IN VITRO MICROPROPAGATION OF *PISTACIA* ROOTSTOCKS

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INTRODUCTION

Pistachio nuts have been planted in the Middle East for a very long time and are gaining more and more popularity in the world. *Pistacia vera* L. is the only species in this genus which produces commercially acceptable large edible nuts. Most of the other *Pistacia* species are used as rootstocks for *P. vera*. Among the important factors limiting the expansion of pistachio plantations based on superior selected lines, is the difficulty of propagation as they are only propagated by the relatively slow method of budding scions to rootstock. *In vitro* mass clonal micropropagation of *Pistacia* has been reported before (1), and this paper presents further work on *Pistacia* rootstocks.

MATERIALS AND METHODS

Seeds of *P. vera* L., *P. mutica* Fisch. & C.A. Mey., *P. khinjuk* Stocks., *P. atlantica* Desf. and *P. palaestina* Boiss. were either germinated aseptically or germinated in vermiculite and grown in plant pots for up to 2 years. The experimental explants were collected as needed. Growing conditions in the glasshouse, sterilization treatments, media preparation, in *in vitro* incubation conditions were as reported by Barghchi and Alderson (2). Murashige and Skoog (MS) medium containing 4 mg/l 6-benzylaminopurine (BAP) was used as standard medium unless specified otherwise. The factors studied were: (a) size of explant; (b) incubation temperature of 20 and 25°C; (c) prewashing of explants in distilled water prior to culture; (d) p. *mutica* and *P. khinjuk* were cultured in MS medium containing naphthaleneacetic acid (NAA) at 0.0, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/l; kinetin at 0.0, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/l in addition to the standard medium; (e) different concentrations of macronutrients (full, ½, and ⅓); (f) initial incubation of some cultures in the dark for up to 4 weeks.

There was a minimum of 10 replicate cultures for each treatment.

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RESULTS

Apical dominance was much stronger in the 1-year-old *P. vera* explants than in the seedling explants and apical buds of 1-year-old plants had better extension growth than axillary buds — (average shoot length 18 and 5 mm, respectively). After a few subcultures there was no difference between the two explants. Removal of the apical bud in the first culture encouraged more axillary buds to develop into shoots. The production of callus or shoots did not appear to be related to explant size. However, explants with three axillary buds showed a slight increase in shoot growth over those with two or one bud (average shoot numbers were 6.77, 5.50, or 4.15, respectively). Shoot growth and proliferation was not affected by pre-washing the explants in water for up to 60 min prior to culture.

Cultures produced more callus at 20° than 25°C, the fresh weight values per culture being 0.623 and 0.310 g, respectively. The lower temperature did not affect number of shoots or shoot growth, but leaves were curled and abnormally thick at 20°C. In general, 25°C was a better incubation temperature.

The use of different concentrations of macronutrients did not produce any beneficial effects; the total shoot length per culture averaged 32.7, 18.6 and 16.5 mm and number of shoots produced averaged 5.30, 6.80 and 3.50, respectively, in full, one-half, and one-third strength macronutrient concentrations.

Apical and axillary buds of *P. mutica* and *p. khinjuk* seedlings produced good shoot growth in the presence of 0.2 to 0.5 mg/l kinetin and NAA. Initial incubation of explants in the dark for 7 to 10 days on a medium containing 0.2 mg/l kinetin and NAA produced plants, complete with roots, within 4 to 6 weeks (Table 1). These plants could be readily established in soil. Cultures on this medium could be stored under the same cultural conditions for a year without any subculture. These two species had best shoot growth and proliferation on MS medium containing 4.0 mg/l BAP (Table 2). However, shoot tip necrosis was common in some shoots when they became greater than 15 mm in length.

Table 1. Comparison of shoot and root growth of seedling explants of *Pistacia mutica* and *P. khinjuk* cultures on MS medium containing 0.2 mg/l kinetin + 0.2 mg/l NAA. (15 cultures per treatment).

Species	Root no.	Total root length (mm)	Shoot no.	Total shoot length (mm)
Pistacia mutica	0.29	2.1	1.14	5.7
P. khinjuk	3.43	17.7	1.00	6.4

Explants from 2-year-old plants had poor establishment in

culture due to severe browning and high levels of phenolics.

Transfer of cultured explants, within the same jar, once the browning of the medium was apparent, improved *in vitro* establishment (*P. atlantica* = 80%, *P. khinjuk* = 60%, *P. mutica* = 80%, and *P. palaestina* = 50%). Axillary bud explants had much lower establishment than apical bud explants in culture.

Table 2. Shoot growth of seedling explants of *Pistacia mutica* and *P. khinjuk* on MS medium supplemented with 4.0 mg/l BAP. (30 cultures per treatment).

Species	Shoot number per culture	Total shoot length per culture	No. of shoots with tip necrosis per culture
Pistacia mutica	3.84	47.3	2.16
P. khinjuk	1.82	22.4	1.50

DISCUSSION

Apical bud explants had better growth and were usually 3 to 5 times larger than axillary bud explants in *in vitro* culture. The basipetal movement of auxin produced on the mother plant and its accumulation in the lower buds, may be responsible for this growth inhibition. Successive subcultures on a medium which prevented apical dominance led to the growth of shoots which were more uniform in size.

The explants with only one axillary bud produced more shoots per bud than those explants with three axillary buds. However, the increased time required for the cutting and preparation of single-bud explants, the further exposure of these explants to desiccation, and the increased oxidation of phenolics at the cut surface, may reduce this small advantage.

The absence of any significant effect of pre-washing suggests that there was no significant leaching of substances from the explant within the time period examined (60 min).

Dilution of macronutrients in MS medium has improved growth in some plants; however, reduced concentration of nutrients can be beneficial to growth only if sufficient nutrients are available. From this study it would appear that a lower macronutrient concentraiton is not beneficial for the shoot growth of *P. vera*. Barghchi and Alderson (1,2) reported that a reduction of macronutrients to half following the shoot proliferation stage improved subsequent root development. This indicates that different stages of growth may have different nutrient requirements.

P. mutica and *P. khinjuk* produced most shoots in MS medium which contained 4.0 mg/l BAP. This medium was optimum for shoot growth and shoot proliferation with *P. vera* and with the commercial cultivars of pistachio also (1,3). In

these previous studies shoot growth improved after a number of further subcultures in the same medium, and it is anticipated that *P. mutica* and *P. khinjuk* would have further improvement after further subcultures.

A practical solution to overcome shoot tip necrosis was to subculture more frequently, which is costly and time-consuming. Shoot tip necrosis is common in suboptimum growth conditions in the *in vitro* culture of many woody plants, and is under further study at the moment.

Shoots cultured on a medium containing kinetin and NAA had a lower shoot growth than those cultured on a medium containing 4 mg/l BAP. The medium with 0.2 mg/l of kinetin and NAA at a lower incubation temperature may be useful for even longer storage of cultured material.

The accumulation of phenolics and other growth inhibitors may occur in plants due to aging and maturation. Rejuvenation of parent material in black locust (*Robinia pseudocacia* L.) improved the establishment and growth of cultures *in vitro* (Barghchi, unpublished). Although two-year-old plants of *Pistacia* were not mature, they had grown through certain stages of aging and maturation; treatments which change the physiological and biochemical state of the explants to a more juvenile form would be expected to improve the establishment and growth *in vitro*. Treatments applied to mature plants of pistachio cultivars to rejuvenate them or to achieve a more active and vigorous growth improve the *in vitro* establishment of explants (4).

LITERATURE CITED

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