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Multiplication of Four *Penstemon* Species in Vitro

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Penstemon (beard tongue) is a native genus of U.S. wildflower that is used for landscape plants (Lindgren, 1984a, 1984b, 1990), for cut flowers (Lindgren, 1986), and for ecological studies (Stubbendieck et al., 1982). Tissue culture techniques could be useful for propagating cultivars and species in this genus as some do not breed true from seed, require special seed germination conditions, or are difficult to propagate using other vegetative methods (Lindgren, 1984b, 1990; Stubbendieck et al., 1982). *Penstemon haydenii* is also the only listed endangered plant species in Nebraska.

The Penstemon spp. *P. digitalis* Nutt. 'Husker Red' (Lindgren, 1984b); *P. grandiflorus* Nutt. 'Prairie Snow' (Lindgren, *1990); P. barbatus* (Cav.) Roth 'Schooley's Yellow' (Lindgren, 1984a), and *P. haydenii* S. Wats (Stubbendieck et al., 1982) were evaluated for their growth response to five levels of N-(phenylmethyl)-1H purine-6-amine (BA).

Lateral buds from greenhouse-grown plants of each of the four species were surface sterilized by sequential immersion in 75% ethanol for 1 min and 0.53% NaOCl for 15 min, followed by rinsing three times in sterile water. Explants were then placed in 120-m] baby food jars with polypropylene closures that contained 30 ml Woody Plant Medium (Lloyd and McCown, 1980) with 0.1% (w/v) Gelrite, 0.3% (w/v) Sigma agar, 2% (w/v) sucrose, 6 mM calcium gluconate, and 0.4 μM BA. Shoots were grown on this medium for 8 weeks, then transferred to Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) without BA and grown for 6 weeks. Shoot sections with one node each were then transferred to 30 ml MS medium, placed vertically, and supplemented with either 0, 0.01, 0.1, 1.0, or 10.0 μM BA. At the end of 4 weeks, shoot tips (1 cm) were transferred to fresh MS media supplemented with the same concentrations of BA and, at the end of 6 weeks, plant growth was measured. All tissue culture media were adjusted to a pH of 5.7. Growth rooms were maintained at 27 ± 1C with 24 h continuous light provided by cool-white fluorescent bulbs (20 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The experimental design was a randomized complete block with five replicates per treatment. One replicate consisted of one jar with one shoot per jar. Statistical comparisons were made between BA levels only within each species.

Linear regression analysis indicated there were significant differences between BA concentrations for number of secondary shoots and roots (Table 1) within all species. However, only *P. barbatus* and *P. haydenii* showed differences in primary shoot length. Secondary shoot counts were generally higher at 1.0 and 10.0 μM BA. However, secondary shoots produced with 10 μM BA were vitreous and not suitable for propagation. Root count was generally highest with the lower BA concentrations.

Microcuttings were excised from the shoot cultures, placed in Techniculture peat plugs (Castle and Cooke Techniculture, Salinas, Calif.) and enclosed in a plastic rooting chamber. Nearly all the shoots of ***P. digitalis*** and *P. barbatus* rooted in the plugs survived when transferred to 'Redi Earth' growing medium (W.R. Grace, Cambridge, Mass.) in 0.04-liter plastic pots in the greenhouse. However, no *P. grandiflorus* or *P. haydenii* shoots rooted in the plugs and, consequently, they did not grow as potted plants.

Penstemon spp. can be grown and propagated in vitro. However, further studies are needed to find successful method(s) of rooting tissue-cultured shoots and establishing them ex vitro, especially for *P. haydenii* that are to be grown for field and greenhouse plantings.

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Table 1. Summary of in vitro growth response of four *Penstemon* spp. to five concentrations of BA.[z]

Species	BA (μM)	Means: Primary shoot length (cm)	Means: Secondary shoots (no.)	Means: Roots (no.)
P. digitalis	0	3.9	0.60	7.6
	0.01	3.8	2.20	5.6
	0.1	5.4	9.0	24.2
	1.0	5.0	41.2	5.6
	10.0	1.9	19.0	0.0
Linear significance		NS	**	*
P. barbatus	0	14.1	0.0	5.6
	0.01	13.6	0.0	5.8
	0.1	12.7	0.8	3.4
	1.0	4.2	12.4	0.2
	10.0	1.2	7.6	0.0
Linear significance[z]		**	**	**
P. grandiflorus	0	2.8	0.0	0.8
	0.01	4.5	0.0	0.0
	0.1	5.7	0.40	0.0
	1.0	3.4	4.40	0.0
	10.0	1.1	7.20	0.0
Linear significance[z]		NS	**	*
P. haydenii	0	12.6	0.0	6.0
	0.01	9.8	0.0	4.2
	0.10	24.4	3.0	0.0
	1.0	11.1	8.0	0.6
	10.0	1.2	8.4	0.0
Linear significance		*	**	**

[z]Comparisons made within each species only in each column for each mean..
NS,*,**Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

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