medium had the lowest average number of leaves (6.50). In addition, Manfalouty plantlet had slightly higher average number of leaves (9.11) compared to Nab El-Gamal plantlet (8.77 leaf/explant).

Plantlet of Manfalouty and Nab El-Gamal cultivars grown on WPM significantly produced the highest average number of nodes (7.5 average of two cultivars) followed by those grown on MS medium (5.8), while NN medium gave the lowest number of nodes (4.4). In addition, Manflaouty plantlet had slightly higher average number of nodes (6.27) compared to Nab El-Gamal plantlet (5.5 average for the three media). On the other hand, internode length took approximately opposite trend for WPM and NN media. NN medium produced significantly the highest average internode length (0.73 cm), while WPM gave the least value (0.53 cm).

The early finding reported by several investigators[7,8,16] on pomegranate are in accordance of the results obtained in the present study. Woody Plant Medium supplemented with BA produced 100% survival for *in vitro* propagated apricot[17]. Furthermore, three berry fruit species (blueberry, blackberry and raspberry) differently response to medium type MS and WPM[18].

Effect of Cytokinins on Proliferation (Shoot/explant): Data in Table (3) cleared that using 1.0 mg/l BA produced the highest rate of proliferation (6.3 shoot/explant), followed by 2.0 mg/l BA (4.2 shoot/explant) as an average for the two cultivars. The least proliferation rate (zero shoot/explant) was obtained from control, while kinetin at 1.0 and 2.0 mg/l

Table 1: Effect of medium type (MS, NN and WPM media) on survival (%) of Manfalouty and Nab El-Gamal pomegranate cultivars.

Medium type (B)	Cultivar (A)		Medium Mean
	Manfalouty	Nab El-Gamal	
MS	60	40	50
NN	60	20	40
WPM	100	60	80
Cultivar Mean	73.3	40	
L.S.D. 5 %	(A) = 26.86	(B) = 15.66	(AB) = 22.15

Table 2: Effect of medium type (MS, NN and WPM media) on plant vegetative growth characteristics of Manfalouty and Nab El-Gamal pomegranate cultivars.

Character	Medium type (B)	Cultivar (A)			L.S.D. 5%
		Manflouty	Nab El-Gamal	Mean	
Plantlet height (cm)	MS	4.28	3.76	4.02	(A) = 0.37
	NN	3.44	3.62	3.51	(B) = 0.41
	WPM	5.10	4.58	4.84	(AB) = N.S
	Mean	4.27	3.99		
Leaf/shoot	MS	9.67	9.67	9.67	(A) = N.S
	NN	6.33	6.67	6.50	(B) = 2.15
	WPM	11.33	10.00	10.67	(AB) = N.S
	Mean	9.11	8.77		
Node/shoot	MS	6.40	5.20	5.80	(A) = N.S
	NN	4.60	4.20	4.40	(B) = 0.92
	WPM	7.80	7.20	7.50	(AB) = N.S
	Mean	6.27	5.50		
Internode length (cm)	MS	0.66	0.56	0.61	(A) = N.S
	NN	0.74	0.72	0.73	(B) = 0.13
	WPM	0.50	0.56	0.53	(AB) = N.S
	Mean	0.63	0.61		

Table 3: Effect of cytokinins (BA and kinetin) at different concentrations on proliferation (shoot/explant) of Manfalouty and Nab El-Gamal pomegranate cultivars.

Cytokinin (B)	Cultivar (A)		Mean
	Manfalouty	Nab El-Gamal	
0.00 (Control)	0.00	0.00	0.00
1.0 mg/l BA	6.80	5.80	6.30
2.0 mg/l BA	4.20	4.20	4.20
1.0 mg/l kinetin	2.20	2.80	2.50
2.0 mg/l kinetin	3.40	3.20	3.30
BA Mean	5.50	5.00	5.30
Kinetin Mean	3.15	3.00	3.10
Cytokinin Mean	4.15	4.00	
L.S.D. 5 %	(A) =N.S	(B) = 0.79	(AB) = N.S

produced 2.5 and 3.3 shoot/explant, respectively. In addition, Manfalouty explant produced insignificantly higher proliferation rate (4.15 shoot/explant) compared to Nab El-Gamal one (4.0 shoot/explant).

Effect of Cytokinins on Plant Vegetative Growth Characteristics: Data in Table (4) indicated that BA at 1.0 and 2.0 mg/l and kinetin at 1.0 mg/l significantly increased the average shoot length (3.17, 3.46 and 3.11 cm), respectively, compared to the control (2.59 cm) for both tested cultivars. Kinetin at 2.0 mg/l produced shoots with slightly higher average length (2.73 cm) compared to control. It could be also noticed that the average shoot length of Manfalouty plantlet was slightly lower compared to Nab El-Gamal plantlet (3.00 vs. 3.22 cm).

The highest average of leaves number was recorded using 1.0 mg/l BA (10.4 and 8.4 leaf/explant) compared to the lowest number of control (1.4 and 1.2 leaf/explant) for Manfalouty and Nab El-Gamal cvs., respectively with an average 9.4 leaf/explant for both tested cultivars followed by 2.0 mg/l BA (7.3 leaf/explant), while 2.0 mg/l kinetin gave the least value (5.4 leaf/explant) compared to other cytokinins treatments.

All concentrations of BA and kinetin significantly decreased the average nodes number compared to control so that untreated plantlet (control) of both tested cultivars produced the highest average of nodes number (7.7 nodes/explant), followed by 1.0 mg/l BA (5.3), then kinetin at 1.0 mg/l (4.7), while kinetin at 2.0 mg/l gave the least average of nodes number (3.7). Moreover, BA treatments produced slightly higher number of nodes (4.8) compared to kinetin treatments (4.2).

Kinetin at 1.0 and 2.0 mg/l significantly decreased the average internode length (0.36 cm) compared to control (0.53 cm) and BA treatments (0.54 cm) for both studied cultivars. The reduction was about 34 and 35% compared to control and BA treatments, respectively. The average internodes length generally ranged from 0.60 cm (Manfalouty plantlet treated with 1.0 mg/l BA) to 0.30 cm (Nab El-Gamal plantlet treated with 2.0 mg/l kinetin).

These findings are in accordance of those reported by[7,8,19,20] on pomegranate cvs. Who found that cytokinins (BA, Zeatin or kinetin) treatments at different concentrations increased survival percentage and proliferation rate and callus formation. Similar findings were suggested on pear[21,22] on apricot[17] and on grape[23,24].

Effect of IBA, NAA and Medium Strength on Rooting Percentage: Data presented in Table (5) showed that both NAA and IBA at 0.25 mg/l significantly produced the highest rooting (100% for HSW and FSW) as an average for Nab El-Gamal plantlet, followed by 0.50 mg/l IBA (90%). For Manfalouty plantlet 0.25 mg/l IBA induced the highest value of rooting (85% average). Concerning the effect of medium concentration, Nab El-Gamal plantlet grown on HSW medium had the highest rooting (97.5%), while the least value (57.5%) was recorded for Manfalouty plantlet grown on FSW medium.

Effect of IBA, NAA and Medium Strength on Roots Number and Length: Data in Table (6) showed that IBA at 0.25 and 0.50 mg/l significantly increased the average number of roots compared to NAA treatments on both strength woody plant media. Nab El-Gamal plantlet treated with 0.50 mg/l IBA and grown on FSW medium produced the highest average number of roots (10.4), followed by those treated with 0.25 mg/l IBA on HSW and FSW media (9.8). In addition, plantlets of Nab El-Gamal cv. produced significantly higher number of roots (8.9 and 8.5) than Manfalouty plantlets (6.6 and 5.8) as an average on HSW and FSW, respectively.

The roots of Manfalouty plantlets were longer (ranged from 4.94 cm to 6.58 cm) than those of Nab El- Gamal plantlets (ranged from 1.0 cm to 3.34 cm). The average roots length of the plantlets grown on HSW medium was longer (5.82 and 2.46 cm) than those grown on FSW medium (5.32 and 2.03 cm) in Manfalouty and Nab El-Gamal cvs., respectively. In addition, NAA at 0.25 mg/l significantly increased the average root length of Manfalouty and Nab El-Gamal plantlet grown on HSW and FSW media compared to other treatments (Table 7).

Similar results were reported in early study[5], who suggested that rooting was successfully obtained from shoot tips of Wardi Red pomegranate grown on MS medium supplemental with 0.50 mg/l of IBA + NAA. The obtained results are also in a harmony with the findings of[6,7,10,16] on pomegranate cultivars, and [12,25] on apple.

In conclusion, the present study outlined on *in vitro* protocol for micropropagation of two major pomegranate cultivars in Egypt. Woody Plant Medium (WPM) proved to produce best vegetative growth characteristics compared to MS and NN ones. BA at 1.0 mg/l induced high significant proliferation rate and shoot quality for two tested cultivars compared to kinetin. To induce rooting NAA at 0.25 mg/l (for Nab El-Gamal) and IBA at 0.25 mg/l (for Manfalouty) while IBA generally proved to produce higher number of roots per shoot. In addition, WPM at half-strength generally produced better plantlets compared to its full-strength.

Table 4: Effect of cytokinines (BA and kinetin) at different concentrations on plant vegetative growth characteristics of Manfalouty and Nab El-Gamal pomegranate cultivars.

Character	cytokinin (B)	Cultivar (A)			L.S.D. 5%
		Manflouty	Nab El-Gamal	Mean	
Shoot length (cm)	0.00 (control)	2.42	2.76	2.59	(A) = N.S
	1.0 mg/l BA	2.72	3.61	3.17	(B) = 0.43
	2.0 mg/l BA	3.22	3.70	3.46	(AB) = N.S
	BA mean	2.97	3.65		
	1.0 mg/l kinetin	3.32	2.89	3.11	
	2.0 mg/l kinetin	2.76	2.69	2.73	
	Kinetin Mean	3.04	2.79		
	Cytokinin Mean	3.00	3.22		
Leaf/shoot	0.00 (control)	1.40	1.20	1.30	(A) = N.S
	1.0 mg/l BA	10.40	8.40	9.40	(B) = 1.27
	2.0 mg/l BA	7.20	7.40	7.30	(AB) = N.S
	BA mean	8.80	8.00		
	1.0 mg/l kinetin	5.00	6.40	5.70	
	2.0 mg/l kinetin	5.40	5.40	5.40	
	Kinetin Mean	5.20	6.00		
	Cytokinin Mean	7.00	6.90		
Node/shoot	0.00 (control)	7.80	7.60	7.70	(A) = N.S
	1.0 mg/l BA	5.60	5.00	5.30	(B) = 0.93
	2.0 mg/l BA	4.40	4.00	4.20	(AB) = N.S
	BA mean	5.00	4.50		
	1.0 mg/l kinetin	4.80	4.60	4.70	
	2.0 mg/l kinetin	3.60	3.80	3.70	
	Kinetin Mean	4.20	4.20		
	Cytokinin Mean	5.20	4.92		
Internode length (cm)	0.00 (control)	0.50	0.56	0.53	(A) = N.S
	1.0 mg/l BA	0.52	0.56	0.54	(B) = 0.10
	2.0 mg/l BA	0.60	0.48	0.54	(AB) = N.S
	BA mean	0.56	0.52		
	1.0 mg/l kinetin	0.40	0.32	0.36	
	2.0 mg/l kinetin	0.36	0.30	0.33	
	Kinetin Mean	0.38	0.31		
	Cytokinin Mean	0.47	0.42		

Table 5: Effect of different concentrations of IBA and NAA with different concentrations of WPM on rooting (%) of Manfalouty and Nab El-Gamal pomegranate cultivars.

	Cultivar (A)	Manfalouty			Nab El-Gamal			Mean
	Medium(B)	HSW	FSW	Mean	HSW	FSW	Mean	
Auxin (C)	0.25 mg/l NAA	80	60	70	100	100	100	85
	0.50 mg/l NAA	60	40	50	90	80	85	67.5
	NAA Mean	70	50		95	90		
	0.25 mg/l IBA	100	70	85	100	100	100	92.5
	0.50 mg/l IBA	100	60	80	100	80	90	85
	IBA Mean	100	65		100	90		
	Auxin Mean	85	57.5		97.50	90		
	L.S.D. 5 %	(A) = 6.63			(B) = 4.43		(C) = 7.01	
		(AB) = 6.27		(AC) = 9.91	(BC) = 9.91		(ABC) = N.S	

Table 6: Effect of different concentrations of IBA and NAA with different concentrations of Woody plant medium on roots number of Manfalouty and Nab El-Gamal pomegranate cultivars.

	Cultivar (A)	Manfalouty			Nab El-Gamal			Mean
	Medium(B)	HSW	FSW	Mean	HSW	FSW	Mean	
Auxin (B)	0.25 mg/l NAA	3.80	3.40	3.60	8.00	5.60	6.80	5.20
	0.50 mg/l NAA	4.60	2.40	3.50	9.40	8.20	8.80	6.10
	NAA Mean	4.20	2.90		8.70	6.90		
	0.25 mg/l IBA	8.40	8.00	8.20	9.80	9.80	9.80	9.00
	0.50 mg/l IBA	9.80	9.40	9.60	8.40	10.40	9.40	9.50
	IBA Mean	9.10	8.70		9.10	10.10		
	Auxin Mean	6.60	5.80		8.90	8.50		
	L.S.D. 5 %	(A) = 0.95			(B) = 0.76		(C) = 1.21	
		(AB) = N.S			(AC) = 1.71	(BC) = 1.71	(ABC) = N.S	

Table 7: Effect of different concentrations of IBA and NAA with different concentrations of Woody plant medium on roots length (cm) of Manfalouty and Nab El-Gamal pomegranate cultivars.

	Cultivar (A)	Manfalouty			Nab El-Gamal			Mean
	Medium(B)	HSW	FSW	Mean	HSW	FSW	Mean	
Auxin (B)	0.25 mg/l NAA	6.58	5.60	6.09	2.96	2.90	2.90	4.50
	0.50 mg/l NAA	5.42	5.32	5.37	2.34	2.00	2.17	3.53
	NAA Mean	6.00	5.46		2.65	2.45		
	0.25 mg/l IBA	5.86	5.44	5.65	3.34	2.20	2.29	3.97
	0.50 mg/l IBA	5.42	4.94	5.18	1.18	1.00	1.09	3.14
	IBA Mean	5.64	5.19		2.26	1.60		
	Auxin Mean	5.82	5.32		2.46	2.03		
	L.S.D. 5 %	(A) = 0.42			(B) = 0.37		(C) = 0.50	
		(AB) = N.S		(AC) = 0.83	(BC) = 0.83		(ABC) = N.S	

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