JKAU: Met., Env., Arid Land Agric. Sci., **Vol. 1**, pp. 67-75 (1410 A.H./1990 A.D.)

In Vitro Culture and Propagation of *Punica granatum* L.

R.S. AL-OBIED, M.A. SHAHEEN*, F.A. SAAD and A.E. SAID
Plant Production Department, College of Agriculture,
King Saud University, Riyadh, Saudi Arabia.

ABSTRACT. Shoot tip explants (1 cm in length) of pomegranate (*Punica granatum* L.) proliferated and produced shoots after 8 weeks of *in vitro* culture. Shoot tip explants were cultured on Murashige and Skoog (MS) basal medium supplemented with a combinations of IAA:BA at ranges of 0.0-1.3 mg/l and 0.0-3.0 mg/l respectively. Shoot multiplication occurred efficiently 4 weeks after the initial growth was recultured. BA alone (0.8 mg/l produced the highest proliferative effect though BA + IAA at 3.0:0.2 mg/l had a relatively high multiplication effect (3.4 shoot/explant). The interaction of IAA and BA showed to have an effect on shoot length and shoot number per culture. The produced shoots were subcultured on half strength MS medium supplemented with 2.0 mg/l IBA and 0.25 g/l activated charcoal. The percentage of rooted cultures was 100%.

Introduction

Pomegranate (*Punica granatum* L.) has been cultivated for fruit production, ornamental and medicinal purposes. It is propagated by vegetative parts, such as hardwood cuttings and suckers. However, few reports on *in vitro* shoot production of Pomegranate have been published (Jaidka and Mehra 1986, and Omura *et al.* 1987). Such method of propagation could reduce the time required for commercial production of large number of lines and/or stock plants from newly released cultivars. *In vitro* mass propagation has been employed for several fruit trees, including apple (Pieniazek 1968, and Werner and Boe, 1980), Peach (Hammerschlag 1979, and Skirivin and Chu 1978) and grapes (Barlass and Skene 1978). Shoot tip culture

*Currently associated with Department of Arid Land Agriculture, Faculty of Meteorology, Environment, and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia.

has been developed for the *in vitro* propagation of woody horticultural plants as apple (Jones 1976, Lane 1978, and Lane and McDougald 1982) and Goosberry (Wainwright and Flegmann, 1985). Murashige and Skoog (1962) medium was found to be suitable for various plant requirements (Kyte 1983, and Rugini and Verma 1981, 1983). This paper describes the production of Pomegranate through bud multiplication *in vitro* using shoot tips as the explant source.

Material and Methods

Shoot tips were collected from 8-years-old pomegranate trees *cv.* "Banati" grown in the Experimental Research Station of the College of Agriculture. The explants were about 1 cm in length. Shoot tips were first washed with tap water and, were then surface-sterilized in 10% commercial clorox solution (5.25% sodium hypochlorite) containing Tween 20 as a wetting agent (one drop /100 ml) for 10 minutes. The tips were rinsed thoroughly three times by sterile distilled water.

Shoot tips were cultured on nutrient medium. The basal medium consisted of the revised medium of Murashige and Skoog (1962) plus 30 g/l sucrose, 100 mg/l inositol, 80 mg/l adenine sulfate, 0.5 mg/l nicotinic acid, 0.5 mg/l pyredoxin, 1.0 mg/l thiamine HCL, 2.0 mg/l glycine.

The growth regulators were BA (N6-Benzylaminopurine) and IAA (Indol-3-acetic acid). BA concentrations were 0.0, 0.2, 0.8, 1.5 and 3 mg/l, whereas IAA concentrations were 0.0, 0.2, 0.4, 0.8 and 1.0 mg/l. The medium pH was adjusted to 5.7 ± 0.1 with 0.1 *N* NaOH or HCl and agar (Bacto-agar) was added at 7 g/l. Twenty-five ml agar medium were distributed per 25×150 mm culture tubes, and these were capped with enclosures (Bellco Kaputs). The medium was autoclaved at 121°C and 15 lbs/inch^2 pressure for 15 minutes. Shoot tips were cultured singly in the 25×150 mm tubes. All cultures were incubated in controlled room at 27°C $\pm$ 2 under 16 hr/day at 1500 lux from cool-white fluorescent lamps (Sylvanic GRO-LUX). Data collected included shoot number, shoot length and leaf number. All IAA and BA combination were replicated 10 times and data were statistically analyzed.

Results and Discussion

Table 1 shows that BA had a significant effect on shoot length, whereas, 0.8 mg/l BA has significantly more effective than the higher and lower concentrations and the control. Furthermore, BA was also significantly more effective in lower concentrations (0.2, 0.8, and 1.5 mg/l) than in the higher concentration (3.0 mg/l) during the first 4 weeks of the treatment. On the other hand, IAA alone had no significant effect on shoot length as compared with control. The interaction between BA and IAA led to a significant difference in shoot length as compared with the control (Table 1). It was, also, clear from the results that when the concentrations of both growth regulators was increased, the shoot length was decreased. In addition, the effects of BA, IAA either alone or in combination. After 8 weeks, consistently followed the trend described for the first 4 weeks (Table 2). Shoot multiplication, however, in BA and IAA either alone or in combination, was not affected during the first 4 weeks of the

TABLE 1. Effect of various combinations of BA and IAA on shoot length (mm) of Pomegranate explant grown on MS medium for 4 weeks.

BA conc. mg/l.	IAA conc. mg/l.					
	0.0	0.2	0.4	0.8	1.0	BA mean
0.0	06.50	10.00	10.43	10.71	12.50	10.03 c
0.2	13.67	28.89	10.00	18.75	13.13	16.89 ab
0.8	23.13	16.11	20.00	23.75	14.63	19.52 a
1.5	12.50	13.75	11.25	15.88	15.00	13.68 ab
3.0	17.40	7.50	11.67	8.00	10.57	11.03 c
IAA mean	14.64 a	15.25 a	12.67 a	15.42 a	13.17 a	

1. Means with the same letters are not significantly different at 0.05, probability level.
2. Average L.S.D. at 0.05 for the treatments means = 3.03.

TABLE 2. Effect of various combinations of BA and IAA on shoot length (mm) of Pomegranate explant grown on MS medium for 8 weeks.

BA conc. mg/l.	IAA conc. mg/l.					
	0.0	0.2	0.4	0.8	1.0	BA mean
0.0	08.63	14.17	21.67	32.14	35.00	22.32 ab
0.2	35.00	42.78	32.50	26.88	15.00	30.43 a
0.8	28.75	33.75	26.88	33.57	38.75	32.34 a
1.5	25.00	37.50	38.33	32.86	34.00	33.54 ab
3.0	17.50	29.00	15.83	22.00	18.57	20.58 b
IAA mean	22.98 a	31.44 a	27.04 a	29.49 a	28.26 a	

1. Means with the same letters are not significantly different at 0.05, probability level.
2. Average L.S.D. at 0.05 for the treatments means = 4.85.

treatment, but it took 6 weeks of treatment to show proliferation of shoots. Furthemore, when explants attained 8 weeks of age, proliferation of shoot became significantly obvious and differed according to the treatment (Table 3). It was found that BA alone induced shoot multiplication and it was also clear that as the concentration of BA was increased the degree of proliferation was increased too was compared with the control. However, BA at 0.8 mg/l was the most effective concentration as

TABLE 3. EEffect of various combinations of BA and IAA on shoot number of Pomegranate explant grown on MS medium for 8 weeks.

BA conc. mg/l.	IAA conc. mg/l.					
	0.0	0.2	0.4	0.8	1.0	BA mean
0.0	1.00	1.00	1.00	1.00	1.00	1.00 b
0.2	1.60	1.33	1.00	1.00	1.00	1.19 b
0.8	4.00	2.13	1.75	1.71	1.63	2.24 a
1.5	2.25	2.50	2.33	1.86	2.00	2.19 a
3.0	2.17	3.40	2.83	2.40	2.60	2.68 a
IAA mean	2.20 a	2.07 a	1.78 a	1.59 a	1.65 a	

1. Means with the same letters are not significantly different at 0.05, probability level.
2. Average L.S.D. at 0.05 for the treatments means = 0.94.

compared with the lower and higher concentration. In contrast, IAA alone was not effective in inducing shoot multiplication. On the other hand, when BA was added with IAA shoots were stimulated to multiply. In addition, when the concentration of both regulators in combination was increased the rate of proliferation was also increased. These results indicated that shoot multiplication probably required only BA in the medium.

Tables 4 and 5 show that the BA concentrations were significantly effective on the number of leaf produced as compared with the BA-free medium. Whereas, IAA not significantly effective on leaf production as compared with IAA free medium. In addition, the interaction between IAA and Ba significantly increased the number of leaves per shoot. It should be noted that the increase in the number of leaves per shoot occurred during the first 4 weeks of the treatments. As the time of treatment was extended to 8 weeks, new shoots that developed had newly developed leaves only in the effective treatments.

In previous work on pomegranate micropropagation, Jaidka and Mehra (1986) reported results which were similar to the present work as far as the shoot multiplica-

TABLE 4. EEffect of various combinations of BA and IAA on leaf number of Pomegranate explant grown on MS medium for 4 weeks.

BA conc. mg/l.	IAA conc. mg/l.					
	0.0	0.2	0.4	0.8	1.0	BA mean
0.0	2.50	5.00	5.43	6.29	4.17	4.68 b
0.2	6.33	10.78	6.60	7.50	8.25	7.89 a
0.8	8.50	8.38	8.43	9.63	8.75	8.74 a
1.5	7.38	10.83	7.13	9.75	8.75	8.77 a
3.0	8.80	8.64	8.33	8.40	6.00	8.03 a
IAA mean	6.70 a	8.73 a	7.18 a	8.31 a	7.18 a	

1. Means with the same letters are not significantly different at 0.05, probability level.

TABLE 5. EEffect of various combinations of BA and IAA on leaf number of Pomegranate explant grown on MS medium for 8weeks.

BA conc. mg/l.	IAA conc. mg/l.					
	0.0	0.2	0.4	0.8	1.0	BA mean
0.0	3.38	6.17	6.83	10.14	8.60	7.02 b
0.2	12.60	13.22	7.67	8.75	7.67	9.98 ab
0.8	14.50	9.63	11.75	14.86	16.25	13.40 a
1.5	11.50	11.63	12.83	12.29	12.80	12.21 a
3.0	11.67	13.40	11.83	12.00	11.20	12.02 a
IAA mean	10.73 a	10.81 a	10.18 a	11.61 a	11.3 a	

1. Means with the same letters are not significantly different at 0.05, probability level.
2. Average L.S.D. at 0.05 for the treatments means = 4.85.

tion was concerned. They used MS and medium supplemented with 2 mg/l BA and 2 mg/l NAA. However, they did not use either growth regulators alone to test out their single effects on shoot multiplication. Moreover, they had not reported the use of various concentrations of BA and NAA combinations except the 1:1 ratio. In contrast, the present work showed that the 1:1 of BA to IAA was not significantly effective in shoot multiplication. Whether the difference between their work and the present one may be due to varietal differences, since they used buds from seedlings of *cv.* Kandhan anar, or to the difference in the chemical form of the auxin used (IAA *vs.* NAA), will have to be reconciled.

Arnold (1982), demonstrated that the presence of IAA at high concentration inhibited shoot proliferation, while BA induced shoot development due to its effect on differentiation of apical and axillary buds. Maheshwari and Sreekrishan (1982), found that the hormone-free medium produced the main shoot only. But when a cytokinin was supplemented to the medium it overcame the apical dominance and led to shoot multiplication. Werner and Boe (1980), and Pieniazek (1968) found that in apple explants, the shoot number was increased by 0.5 mg/l BA. Similarly, Bhojwani *et al.* (1984), on pear (*Pyrus pyrifolia*) reported that 1.0 mg/l BA with MS medium led to 4-5 shoots formation. Babic and Mirjana (1984) showed that 1-3 mg/l BA gave the best result for shoot number increase in blackberry micropropagation, while the higher concentrations of BA produced only shorter stems. Kunisaki (1980), found that BA at 0.2-1.0 mg/l increased shoot proliferation in *Anthurium* but in the higher concentrations shoots were dwarfed and callus was also formed.

The combination of cytokinin and auxin was found effective in shoot proliferation and shoot length, when blackberry shoot-tips were cultured on medium supplemented with low concentrations of BA and IBA or NAA (Chalupa 1981). Hasegawa (1986), obtained 6 multiple shoots from rose shoot tip on MS medium containing 0.3 mg/l IAA and 1.3 and 10 mg/l. However, Hildebrandt and Harney (1984), indicated that new shoot initiation and Deutzia × lemoinei was obtained on MS medium containing 0.1 mg/l BA and 0.1 mg/l IAA.

From the aforementioned discussion, it is clear that cytokinins and auxins are important for the micropropagation and tissue culture. The requirements of plants are also different as far as the concentration of these regulators is concerned (Hanh *et al.* 1981, Stoltz 1984, and Dhawan and Bhojwani 1985). The shoot length in Hydrangea was found to decrease by the increase of hormone concentration (Stoltz 1984). Similar results for *Anthurium andereanum* were reported by Kunisaki (1980). In addition, the effect of plant hormones on leaf development is not well understood. Auxins are probably effective in stimulating leaf vein growth, while cytokinins may induce the mesophyll tissue development (Wareing and Phillips 1985).

The produced shoots were subcultured on half strength MS medium supplemented with 2.0 mg/l IBA and 0.25 g/l activated charcoal. Percentage of rooted cultures was 100%. The rooted shoots were transferred to free living conditions in peat and perlite mixture (1:1) Fig (1).

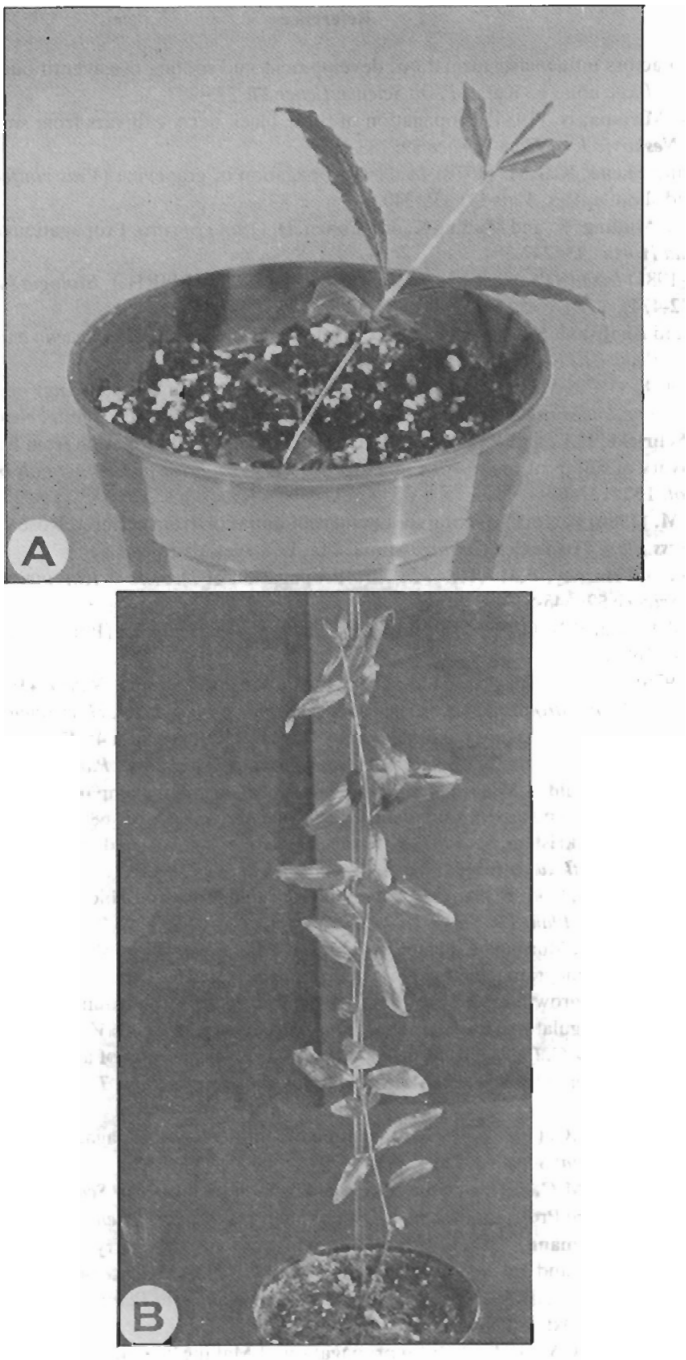

FIG. 1. Pomegranate plant grown on the soil medium (Peat and Perlite mixture 1:1) for a) 2 months, (b) 4 months.

References

Arnold, S.V. Factors influencing formation, development and rooting of adventitious shoots from embryos of *Picea abies* L. Karst, *Plant Science Letter* **27:** 275-287.

Babic, V. and **Mirjana, N.** (1984) Propagation of three black berry cultivars from small apical buds *in vitro*, *Neskovic J. of Hort Science* **59:** 183-185.

Barlass, M. and **Skene, K.G.M.** (1978) *In vitro* propagation of grapevine (*Vitis vinifera* L.) from fragmented shoot apices, *Vitis* **17:** 335-340.

Bhojwani, S.S., Mulling, K. and **Mulling K.** and **Cohen, D.** (1984) *In vitro*. Propagation of *Pyrus pyrifolia*. *Scientia Hortic.* **23:** 247-254.

Chalupa, V. (1981) *In vitro* Propagation of Brich (*Betula verrucose* EHRH.), *Biologia Plantarum (Parha)* **23:** 472-474).

Dhawan, V. and **Bhojwani, S.S.** (1985) *In vitro* vegetative propagation of *Leucaena leucocephala* (Lam) de Wit, *Plant Cell Reports* **4:** 315-318.

Hammerschlag, F. (1979) *Micropropagation of Peach: Stages I and II. Proceedings of the 55th Cumberland-Shenandoah Fruit Workers Conference*, Harpers Farry, West Virginia, November 15-16.

Hanh, T.T., Schricke, H.L. and **Van, T.T.** (1981) Direct *in vitro* bud formation from fragments and thin cell layers of different organs of the winged bean (*Psophocarpus tetragonolobus*), *Z. Pflanzen Physiol.* **102:** 127-139.

Hasegawa, P.M. (1986) Factors affecting shoot and root initiation from cultured Rose shoot tips, *6th Int. Congress Plant Tissue Cell Culture*, Minnesota, U.S.A. (Abstract).

Hildebrant, V. and **Harney, P.M.** (1984) *In vitro* Propagation of *Deutzia* × *lemoinei* var., *compacta*. *J. Hort. Science* **59:** 545-548.

Jaidka, K. and **Mehra, P.N.** (1986) Morphogenesis in *Punica granatum* L. (Pomegranate), *Can. J. Bot.* **64:** 1644-1653.

Jones, O.P. (1976) Effect of phloridzin and phloroglucinol on apple shoots, *Nature* **43:** 163-166.

Kunisaki, J.T. (1980) *In vitro* propagation of *Anthurium andereanum* Lind, *Hort Science* **15:** 508-509.

Kyte, L. (1983) *Plants from test tubes*, Timber Press, Portland, Oregon, pp. 45-47.

Lane, W.D. (1978) Regeneration of apple plants from shoot meristem tips, *Plant Sci. Lett.* **13:** 281-286.

Lane, W.D. and **McDougald, J.M.** (1982) Shoot tissue culture of apple: Comparative response of five cultivars to cytokinin and auxin, *Canadian Journal of Plant Science* **62:** 689-694.

Maheshwari, R. and **Sreekrishan, S.** (1982) The apical control of lateral bud development in excised shoot tips of *Cuscuta reflexa* cultured *in vitro*, *Physiol. Plant* **56:** 474-481.

Murashige, T. and **Skoog, F.** (1962) A revised medium for rapid growth and bio-assays with tobacco tissue cultures, *Physiol. Plant* **15:** 473-497.

Omura, M., Matsuta, N., Moriguchi, T. and **Kozaki, I.** (1987) Adventitious shoot and plantlet formation from cultured pomegranate leaf explants, *Hort Science* **22:** 133-134.

Pieniazek, J. (1968) The growth *in vitro.*, of isolated apple-shoot tips from young seedlings on media containing growth regulators. *Bull. de Acad. Polonaise Des. Sciences O.V.* **16**(3): 179-183.

Rugini, E. and **Verma, D.C.** (1981) Micropropagation and cell suspension of a difficult-to-propagate almond (*Prunus amyqdalus* Batsch) cultivar, *Proc. 5th Congr. Plant Tissue and Cell Culture*, pp. 741-742.

Rugini, E. and **Verma, D.C.** (1983) Micropropagation of a difficult-to-propagate almond (*Prunus amyqdalus* Batsch), *Plant Sci. Lett.* **28:** 273-281.

Skirivin, R.M. and **Chu, M.C.** (1978) Tissue culture of Peach shoot tips, *Hort Science* **13:** 349 (Abstr.), 24.

Stoltz, L.P. (1984) *In vitro* Propagation and growth of Hydrangea, *Hort Science* **19:** 717-719.

Wainwright, H. and **Flegmann, A.W.** (1985) micropropagation of gooseberry (*Rives uvacrispa* L.) II: *In vitro* proliferation and *in vivo* establishment, *Journal of Hort. Science* **60:** 485-491.

Wareing, P.F. and **Phillips, I.D.J.** (1985) *Growth and Differentiation in Plants*. 3rd Edition, pp. 125-126, Pergamon Press, Oxford, N.Y. and Toronto.

Werner, E.M. and **Boe, A.A.** (1980) *In vitro* propagation of Malling 7 apple root stock. *Hort Science* **15:** 509-510.

زراعة الرمان وإكثاره معمليا

راشد سلطان العبيد ، محمد عبد الرحيم شاهين ، فيصل عبد الله سعد وعبد الغفار الحاج سعيد
قسم الإنتاج النباتى – كلية الزراعة – جامعة الملك سعود – الرياض – المملكة العربية السعودية .

أمكن إكثار القمم النامية الخضرية (بطول ١ سم) للرمان وأنتجت نموات خضرية (أفرع خضرية) بعد أسابيع من زراعتها فى الأنابيب . وقد زرعت هذه القمم النامية على بيئة موراشيجى واسكوج الأساسية المزودة بمزيج من أندول حمض الخليك (IAA) أدنين البنزيل (BA) بنسب تتراوح من صفر –١ مجم/لتر وصفر –٣ مجم/لتر على التوالى .

وكان إنتاج الأفرع الخضرية وفيرا عندما نقلت المزرعة الأولية إلى بيئة طازجة بعد ٤ أسابيع .. استخدام أدنين البنزايل بمفرده (بتركيز ٠,٨ مجم/لتر) أدى إلى أعلى تأثير في التفريع ، ورغم ذلك فإن استخدام أدنين البنزيل وإندول حمض الخليك (بنسبة ٣ : ٠,٢ مجم/لتر) أدى إلى زيادة نسبية فى عملية التفريع (٣,٤ فرع/منفصل نباتي) . وكان لاستخدام أدنين البنزيل مع إندول حمض الخليك عموما تأثير آخر على طول الفرع وعدد الأفرع لكل مزرعة .

ولقد زرعت الأفرع الخضرية الناتجة على بيئة موراشيجى واسكوج الأساسية المخففة للنصف مزودة بتركيز ٠,٢ ملجرام/لتر من الـ IBA وتركيز ٠,٢٥ جرام/لتر من الفحم المُنَشَّط . ووصلت نسبة التجذير في الزراعات إلى ١٠٠٪ .