HortScience 33(2):339–341. 1998.

Shoot Proliferation and Rooting in Vitro of *Pulmonaria*

Dennis P. Stimart[1], John C. Mather[2], and Kenneth R. Schroeder[3]
Department of Horticulture, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706

Additional index words. lungwort, micropropagation, propagation

Abstract. **Expanding shoot tips of *Pulmonaria* 'Roy Davidson' and *Pulmonaria saccharata* 'Margery Fish' were cultured in vitro on a modified Murashige and Skoog medium containing BA to establish proliferating cultures for use in comparing BA concentrations on shoot proliferation and rooting. The optimum level for shoot proliferation was 8.8 μM BA. Greatest rooting was on medium without BA. Genotype and time in culture influenced shoot and root counts. Chemical names used: N⁶-benzyladenine (BA)**

Pulmonaria, commonly known as lungwort, belongs to the Boraginaceae and is native throughout Europe and into Asia. Only four of 12 species are attractive for the shade garden (Armitage, 1989; Lovejoy, 1993). The garden species and cultivars are of European origin and are generally herbaceous, although some are evergreen or nearly so where winters are mild. Some have been valued as garden plants for hundreds of years, but only recently have become of interest in North America. The primary ornamental value of some lungworts is their long-lived, long-stemmed, colorful flowers and silver-spotted foliage. Flowers often open pink and turn blue before dropping.

Pulmonaria is propagated most commonly by seed or division (Armitage, 1989; Lovejoy, 1993). Seed is sown primarily to select new cultivars, which are maintained by division every 3 to 4 years. Plants are shipped as bareroot stock, but do not store well (R. Klehm, Klehm Nurseries, South Barrington, Ill., pers. communication). The introduction of a new crop involves development of techniques for propagation, growing, and postharvest handling. Based on the need to develop large clonal populations and to improve postharvest handling, the objective of this study was to establish a rapid in vitro culture system to substitute for conventional propagation and handling methods.

Materials and Methods

Shoots of *Pulmonaria* x 'Roy Davidson' and *Pulmonaria saccharata* Mill. 'Margery Fish' were excised from plants actively growing in the greenhouse during Dec. 1994. The outer leaves were peeled from expanding shoot tips and discarded down to a leaf length of ≈5 mm. Shoot tips were stirred for 15 min in 5% Clorox (Clorox Co., Oakland, Calif.) with 1 drop/100 mL Tween 20 and rinsed three times in sterile water. The distal 2 to 3 mm of each shoot was discarded to eliminate tissue damaged during disinfestation.

Basal culture medium consisted of Murashige and Skoog (MS) salts (Murashige and Skoog, 1962) supplemented with 0.56 mM myo-inositol, 1.2 μM thiamine HCl, and 88 mM sucrose (basal medium). The medium was adjusted to pH 5.7 prior to autoclaving and was gelled with 7 $g \cdot L^{-1}$ Difco Bacto agar. GA-7 containers with 75 mL of medium were autoclaved for 20 min at 120 °C and 104 kPa.

Cultures were maintained in a growth chamber at 25 °C under cool-white fluorescent light (30 $\mu mol \cdot s^{-1} \cdot m^{-2}$) from 0800 to 2400 HR and in darkness from 2400 to 0800 HR.

Aseptic cultures were established as clonal proliferating shoot populations after five subcultures at 4-week intervals on basal medium with 4.4 μM N⁶–benzyladenine (BA). After 20 weeks of subculture, proliferating shoots were divided into single shoots and subcultured on basal medium supplemented with 0, 0.4, 0.8, 4.4, 8.8, or 44.4 μM BA. Shoots were placed in an upright position on the medium surface with the basal 5 to 6 mm inserted into the medium and subcultured on fresh medium of the same composition after 4 weeks. At the end of the first and second 4-week culture periods, numbers of shoots and roots per cultured shoot were recorded. A shoot was counted only after it had developed a whorl of at least three to four leaves >10 mm long and assumed the morphology of an organized shoot; counts were made on 2 and 30 May 1995 for 'Roy Davidson' and 12 Apr. and 10 May 1996 for *P. saccharata* 'Margery Fish'. Additionally, shoot and root counts for 'Roy Davidson' were made on 16 Apr. and 14 May 1996. Between the 1995 and 1996 data collecting periods, proliferating shoots of 'Roy Davidson' were maintained on MS medium with 4.4 μM BA and subcultured at 4-week intervals.

Five replications were used per treatment with four samples per replication. A replication consisted of a GA-7 container and a sample consisted of one shoot. All experimental treatments were arranged in completely random designs, and data were analyzed using the General Linear Models Procedure (SAS Institute, Cary, N.C.).

Results and Discussion

The year × subculture × BA interaction for 'Roy Davidson' (Table 1) and the subculture × BA interaction for 'Margery Fish' (Table 2) were significant for both shoot and root counts. For 'Roy Davidson', shoot counts remained low at BA concentrations <4.4 μM, then increased; the highest concentration (44.4 μM) was less effective than lower concentrations in some cases (Fig. 1A). Response in year 2 was

Table 1. Analysis of variance for effects of year, subculture and BA concentration on shoot and root counts of *Pulmonaria* 'Roy Davidson' in vitro.

		Mean square	
Source of variation	df	Shoot count	Root count
Year (Y)[z]	1	114.18***	108.32***
Subculture (S)[y]	1	6.50[NS]	7.41[NS]
BA	5	511.98***	82.11***
Y × S	1	41.91***	1.61[NS]
Y × BA	5	39.38***	36.88***
S × BA	5	4.64[NS]	5.27[NS]
Y × S × BA	5	75.07***	16.87***
Error	297	4.41	2.50

[z]1995 and 1996.
[y]Subcultured on 2 May and 30 May 1995, and on 6 Apr. and 14 May 1996.
[NS], ***Nonsignificant and significant at $P < 0.001$.

Table 2. Analysis of variance for effects of subculture and BA concentration on shoot and root counts of *Pulmonaria saccharata* 'Margery Fish' in vitro.

		Mean square	
Source of variation	df	Shoot count	Root count
Subculture (S)[z]	1	159.87***	129.27***
BA	5	241.00***	300.14***
S × BA	5	41.66***	43.62***
Error	221	2.85	2.75

[z]Subcultured on 12 Apr. and 10 May 1996.
***Significant at $P < 0.001$.

Received for publication 30 June 1997. Accepted for publication 22 Oct. 1997. Use of trade names does not imply endorsement of the products named or criticism of similar ones not named. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.
[1]Professor.
[2]Research Program Manager II.
[3]Graduate Research Assistant.

generally higher than in year 1. Shoot counts for 'Margery Fish' increased as BA concentration increased from 0 through 8.8 μM BA, then decreased at 44.4 μM BA (Fig. 2A). At higher concentrations of BA, shoot counts were greater in subculture 1 than in subculture 2. Shoot counts for both cultivars tended to be highest at 8.8 μM BA.

Root counts for both cultivars decreased steadily as BA concentration increased, and no roots formed at 44.4 μM BA (Figs. 1B and 2B). Root counts were higher for 'Roy Davidson' in year 2 than in year 1 and for 'Margery Fish' in subculture 2 than in subculture 1 (Fig. 2B). Overall, 'Margery Fish' tended to produce higher root counts than did 'Roy Davidson'.

The inverse relationship between shoot and root counts (Figs. 1 and 2) has been observed with many other micropropagated plants (George and Sherrington, 1984). Pulmonarias rooted in vitro without addition of exogenous auxin to the culture medium; thus endogenous auxin concentration was sufficient to support adventitious root formation when cytokinin was omitted. Conversely, endogenous cytokinin concentration of shoots was apparently too low to overcome apical dominance, resulting in low shoot proliferation. Thus, a supply of exogenous cytokinin was required for shoot proliferation in vitro.

Subculture has increased or decreased shoot count, decreased shoot size, or caused vitreous shoot formation (George and Sherrington, 1984; Stimart, 1986). Also, rooting of microcuttings can either be enhanced or reduced by subculture. In our study, shoot and root counts increased following subculture with no apparent change in size or quality. Formation of roots and shoots is under hormonal control; thus the requirement for auxin and cytokinin apparently changed with subculture. Extended micropropagation of plants often alters hormone requirements.

Leaf size obviously decreased as BA concentration increased, although no data were collected. Leaves in cultures with 0 to 4.4 μM BA were larger and more brittle than those in cultures with higher concentrations of BA (Fig. 3A). Explants were optimum size for shoot handling when cultured on 8.8 μM BA (Fig. 3B).

About 25 rooted plants of 'Roy Davidson' and 'Margery Fish' were grown in the greenhouse for about 60 d. Plants grew rapidly and appeared morphologically identical with control plants (data not presented).

This study demonstrates that BA can promote multiple shoot development of *Pulmonaria* in vitro; the optimum is 8.8 μM BA. At this concentration, shoot count was high and shoot size optimum for reducing breakage and facilitating explant handling. Greatest rooting occurred without BA. Micropropagation appears to be an appropriate method for rapid increase of *Pulmonaria*.

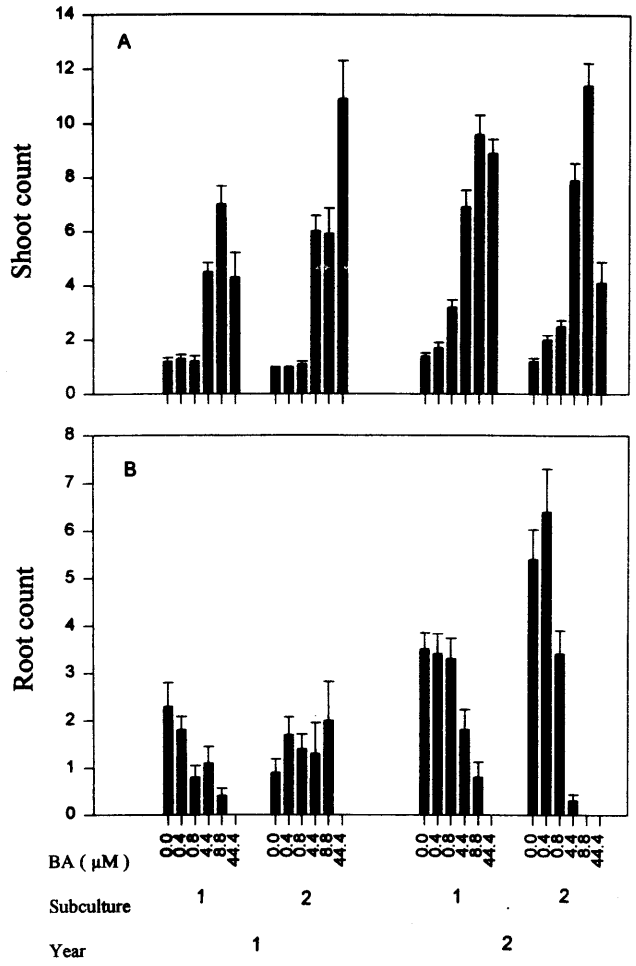

Fig. 1. (**A**) Shoot and (**B**) root counts per explant on *Pulmonaria* 'Roy Davidson' in vitro in response to year, subculture and BA concentration. Vertical bars represent ± SE.

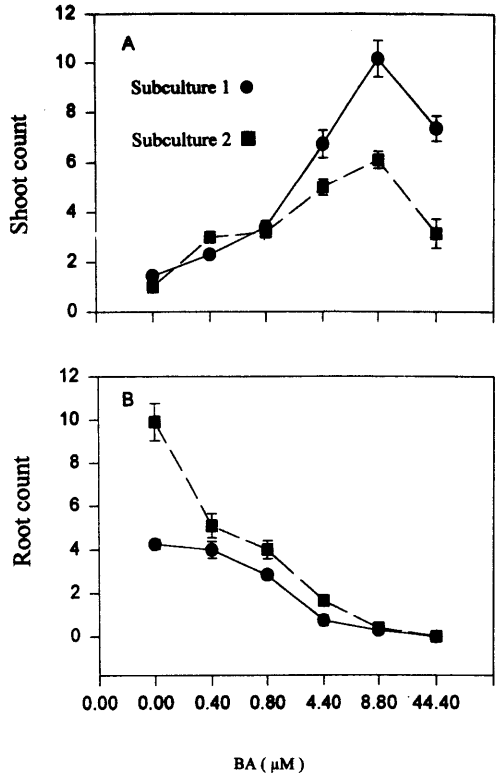

Fig. 2. Mean shoot and root counts per explant on *Pulmonaria saccharata* 'Margery Fish' in vitro as influenced by subculture and BA concentration. Vertical bars represent ± SE.

Literature Cited

Armitage, A.M. 1989. Herbaceous perennial plants. Varsity Press, Athens, Ga.

Fig. 3. Axillary shoot proliferation of *Pulmonaria* 'Roy Davidson' on MS medium supplemented with (**left**) 4.4 μM or (**right**) 8.8 μM BA.

George, E.F. and P.D. Sherrington. 1984. Plant propagation by tissue culture. Eastern Press, Reading, Great Britain.

Lovejoy, A. 1993. Pulmonarias. Horticulture 71(3):54–58, 80.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

SAS Institute. 1990. SAS/STAT users' guide. Ver. 6.09. 4th ed. vol. 2. SAS Inst., Cary, N.C.

Stimart, D.P. 1986. Commercial micropropagation of florist flower crops, p. 301–315. In: R.H. Zimmerman (ed.). Tissue culture as a plant production system for horticulture crops. Martinus Nijhoff/W. Junk, Dordrecht, The Netherlands.