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Micropropagation of *Baptisia* 'Purple Smoke'

James R. Ault and Kayri Havens
Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, IL 60022

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Abstract. **Shoot explants from actively growing, greenhouse-maintained plants of *Baptisia* 'Purple Smoke' were cultured in vitro for shoot initiation on Murashige and Skoog (MS) basal medium containing vitamins and supplemented with 30 g·L^{-1} sucrose, 8.87 μM BA, and 4.14 μM K-IBA. All subsequent media were supplemented with 2.47 mM NaH_2PO_4 to enhance shoot growth. Single-node explants were subcultured for shoot multiplication on MS medium with either no plant growth regulator or with 2.22, 4.44, 8.87, 17.74, or 35.48 μM BA in combination with 0.0 or 4.14 μM K-IBA. Explants produced a maximum of 4.1 shoots on the medium with 2.22 μM BA. Shoots rooted on all concentrations of K-IBA (2.07, 4.14, 10.36, or 20.72 μM) and K-NAA (2.23, 4.46, 11.15, or 22.29 μM) tested. Maximum rooting was 100% on MS medium with 11.15 μM K-NAA; however, this treatment induced copious stem callusing. Rooted shoots were greenhouse-acclimatized for 2.5 weeks. Overall survival was 86%. For optimal rooting and subsequent acclimatization, treatment with 2.23 μM K-NAA is recommended; this resulted in 83% rooting and 87% acclimatization. Chemical names used: N^6 benzyladenine (BA); potassium salt of indole-3-butyric acid (K-IBA); potassium salt of 1-naphthalene acetic acid (K-NAA).**

Baptisia is a genus of herbaceous perennials known as false or wild indigos. These ornamental members of the Fabaceae are grown for their conspicuous and colorful flowers, attractive foliage, and ornamental fruit. The species more commonly cultivated are *B. alba* L. Vent., *B. australis* (L.) R. Br. ex Ait. f., *B. bracteata* Muhl. ex Ell., and *B. sphaerocarpa* Nutt.

Baptisia 'Purple Smoke' is a new cultivar introduced in 1996. This showy perennial with deep blue flowers and gray-green foliage was discovered at the North Carolina Botanical Garden (Beattie, 1998) and is thought to be a chance hybrid of *B. australis* and *B. alba*. As a clonal selection, it must be vegetatively propagated. Young shoot tip cuttings can be rooted in the spring, but overwintering rooted cuttings has proven difficult (J. Ault, unpublished data; R. Gardner, personal communication). Limited amounts of stock material are restricting distribution of this plant.

A preliminary micropropagation study was initiated. The only other known report on the micropropagation of *Baptisia* is on *B. arachnifera* Duncan (Pinnell and Dirr, 1986).

Materials and Methods

Culture initiation. Young, actively growing shoot tips were harvested from plants maintained in a greenhouse. After leaf removal, stem segments of three to five nodes each were surface-disinfested in a solution of 1.0% sodium hypochlorite and 0.1% Tween 80 for 12 min, then rinsed twice for 5 min in sterile distilled water. Single-node explants ≈1.0 cm in length were individually placed basal end down in 25 × 150-mm culture tubes containing 10 mL of MS (Murashige and Skoog, 1962) basal salts and vitamins medium, 30 g·L^{-1} sucrose, 8.87 μM N^6 benzyladenine (BA), and 4.14 μM K-IBA. Medium pH was adjusted to 5.8 prior to adding 7.0 g·L^{-1} Sigma A 1296 agar (Sigma Chemical Co., St. Louis). Culture tubes were sealed with polypropylene caps and autoclaved at 121 °C for 15 min.

After explant transfer, culture tubes were sealed with Parafilm, then placed upright and maintained at 22 °C under a 14-h photoperiod with a photosynthetic photon flux (*PPF*) of 15 to 40 μmol·m^{-2}·s^{-1} [measured with a LI-190SA Quantum Sensor (LI-COR, Lincoln, Nebr.)] provided by two 40-W cool-white fluorescent lamps.

The primary explants were cultured for 6 to 8 weeks, then axillary shoots were excised and cultured on fresh medium for another 5 weeks. To enhance shoot growth, 2.47 mM NaH_2PO_4 was incorporated in the medium for the second and all subsequent culture periods.

Shoot proliferation. Single-node explants were excised from the shoots generated in vitro, and cultured on MS medium with either no plant growth regulators (PGRs) or with one of 10 combinations of BA and K-IBA (Table 1). After 6 weeks of culture, shoots were harvested, and the number of axillary shoots and the lengths of primary and axillary shoots recorded.

Rooting. The first rooting study was initiated at the same time as the shoot proliferation study. Individual, unrooted shoots 3 to 5 cm tall were excised and cultured on MS medium with either no PGRs or with one of four concentrations of K-IBA or K-NAA (Table 2). Percent rooting and percentage of rooted shoots with callus were recorded after 6 weeks.

In the second study, shoots were cultured on the proliferation medium for three culture periods. Shoots were then excised and cultured on MS medium with either no PGRs or with 0.44, 2.23, or 4.46 μM K-NAA. The basal portions of additional shoots were dipped for 5 s in either 0.44 or 2.23 mM K-NAA solution (prepared by dissolving K-NAA in distilled water, then autoclaving at 121 °C for 15 min) prior to culture on PGR-free medium. Percent rooting and percent rooted shoots with callus were recorded for all treatments after 6 weeks of culture.

Shoot acclimatization. Rooted shoots from the second rooting study were removed from tissue culture after 6 weeks, rinsed free of tissue culture medium, and planted in 72-cell plug trays containing 2 peat : 1 perlite (v/v). Trays were covered with clear plastic domes, then placed in a shaded greenhouse (min. 18 °C) 5 cm above a warm water-circulation tubing system (21 °C bottom heat). Maximum *PPF* = 130 μmol·m^{-2}·s^{-1}. Domes were vented twice daily for ≈15 to 30 s. After 2 weeks, the domes were propped open ≈5 cm on all sides, then removed 4 d later when the flats were moved to a second greenhouse (maximum *PPF* = 320 μmol·m^{-2}·s^{-1}). Percent survival was recorded 3 weeks after plants were removed from tissue culture.

Statistical analysis. Forty explants were randomly assigned to each shoot proliferation treatment and to each rooting treatment. The shoot proliferation and rooting experiments were conducted once. Data were subjected to analysis of variance, and means separated according to the Student–Newman–Keul test (Sokal and Rohlf, 1969). Percent rooting and shoot callusing were analyzed with the G

Table 1. Effects of benzyladenine (BA) and K-IBA on primary shoot length, and on the number and length of axillary shoots produced in vitro from single-node explants of *Baptisia* 'Purple Smoke' after 6 weeks of culture on MS medium (n = 37 to 40).

BA		Mean primary shoot length (cm)		Axillary shoots: Mean no.		Axillary shoots: Mean length (cm)	
(μM)	K-IBA (μM):	0.0	4.14	0.0	4.14	0.0	4.14
0.0		6.0 a[z]	---	0.0	---	---	---
2.22		3.6 b	5.8 a	4.1 a	3.7 a	1.2 a	1.3 a
4.44		3.4 b	4.1 b	2.1 b	4.0 a	1.1 ab	1.2 a
8.87		2.7 c	2.5 cd	2.1 b	1.8 b	0.9 bc	0.9 bc
17.74		2.1 cd	2.6 cd	1.9 b	2.5 b	0.9 bc	0.7 cd
35.48		1.7 d	1.8 cd	1.1 c	0.7 c	0.6 cd	0.4 d

[z]Mean separation within paired columns by Student–Newman–Keul test, $P \leq 0.05$.

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Table 2. Effects of K-IBA and K-NAA on the rooting of in vitro–produced shoots of *Baptisia* 'Purple Smoke' after 6 weeks of culture on MS medium (n = 40).

K-IBA (μM)	K-NAA (μM)	Rooting (%)	Rooted shoots with callus (%)
---	---	40	0
2.07	---	48	0
4.14	---	50	0
10.36	---	67*	0
20.72	---	35	0
---	2.23	98**	23*
---	4.46	90**	100**
---	11.15	100**	100**
---	22.29	97**	100**

*, **Significantly different from the control (no plant growth regulators) at $P \leq 0.05$ or 0.01, respectively, by the G statistic.

Table 3. The effect of K-NAA in the medium vs. as a quick dip on the rooting of *Baptisia* 'Purple Smoke' shoots produced in vitro, and on subsequent shoot acclimatization in the greenhouse (n = 40).

Treatment	K-NAA	Rooting (%)	Rooted shoots with callus (%)	Shoot survival (%)
Medium	0.00	48	0	94 (16)z
	0.44 μM	73*	66**	96 (28)
	2.23	83**	24**	87 (31)
	4.46	90**	37**	58 (33)
Quick dip	0.44 mM	68	67**	91 (23)
	2.23	63	84**	100 (22)
				NS

zNumber of shoots transferred to greenhouse in parentheses.
NS, *, **Nonsignificant or significantly different from the control (no auxin) at $P \leq 0.05$ or 0.01, respectively, by the G statistic.

statistic, comparing the individual auxin treatments to the control (no PGR). Percent survival was analyzed with the Chi-square test. Data were analyzed with CoStat statistical software (CoHort Software, Berkeley, Calif.).

Results and Discussion

Culture initiation. Of the 152 original explants, 135 produced shoots 1 to 10 cm tall from axillary buds. All of the shoots had a large basal callus, which was excised and discarded with subsequent subcultures. No roots were formed.

Shoot proliferation. Explants on PGR-free medium produced one unbranched shoot each, indicating strong apical dominance (Table 1). Addition of BA to the medium, with or without K-IBA, stimulated the production of axillary shoots regardless of concentration. Primary shoot length and the number and length of axillary shoots decreased as concentrations of BA increased. All shoots produced callus on all treatments except the control.

A pairwise comparison of the BA treatments with and without K-IBA indicated that K-IBA at the level tested generally had no significant effect on primary shoot length or the number or length of axillary shoots, as only two of the 15 comparisons were significant (Table 1). Satisfactory shoot multiplication by a cytokinin without the incorporation of an auxin in the medium has been reported for other members of the Fabaceae, including *Lupinus texensis* Hook. (Upadhyaya et al., 1992) and *Gymnocladius dioicus* L. (Geneve et al., 1990).

Benzyladenine at 2.22 and 4.44 μM with or without K-IBA induced significantly more and longer axillary shoots than did the other treatments. For shoot multiplication, 2.22 μM BA is recommended; K-IBA at the level tested appears unnecessary, as previously discussed, and the use of 2.22 instead of 4.44 μM BA may be advisable, as high BA concentration can inhibit subsequent shoot rooting in other taxa, e.g., *Vitis rotundifolia* Michx. (Lee and Wetzstein, 1990). By comparison, optimal shoot multiplication for *Baptisia arachnifera* occurred on MS medium with 33.3 μM BA and 14.3 μM IAA (Pinnell and Dirr, 1986). Satisfactory shoot multiplication for *Baptisia bracteata* occurred on a modified MS media with 4.44 or 6.66 μM BA and 0.29 or 0.57 μM IAA (P.E. Read, unpublished data). For shoot multiplication, optimum PGR concentration may vary with taxon of *Baptisia*.

Rooting. K-NAA was more effective in inducing rooting than was K-IBA (Table 2). Only one K-IBA treatment significantly increased percent rooting.

The morphological response to K-NAA vs. K-IBA treatments differed markedly (data not shown). In general, K-IBA produced fewer, but thicker, more laterally branched and longer roots than did K-NAA. Roots on K-IBA treated shoots arose only from the stem portion in the medium, and had no root hairs, whereas those on the K-NAA treated shoots occurred both in and immediately above the medium. Roots formed above the medium produced many root hairs. Variation in root morphology in response to different PGR treatments has been reported for other taxa, such as *Penstemon serrulatus* Menzies ex Smith (Wysokinska, 1993). All four K-NAA treatments significantly stimulated callus production on rooted shoots (Table 2); the quantity of callus formed appeared to be greatest for the two highest K-NAA treatments (data not shown).

The rooting percentages for the K-NAA treatments were sufficiently high to justify recommending this PGR for rooting of 'Purple Smoke'. However, the production of callus on the rooted shoots could inhibit subsequent shoot acclimatization. Therefore, the second rooting experiment was initiated.

Treatment with 0.44, 2.23, or 4.46 μM K-NAA in the medium significantly increased percent rooting, whereas the quick dips did not (Table 3). The additional labor involved precludes recommending the dips.

Rooted shoots from all K-NAA treatments produced callus. However, the percentage of rooted shoots with callus (Table 3), and the volume of callus produced per rooted shoot (subjective evaluation), were lower than for the NAA treatments in the first rooting study.

Shoot acclimatization was not significantly affected by treatment (Table 3). Overall shoot survival was 86% (131/153 shoots). In summary, 2.22 μM BA is recommended for shoot multiplication, and 2.23 μM K-NAA is recommended for rooting and subsequent acclimatization of rooted shoots.

Literature Cited

Beattie, D. 1998. Short forum on new plants: *Baptisia* x 'Purple Smoke'. Perennial Plants 6:3–4.

Geneve, R.L., S.T. Kester, and S. El-Shall. 1990. In vitro shoot initiation in Kentucky coffeetree. HortScience 25:578.

Lee, N. and H.Y. Wetzstein. 1990. In vitro propagation of muscadine grape by axillary shoot proliferation. J. Amer. Soc. Hort. Sci. 115:324–329.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–479.

Pinnell, M.M. and M.A. Dirr. 1986. An overview of Georgia's endangered species program. HortScience 21:863.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. 1st ed. W.H. Freeman, San Francisco.

Upadhyaya, A., T.D. Davis, D. Sankhla, and N. Sankhla. 1992. Micropropagation of *Lupinus texensis* from cotyledonary node explants. HortScience 27:1222–1223.

Wysokinska, H. 1993. Micropropagation of *Penstemon serrulatus* and iridoid formation in regenerated plants. Plant Cell Tiss. Organ Cult. 33:181–186.