JKAU: Met., Env., Arid Land Agric. Sci., **Vol. 2**, pp. 47-53 (1411 A.H./1991 A.D.)

In vitro Callus Formation from Pomegranate (*Punica granatum* L.) Shoot Tips

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ABSTRACT. Callus was initiated from cultured shoot tips (1 cm in length) of pomegranate (*Punica granatum* L.) cv. "Banat" on a basal medium containing Murashige and Skoog (MS) salts, vitamins, glycine, i-inositol, sucrose, agar and IAA/BA at concentrations of 00/00, 0.1/1.0, 0.3/3.0, 0.5/5.0 and 1.0/10.0 mg/L. Callus growth was better in light than in darkness. Also, callus formation occurred on MS medium containing benzylaminopurine (BA) at concentrations of 0, 2.5, 5 and 7.5 mg/L. The best callus growth was in the medium containing 5 mg/L BA.

When shoot tips were cultured on MS medium supplemented with 0, 2, 4 and 6 mg/L NAA, callus was formed and then subcultured twice. The formed callus was maintained for 105 days.

Introduction

Pomegranate (*Punica granatum* L.) is one of the most important fruit crop in Saudi Arabia and other countries in the Mediterranean zone. It can be propagated by conventional methods, such as, hardwood cuttings and layring. Little information is available on *in vitro* culture methods for the propagation of pomegranate. Jaidka and Mehra (1986), reported some callus induction from shoot tips, nodes and roots of pomegranate seedling. The formation of callus from leaf explant of pomegranate has been reported (Omura *et al.* 1987). The potential of mass *in vitro* propagation has been demonstrated for many plants (Papachatzi *et al.* 1980, Cellarova *et al.* 1982 and Jaiswal and Naryan 1985).

No attempt was found in the literature to use the *in vitro* culture method for propagation of pomegranate in Saudi Arabia. The following investigation described the

ability of the induction and maintenance of callus from shoot tips of pomegranate clone.

Material and Methods

Shoot tips (1 cm long) were obtained from 8-years old pomegranate trees of the cultivar "Banat" grown in the Experimental Research Station of the Faculty of Agriculture, King Saud University.

Explants were surface sterilized in a solution of 10% clorox (5.25% sodium hypochlorite) and 0.1% Tween – 20 (Polyoxethylene sorbitan monolaurate) for 10-12 min and rinsed three times with sterile distilled water.

The cultured medium consisted of Murashige and Skoog salts (1962) at 1X concentration supplemented with 3% sucrose, thiamine – HCl, 0.5, pyridoxine – HCl, 0.5, nicotinic acid, 0.5, glycine, 2.0, 1-inositol, 100 and adenine sulfate, 80 mg/L, pH was adjusted to 5.7 and 7 g/L agar was added. All media were autoclaved for 15 min at 121°C and 1.2 kg/cm^2 (15 psi). Individual shoot tip was cultured into 25 × 150 mm glass culture tubes containing 25 ml of the medium.

Treatment consisted of MS medium containing IAA (Indole-3-acetic acid)/ BA(N6-benzylaminopurine) at concentrations of 00/00, 0.1/1.0, 0.3/3.0, 0.5/5.0 and 1.0/10.0 mg/L. BA alone was added to another set of the media at concentrations of 0, 2.5, 5 and 7.5 mg/L. A third set of media was prepared and supplemented with NAA at 0, 2, 4 or 6 mg/L.

Cultures were maintained in controlled environment room at 27 ± 2°C under cool – white fluorescent lamps (Sylvania GRO-LUX) and 16 hr daylength. Moreover, some cultures of each treatment were maintained under dark condition. Visual observations were recorded using (–) and (+) signs. Plus (+) sign was given to the explant formed callus. While, two plus (++) were given to the explant formed callus, twice in amount, as callus given one plus. Three plus signs (+++) were given to the explant callus, formed troble the amount, as those given one plus, while, minus (–) was given to nonformed callus. For computing these signs, they were transformed into numbers as follows: – = 0, + = 1, ++ = 2 and +++ = 3, and data were statistically analyzed.

Results and Discussion

In dark conditions, the greatest amount of callus was obtained on the medium containing a combination of 0.3 mg/L IAA with 3.0 mg/L BA and the least amount of callus was obtained on the hormone free medium (Table 1). The callus was brown yellowish or light brown in color with soft structure, but at 0.1 mg/L IAA with 1.0 mg/L BA the callus was semi-compact. In light condition the maximum callus production was obtained on medium containing 1.0 mg/L IAA plus 10 mg/L BA and the least amount of callus occurred on hormone free medium and on medium containing 0.1 mg/L IAA plus 1.0 mg/L BA (Table 2). The colour of the callus varied according to

IAA/BA treatments, but the structure was compact in all treatments, except the one that contained 0.3 mg/L IAA and 3.0 mg/L BA which had a soft callus.

TABLE 1. The effect of IAA:BA ratio on callus formation and growth pomegranate explants grown on MS medium for 10 weeks in the dark conditions.

Concent. mg/L IAA/BA	Callus		
	Mean	Amount	Colour
0.0	0.60	+	B (S)*
0.1/1.0	0.40	+	B/W (C)
0.3/3.0	1.60	+++	B (S)
0.5/5.0	1.40	++	B/Y (S)
1.0/10.0	1.40	++	B/Y (S)

C = Compact callus, S = Soft callus.
+, ++, +++, were used as indicator for the amount of callus.
B = Brown, W = White, Y = Yellow.
L.S.D. at 0.05 = 0.7226.

TABLE 2. The effect of IAA:BA ratio on callus formation and growth pomegranate explants grown on MS medium for 10 weeks in the light conditions.

Concent. mg/L IAA/BA	Callus		
	Mean	Amount	Colour
0.0	0.80	+	Y/G (C)
0.1/1.0	1.00	+	G/B (C)
0.3/3.0	1.60	++	B/Y (S)
0.5/5.0	1.20	+	Y/B (C)
1.0/10.0	1.80	+++	Y/G (C)

C = Compact callus, S = Soft callus.
+, ++, +++, were used as indicator for the amount of callus.
B = Brown, W = White, Y = Yellow.
L.S.D. at 0.05 = 0.6981.

Table 3 shows that the best callus growth was obtained in the medium containing 5 mg/L BA followed by that containing 7.5 mg/L BA, while the control gave the least growth. The effect of BA on the treated callus was similar in most treatments where the developed colour was green. However, BA at 5 mg/L led to the formation of greenish yellow colour.

Table 4 indicates that in the first subculture there was a slight difference in the callus growth between the treatments. The colour of callus was greenish with compact

TABLE 3. Affect of BA concentration on callus formation and growth of pomegranate explants grown on MS medium for 8 weeks.

BA concentration mg/L	Callus		
	Mean	Amount	Colour
0.0	1.00	+	G + B (C)
2.5	1.167	+	G (C)
5	1.167	+ + +	G/Y (C)
7.5	1.500	+ +	B + G (C)
10	1.167	+	B + G (C)

C = Compact callus, S = Soft callus.
+, + +, + + +, were used as indicator for the amount of callus.
G = Green, B = Brown, W = White, Y = Yellow.
L.S.D. at 0.05 = 0.60631.

texture. The amount of callus increased after the second subculture in all treatments. When the callus was subcultured in the *3rd* fresh media, the concentration of 6.0 mg/L NAA gave the best callus growth as compared with other media. In comparison between the subcultures (Table 4), the growth of callus through the subcultures was improved, but no differentiation occurred.

TABLE 4. The effect of NAA concentration and subculture on callus growth of pomegranate grown on MS medium.

NAA Conc. mg/L	Subculture 1 (28 days)			Subculture 2 (45 days)			Subculture 3 (105 days)		
	Callus								
	Mean	Amount	Colour	Mean	Amount	Colour	Mean	Amount	Colour
0.0	1.50	+ +	G/WC	1.667	+ +	GC	1.00	+	Y/GC
2.0	1.17	+	G/WC	1.83	+ + +	GC	2.00	+ +	G/YC
4.0	1.00	+	G + BC	1.67	+ +	G/WC	1.83	+ +	G/WC
6.0	1.50	+ +	G + BC	1.67	+ +	G/YC	2.50	+ + +	G + BC
Subcultural comparing	b			a			a		

G = Green, B = Brown, C = Compact Callus, G/W Greenish White, G / Y = Greenish Yellow.
+, + +, + + + were used as indicators for the amount of callus.
The letters (a and b) were used as indicators for significant differences.
L.S.D. at 0.05 1*st* subculture = 0.65. 2*nd* subculture = 0.70. 3*rd* subculture = 1.02.

The results indicates that the callus growth under light condition was better than the callus growth in darkness. However, the callus growth in light condition might need higher concentration of the growth regulators than in darkness and the green

colour of callus under light was most likely due to the effect of light on chlorophyll pigment formation.

NAA in the initial media had no significant effect on callus growth, although its concentration was increased threefold. It might be that the lack of effect was due to the establishment of the callus in the initial media. Through further subculturing, and as time passes on, the callus responded to the presence of NAA and this observed response in subcultures 2 and 3 lasted throughout the subculturing process.

In other studies on callus indication, Jaidka and Mehra (1986) used pomegranate seedlings and reported that callus formation and differentiation into a complete plantlets was achieved by the equal ratio of BA and IAA (2:2 mg/L). Also, Omura, *et al.* (1984) obtained pomegranate plantlets from callus when leaf explants were cultured on MS medium supplemented with a range of 0.1-2.3 mg/L BA and 0.01-0.9 mg/L NAA. These positive results could not be confirmed with the present study using the "Banati" cultivar, which may indicate a genetical variation between their plant specimen (seedling) and the cv. "Banati" used in the present work. Similar differences have been reported in various callus differentiation studies (Anderson 1980, James *et al.* 1980, Snir, 1981 and Welander 1985). On the other hand, it was found that the auxins such as 2, 4-D, NAA and IAA promoted callus formation (Bermudez *et al.* 1984). In other studies, the high concentration Cytokinin (BA) promoted the callus formation (Stoltz 1984 and Yang *et al.* 1986). However, in another study, Nickerson and Hall (1976), found that the callus formation in dark was better than in light. But in the present work the callus growth was better in the light than in dark conditions.

From the results mentioned above, it can be concluded that the callus formation occurred on MS medium supplemented with auxin or cytokinin. Also, the maintenance of callus had been done on MS media enriched with NAA. But the differentiation of the callus into plantlet has not been resolved. This point should be considered for further work by researchers to produce plantlets and to perform cytogenetic examinations for identifying variations for breeding purposes.

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إنتاج الكالوس من الأنسجة القمية للأفرخ في الرمان بطريقة زراعة الأنسجة

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المستخلص . أجريت هذه الدراسة لبحث تكون الكالوس عند زراعة القمم لأفرخ الرمان البناتي (بطول ١ سم) على بيئة موراشيجي وسكوج والتي تحتوي على الأملاح والفيتامينات والجلايسين والأينوسيتول والسكروز والأجار وأضيف حمض اندول الخليك والبنزايل أدينين بتركيز صفر/صفر ، ١/٠,١ ، ٣/٠,٣ ، ٥/٠,٥ ، ١٠/١ ملليجرام/لتر . وقد كان الكالوس المتكون تحت ظروف الضوء أفضل منه في حالة الظلام .

كما تكون الكالوس أيضا عند الزراعة في بيئة موراشيجي وسكوج المحتوية على البنزايل أدنيين بتركيز صفر ، ٣,٥ ، ٥ ، ٧,٥ ملليجرام/لتر وكان أفضل نمو للكالوس في البيئة المحتوية على ٥ ملليجرام/لتر من البنزيل أدنيين .

وعندما زرعت قمم الأفرخ على بيئة موراشيجي وسكوج المضاف إليها نفثالين حمض الخليك بتركيز صفر ، ٢ ، ٤ ، ٦ ملليجرام/لتر تكون الكالوس وأمكن نقله مرتين والاحتفاظ به على البيئة لمدة ١٠٥ يومًا .