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Research note

Regeneration of *Elaeagnus angustifolia* from leaf segments of in vitro-derived shoots

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Abstract

Shoot regeneration was achieved from in vitro-produced leaves of *Elaeagnus angustifolia* L. Half-leaf explants from the terminal part of the shoot produced more shoots than explants from the basal part of the in vitro-derived shoots on agar-solidified WPM medium supplemented with 1 μM benzyladenine (BA). In liquid medium of the same formulation, compact shoots that did not elongate were formed on the explants. Leaf cross-section explants (1 mm thick) produced shoots both on solid and liquid medium with 1 μM BA, whereas again compact shoots were formed with 10 μM BA. Further shoot development on these explants was promoted by their transfer to fresh solid medium containing 1 μM BA and 1 μM gibberellic acid (GA_3).

Abbreviations: BA – benzyladenine, GA_3 – gibberellic acid, WPM – woody plant medium

Elaeagnus angustifolia L. (oleaster), an ornamental tree, is suitable for seaside planting because of its tolerance to saline and alkaline soils. In vitro regeneration could be used for mass propagation and/or genetic improvement. So far, shoot regeneration in vitro has been reported for *Elaeagnus* (Bertrand & Lalonde 1985; Economou & Spanoudaki 1988) with an initial 3.5-fold multiplication rate from shoot-tip explants that increased to 6.7-fold during the second passage (Economou & Spanoudaki 1988). Leaf segment explants could be more productive in shoot regeneration than shoot-tip explants. Use of in vitro-derived leaves will eliminate the need for disinfestation. Shoot regeneration from leaf segment explants in woody species has been reported, among others, for *Rubus* (Swartz et al. 1990), *Vaccinium* (Billings et al. 1988), *Vitis* (Stamp et al. 1990), *Malus* (Fasolo et al. 1989; Swartz et al. 1990), *Pyrus* (Leblay et al. 1991) and *Populus* (Ostry et al. 1990).

The aim of this work was to obtain high shoot regeneration rates from explants of in vitro-derived leaves of *Elaeagnus*. Among the factors tested were explant source and type, cytokinin concentration and medium form.

WPM medium (Lloyd & McCown 1980), modified by replacing Na_2EDTA and $FeSO_4$ with FeNa-DTPA (Economou & Spanoudaki 1988), was used. For shoot formation the cytokinin BA was incorporated into the medium.

In vitro proliferating shoots, with leaves 5 to 6 mm long and 3 to 4 mm wide, from the first passage of *Elaeagnus angustifolia* shoot-tip cultures, were used as explant source. Explants were half-leaves, obtained by cutting the lamina into distal and proximal halves, and leaf segments obtained by cutting 1 mm thick cross sections containing a portion of the main vein. With half-leaves, explants from the terminal part of the in vitro-derived shoots were treated separately from those from the basal part of the shoot to compare their organogenic capacity.

In cultures with explants of half-leaf segments, BA was tested at 0.1, 1 or 10 μM both in agar-solidified and liquid medium. On agar-solidified medium, the explants were placed horizontally, 2 per 3 × 10 cm

test tube, with the abaxial side touching the medium. For liquid medium, 100 ml Erlenmeyer flasks containing 25 ml medium and 5 explants each were placed on a shaker (100 rpm). Both test tubes and Erlenmeyer flasks were sealed with aluminum foil. After 8 weeks of culture, shoots formed on the explants were counted. Explants with compact shoots produced were transferred to solid medium containing 1 μM BA for further shoot elongation.

In cultures with leaf cross-section explants, BA was tested again at concentrations of 0.1, 1 or 10 μM. The explants, from the terminal part of the in vitro-derived shoots, were cultured on solid medium or in liquid medium either shaken (100 rpm) or on filter-paper bridges. Shoot formation on the explants was evaluated after 6 weeks. Leaf segments with compact shoots formed were again cultured on solid medium containing 1 μM BA plus 1 μM GA_3 for further shoot elongation.

Cultures of all experiments were maintained at 24 ± 1°/21 ± 1°C day/night temperature on a 16-h photoperiod (52 μmol $m^{-2}s^{-1}$ (400–700 nm), cool-white fluorescent lamps). Each experiment was conducted twice. Statistical analysis for the number of shoots formed was based on analysis of variance while mean separation was based on the HSD test.

In solid medium both proximal and distal half-leaf explants excised from the terminal part of the shoot responded (100%) only to medium containing 1 μM BA and produced new shoots (3.4 and 4.1 shoots per explant, respectively), while explants from the basal part of the shoot formed a negligible number of new shoots with 1 μM BA (Table 1). No shoots were formed on solid medium with 0.1 or 10 μM BA. In cultures with shoots, these arose from the cut edge of the leaf explant. There were no differences in the length of the shoots produced from the various explant types used. In liquid medium extremely compact shoots were formed in the wounded tissues. Such shoots were observed on explants from the terminal part of the shoot and only at concentrations of 1 and 10 μM BA (in 94–100% of the explants), while on explants from the basal part of the shoot, shoots were formed mainly on medium containing 10 μM BA (in 80–82% of the explants) and none at 0.1 μM BA. Explants in 0.1 μM BA increased in size without signs of shoot formation. When explants with such compact shoots from liquid medium were cultured afterwards on solid medium containing 1 μM BA, shoots developed (4.3 shoots per explant with an average length of 11 mm) only from those that were treated with 1 μM BA in the liquid medium.

The leaf cross-section explants, on the other hand, regenerated shoots (2.4–3.6 shoots per explant) both on solid (agar) and liquid (shaken or filter-paper bridge) medium at a concentration of 1 μM BA (Table 2). At this concentration of BA, there were no differences between the medium forms in number of shoots produced, while the length of shoots was greater in the explants on solid than in liquid medium. At concentrations of 10μM BA, however, shoots that formed on the explants were compact in all medium forms tested. These shoots elongated (average length 11 mm) when explants were transferred to fresh solid medium containing 1 μM BA and 1 μM GA_3. Visual examination revealed that shoots originated from the cut edges of the leaf segments without any callus formation.

In this work regeneration of multiple adventitious shoots was achieved in *Elaeagnus* half-leaf segments taken from the terminal part of in vitro-derived shoots and cultured for 8 weeks on solid modified WPM medium containing 1 μM BA. Similarly, shoots were produced from micropropagated leaves that were cut in halves perpendicular to the midrib in *Vaccinium corymbosum* (Billings et al. 1988) and *Pyrus communis* (Leblay et al. 1991). The concentration of BA and the form of the medium affected shoot formation in *Elaeagnus*. BA at a concentration of 1 μM in solid medium was effective in inducing shoot proliferation while at concentrations of 0.1 or 10 μM no response occurred. In liquid medium 1 and 10 μM BA produced compact shoots that elongated when transferred to agar medium. Also the explant source had a strong influence in determining the number of developing shoots. Leaf explants from the terminal part of the in vitro-derived shoots were more responsive and produced more shoots than explants from the basal part of the shoot. This is in agreement with the results reported by Fasolo et al. (1989) for apple cultivars (*Malus domestica*). The high regenerative ability of the leaves from the terminal part of the shoot might be attributed to a lower degree of differentiation. The type of half-leaf explant (proximal or distal leaf segment) did not influence significantly the number of shoots produced per explant. The shoots were mainly developed on the cut surface of the leaf lamina without any callus formation and this agrees with the report of Stamp et al. (1990) on organogenesis of grape (*Vitis vinifera*).

Multiple adventitious shoots were also produced from leaf cross sections (1 mm thick), again from leaves of the top part of the shoot, cultured on either solid or liquid medium (shaken or filter-paper bridge) with the inclusion of 1 μM BA in the medium. Shoot

Table 1. Effect of BA and explant source on shoot formation from half-leaf explants of *Elaeagnus* on solid and liquid medium

Explant source		BA (μM)							
		Solid medium				Liquid medium			
		1		10		1		10	
Shoot	Leaf	Number of shoots	Length of shoots (mm)	Number of shoots	Length of shoots (mm)	Number of shoots	Length of shoots (mm)	Number of shoots	Length of shoots (mm)
Terminal	Distal	4.1 ± 0.7[a]	6.8 ± 0.7	0	-	4.3 ± 0.6	1.3 ± 0.3	5.4 ± 0.5	1.1 ± 0.1
	Proximal	3.4 ± 0.7	8.4 ± 1.0	0	-	5.9 ± 0.9	1.1 ± 0.2	5.9 ± 0.6	1.2 ± 0.2
Basal	Distal	0.2 ± 0.1	6.7 ± 0.5	0	-	0.5 ± 0.2	0.9 ± 0.1	4.5 ± 0.5	1.0 ± 0.1
	Proximal	0.4 ± 0.2	6.5 ± 0.8	0	-	0.7 ± 0.3	1.0 ± 0.1	5.8 ± 0.6	1.1 ± 0.1

[a]Mean of 20 cultures per treatment ± standard error.

Table 2. Effect of BA and form of medium on shoot formation from leaf cross-section explants of *Elaeagnus*.

Medium form	BA (μM)					
	0.1		1		10	
	Number of shoots	Length of shoots (mm)	Number of shoots	Length of shoots (mm)	Number of shoots	Length of shoots (mm)
Solid (agar)	0	-	3.6 ± 0.6[a]	6.8 ± 0.5	9.9 ± 1.4	0.9 ± 0.2
Liquid (shaken)	0	-	2.4 ± 0.4	3.2 ± 0.2	8.1 ± 1.3	1.1 ± 0.1
Liquid (filter-paper bridge)	0	-	2.5 ± 0.4	3.5 ± 0.2	9.7 ± 1.2	1.2 ± 0.2

[a]Mean of 40 cultures per treatment ± standard error.

regeneration from leaf strips, 0.4–1 mm thick, was also reported for *Populus* (Ostry et al. 1990) and fruit trees (Welander 1986). Again in this type of explant, BA had a profound effect on adventitious shoot regeneration. The explants did not form shoots at 0.1 μM BA while they formed compact shoots with the addition of 10 μM BA in the medium. Further shoot development occurred on these explants upon transfer to fresh solid medium containing 1 μM BA and 1 μM GA_3.

This work showed an efficient way of adventitious shoot formation from leaf explants excised from leaves of the terminal part of in vitro-derived shoots of *Elaeagnus*. However, further reseach is needed before the system can be recommended for either micropropagation or generation of somaclonal variants.

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References

Bertrand LJ & Lalonde M (1985) In vitro propagation and nodulation by *Frankia* of actinorhizal Russian olive (*Elaeagnus angustifolia* L.). Plant Soil 87: 143–152

Billings SG, Chin CK & Jelenkovic G (1988) Regeneration of blueberry plantlets from leaf segments. HortScience 23: 763–766

Economou AS & Spanoudaki MJ (1988) Regeneration in vitro of oleaster (*Elaeagnus angustifolia* L.) from shoot tips of mature tree. Acta Hort. 227: 363–368

Fasolo F, Zimmerman RH & Fordham I (1989) Adventitious shoot formation on excised leaves of in vitro grown shoots of apple cultivars. Plant Cell Tiss. Org. Cult. 16: 75–87

Leblay C, Chevreau E & Raboin LM (1991) Adventitious shoot regeneration from in vitro leaves of several pear cultivars (*Pyrus communis* L.). Plant Cell Tiss. Org. Cult. 25: 99–105

Lloyd GB & McCown BH (1980) Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Comb. Proc. Intl. Plant Prop. Soc. 30: 421–437

Ostry ME, Skilling DD, Lee-Stadelmann OY & Hackett WP (1990) Recovery of somatic variation in resistance of *Populus* to *Septoria musiva*. In: Rodriguez R, Tames RS & Durzan DJ (Eds) Plant Aging: Basic and Applied Approaches (pp 113–116). Plenum Press, New York

Stamp JA, Colby SM & Meredith CP (1990) Direct shoot organogenesis and plant regeneration from leaves of grape (*Vitis* spp). Plant Cell Tiss. Org. Cult. 22: 127–133

Swartz HJ, Bors R, Mohamed F & Naess SK (1990) The effect of in vitro pretreatments on subsequent shoot organogenesis from excised *Rubus* and *Malus* leaves. Plant Cell Tiss. Org. Cult. 21: 179–184

Welander M (1986) Plant regeneration in leaf segments of shoots raised in vitro from mature trees. In: Somers DA, Gengenbach BG, Biesboer DD, Hackett WP & Green CE (Eds) 6th Intl Congress of Plant Tissue and Cell Culture, Abstracts (p. 267). University of Minnesota, Minneapolis (abst)