tal to growth of potato plantlets (Table 1). Lanolin and vegetable shortening caused significant reductions in plant height and root weight. Similar results were obtained by Wardle et al. (1983) with cauliflower, a result that he attributed to reduced relative humidity. Thickening of the leaves and stems was also noticed. The paraffin treatment caused a significant reduction in root weight, but did not otherwise affect growth.

There were no apparent effects on stem growth characteristics of 'Russet Burbank' or 'Centennial Russet' as a result of the paraffin treatment. Statistically, there was also no effect on root growth, although variability was quite high (data not shown).

The paraffin medium overlay used in the third experiment reduced relative humidity from 100% to 70%. Reduction in water loss

(Fig. 1) for the potato plantlets grown under reduced humidity was comparable to that noted by Brainerd et al. (1981) and Brainerd and Fuchigami (1981) for in vitro-grown plum and apple plantlets that had been hardened for several days in the greenhouse.

The use of paraffin as a medium overlay improved resistance of detached leaves to water loss. Reduced relative humidity in vitro may result in improved cuticle formation or stomatal function; however, a medium overlay can reduce root growth.

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Plant Recovery from Sweetpotato Somatic Embryos

Raymond P. Chée, Jonathan R. Schultheis, and Daniel J. Cantliffe
Vegetable Crops Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611

Additional index words. somatic embryogenesis, *Ipomoea batatas*, synthetic seed, micropropagation, tissue culture

Abstract. Plant formation from somatic embryos in response to BAP, NAA, and sucrose was studied in sweetpotato [*Ipomoea batatas* (L.) Lam.]. A maximum of 15% embryos at the torpedo stage of development formed plants of agar-solidified basal medium containing 3% sucrose and no growth regulators. The percentage of embryos forming shoots was increased to 53% by 4 μ M BAP, but BAP reduced whole plant formation and promoted callusing at the root axis end of embryos. The frequency of plant development was increased to 38% by adding 0.1 μ M NAA to the basal medium. Reducing sucrose concentration to 1.6% in basal medium increased the frequency of plant development to 32%. Chemical names used: 6-benzylaminopurine (BAP; α -naphthaleneacetic acid (NAA).

The production of synthetic sweetpotato seed via somatic embryogenesis has been studied as a means for planting large areas with this species (Cantliffe et al., 1988). Patterns of sweetpotato somatic embryony have been reported, and embryos capable of producing plants have been described (Chée and Cantliffe, 1988a). The success of synthetic seed systems depends on efficient somatic embryo germination. However, the efficient germination of somatic embryos has rarely been investigated (Ammirato, 1983; Vasil, 1986). The responses of somatic embryos to auxin and cytokinins have varied with species (Stamp and Henshaw, 1982; Pérez et

al., 1983; Lazzeri et al., 1987). In cassava, rapid leaf and root development was obtained from embryos subcultured onto media containing BAP and 2,4-D (Stamp and Henshaw, 1982). Germination of soybean embryos was not affected by NAA, BAP, kinetin, and zeatin either alone or together in various combinations (Lazzeri et al., 1987).

Shoot and root development from filbert embryos was not affected by BAP concentration (Pérez et al., 1983). Likewise, somatic embryo germination in response to sucrose concentration has varied with species (Ahloowalia and Maretzki, 1983; Heirwegh et al., 1985; Arnold and Hakman, 1988). In sugarcane cultures, 3% sucrose promoted plant formation from embryos when compared to sucrose at 6% or 1.5% (Ahloowalia and Maretzki, 1983). In norway spruce, sucrose at 2% promoted shoot formation from embryos when compared to sucrose at 1% and 3% (Arnold and Hakman, 1988). In chicory, lowering sucrose from 2% to 1% had no positive effect on embryo germination (Heirwegh et al., 1985). Because of the differential effects of BAP, NAA, and sucrose on shoot and root development from somatic embryos of various species, we studied their effects on sweetpotato somatic embryo germination.

Embryogenic callus of sweetpotato cultivar White Star obtained from shoot apices was proliferated on basal medium supplemented with 10 μ M 2,4-D and 1 μ M BAP (Chée and Cantliffe, 1988b). The basal medium was composed of the inorganic salts of Murashige and Skoog (Murashige and Skoog, 1962), 500 μ M myoinositol, 5 μ M thiamine-HCl, 10 μ M nicotinic acid, 5 μ M pyridoxine-HCl, 3% w/v sucrose and 0.6% w/v 'Phytagar' (GIBCO Laboratories, Grand

Table 1. Response of sweetpotato somatic embryos to BAP.*

Criterion	Embryos responding (%)								F	R ²
	BAP (μM)									
	0	0.1	1	2	4	5	8	10		
Shoot growth	21	17	33	38	53	36	20	3	Q**	0.53
Root growth	53	46	13	10	7	6	0	7	Q*	0.50
Shoot and root growth	13	7	0	3	3	0	0	0	L*	0.37
Basal callus growth	4	4	20	32	39	29	58	29	Q*	0.41

*Results at 21 days; statistical analysis by the component of variance method; F = F test of significance, Q = quadratic, L = linear, R² = determination coefficient; data are means of three blocks of 10 embryos.

***Significant at P = 0.05 or 0.01, respectively.

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Table 2. Response of sweetpotato somatic embryos to NAA.^a

Criterion	Embryos responding (%)							F	R ²
	NAA (μM)								
	0	0.1	0.2	1	2	5	10		
Shoot growth	17	41	38	33	25	26	13	L*	0.23
Root growth	47	76	78	80	74	51	20	L**	0.49
Shoot and root growth	3	38	33	28	23	14	5	L**	0.39
Basal callus growth	3	8	8	5	9	3	53	Q*	0.48

^aResults at 21 days. Statistical analysis by the component of variance method; regression analysis for NAA treatments only, F = F test of significance, L = linear, Q = quadratic, R² = determination coefficient; the contrasts "0 vs. 0.1 μM NAA" were significant at P = 0.01 for root growth and callus growth, and at P = 0.05 for embryos with both shoot and root growth; data are means of four blocks of 10 embryos.

***Significant at P = 0.05 or 0.01, respectively.

Table 3. Response of sweetpotato somatic embryos to sucrose.^a

Criterion	Embryos responding (%)					F	R ²
	Sucrose (% w/v)						
	3.2	1.6	0.8	0.4	0		
Shoot growth	20	32	25	25	0	Q**	0.43
Root growth	48	63	55	50	2	Q**	0.58
Shoot and root growth	15	32	20	10	0	Q**	0.56
Basal callus growth	0	0	0	0	0	NR	NR

^aResults at 21 days. Statistical analysis by the component of variance method; F = F test of significance, Q** = quadratic at P = 0.01, R² = determination coefficient, NR = not relevant; data are means of six blocks of 10 embryos.

Island, N.Y.). The pH was adjusted to 5.8 using 1 N NaOH before autoclaving at 121°C and 1.1 kg·cm⁻² for 15 min. Somatic embryos developed from embryogenic calli were transferred to basal medium without growth regulators (Chée and Cantliffe, 1988a). After 2 to 3 weeks, embryos that were arrested at the torpedo stage of development (Chée and Cantliffe, 1988a) were subcultured individually onto 25 ml of basal medium in 25 × 100-mm plastic petri dishes with the addition of either BAP or NAA or various sucrose concentrations. Incubation was at constant 27°C with 10 hr light/14 hr dark photoperiods. Irradiance of 22 W·m⁻² was provided by cool-white fluorescent tubes. At 21 days of culture, the embryos were scored for shoot growth, root growth, and growth of both on the same plantlet. Consequently, embryos that produced plantlets contributed to both shoot and root scores. Shoots were 3 to 15 mm long and consisted of an apical bud with at least one separated leaflet. Roots were 2 to 30 mm long. The percentage of embryos in each of the three germination classes was calculated and the data analyzed using the component of variance method after arcsin-square root transformation.

Embryo germination on basal medium began with either root initiation within 7 days or shoot growth within 14 days. The roots produced were either tap roots or one or more adventitious roots. Hypocotyl and cotyledon elongation either preceded root initiation and shoot growth or was concomitant with root initiation. After 21 days of culture on basal medium (3% sucrose, except 3.2% in Table 3) the percentages of rooted embryos (47%

to 53%) and the percentages of embryos with shoots (17% to 21%) were similar among experiments (Tables 1–3). However, the percentages of embryos producing both roots and shoots ranged from 3% to 15% (Tables 1–3). The root poles of up to 4% of the embryos grown on basal medium became nodulated with a hard green callus (Tables 1–3). This callus, which remained an integral part of the embryos, differed greatly from embryogenic callus, which is yellow, comparatively friable, and grows away from the source explant (Chée and Cantliffe, 1988b).

The percentage of embryos forming shoots increased with increasing BAP concentrations to a maximum (53%) at 4 μM BAP (Table 1). Higher BAP levels resulted in reduced shoot formation. However, root and whole plantlet formation decreased with the addition of BAP, since organized growth of the root axis was disrupted and callus was formed. Thus, BAP was not useful in promoting somatic embryo germination.

The percentage of embryos that developed shoots and both shoots and roots was promoted by adding 0.1 μM NAA to basal medium; thereafter, plant formation decreased with increasing NAA concentrations (Table 2). Rooting was maximal at 1 μM NAA. Organized growth of the root axis was disrupted by callus formation at 10 μM NAA. Thus, embryo germination was improved using 0.1 μM NAA with a 141% increase in shoot development, a 62% increase in rooting, and a nearly 1200% increase in whole plants formed.

The percentages of embryos forming shoots and/or roots initially increased and then de-

creased with decreasing sucrose concentrations, with a maximum at 1.6% (Table 3). Thus, reducing sucrose from 3.2%, near the concentration in basal medium, to 1.6% caused a 60% increase in shoot production, a 31% increase in rooting, and about a 1100% increase in whole plants formed. In micropropagation of grapevines, improved rooting of subcultured shoot tips on media with reduced sucrose concentrations was attributed to increased shoot vigor (Chée and Pool, 1988).

Our previous study of sweetpotato somatic embryo development patterns demonstrated that all embryo developmental stages were able to produce roots but not shoots (Chée and Cantliffe, 1988a). Consequently, shoot development was the limiting factor for plant formation from somatic embryos in sweetpotato. In this study, our attempt to promote vigorous shoot growth with a cytokinin was successful but led to callus growth at the root pole of the embryos, thus inhibiting whole plant formation. Increased whole plant formation depended on the ability of embryos to develop a shoot axis, since the frequency of root development exceeded that of shoot development in all treatments. Consequently, in sweetpotato, synthetic seed delivery to the field must address the production of propagules with shoot apices primed for vigorous growth.

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Vessel Type, Closure, and Explant Orientation Influence in Vitro Performance of Five Woody Species

M.T. McClelland and M.A.L. Smith

Department of Horticulture, University of Illinois, Urbana, IL 61801

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Abstract. Effects of three variables (vessel type, closure, and explant orientation) on microcutting quality were investigated using five woody species [low shadblow, *Amelanchier spicata* (Lam.) C. Koch (Syn. *A. humilis* Wieg.); red maple, *Acer rubrum* L. 'Red Sunset'; border forsythia, *Forsythia × intermedia* Zab. 'Sunrise'; apple, *Malus × domestica* Borkh. 'McIntosh'; river birch, *Betula nigra* L.]. Uniform shoot explants were oriented vertically or horizontally in three vessel types (60-ml glass culture tubes, 200-ml glass baby food jars, and 350-ml polypropylene GA7 vessels) with and without a Parafilm seal. Visual density per explant obtained by image analysis was increased in larger vessel types, and significantly more shoots were produced from horizontally placed explants. Closure treatments influenced microshoot quality, but trends were species specific. Overall, horizontal explant orientation in larger vessels without parafilm maximized shoot response for most of the species studied. In vitro rooting of microcuttings was significantly enhanced in larger vessels.

Shoot length, proliferation rate, and fresh weight of micropropagated plants have varied according to culture vessel type (Mackay and Kitto, 1988; Monette, 1986). Mackay and Kitto (1988) demonstrated the ratio of explant number to volume of medium could cause differences in fresh weight and proliferation rates, and vessel closure treatments affected culture proliferation rates, shoot yield, and plant morphology according to other reports (Ivanicka, 1987; Webb et al., 1986). The gaseous atmosphere in vitro, and, consequently, the quality of micropropagated plants, can be significantly affected by closure around vessel caps (Lentini et al., 1988). Explant orientation may modify both the number and length of proliferated shoots (Mackay and Kitto, 1988; Sutter and Barker, 1985). This study examines the effects of vessel type, closure, and explant orientation on in vitro microshoot development and rooting capacity for a range of commercially micropropagated woody plant genera, using production methods that parallel commercial

standards.

Plant material. Established, proliferating shoot cultures of *Amelanchier*, *Acer*, *Forsythia*, *Malus*, and *Betula* grown in GA7 vessels (Magenta Corp., Chicago) were used as explant sources for all experiments. Before treatment, stock microcultures from the previous subculture were grown for 4 to 8 weeks. Uniform two- to three-node explants were taken from cultures; leaves, petioles, and apical buds were removed. Explants were ≈ 1.0 to 2.5 cm across, depending on the

genus. Explants of *Amelanchier*, *Acer*, and *Forsythia* were subcultured onto a modified MS salts medium (Murashige and Skoog, 1962) containing (in μM) 1.0 *N*-(phenylmethyl)-1*H*-purine-6-amine (BA), 0.05 *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (TDZ), and 0.05 1*H*-indole-3-butyric acid (IBA), with (in $\text{g}\cdot\text{liter}^{-1}$) 25 sucrose, 100 myoinositol, and (in $\text{mg}\cdot\text{liter}^{-1}$) 5.0 nicotinic acid, 1.0 pyridoxine-HCl, and 0.5 thiamine-HCl (Kerns and Meyer, 1986). The pH was adjusted to 5.8; 7.0 g Difco bacto agar/liter was added before autoclaving. Explants of *Malus* were subcultured onto a similar medium containing 8.8 μM BA and 0.5 μM 1-naphthaleneacetic acid (NAA). Explants of *Betula* were subcultured onto Woody Plant Medium (WPM; Lloyd and McCown, 1980) containing 2.2 μM BA. Cultures were maintained at $22 \pm 2^\circ\text{C}$ under a 24-hr photoperiod of 50 to 60 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ provided by cool-white fluorescent lamps.

Physical microenvironmental treatments. Explants were subcultured into either 350-ml GA7 polypropylene vessels, 200-ml glass jars (baby food), or 60-ml glass culture tubes with medium surface areas of 39.6, 23.2, or 3.7 cm^2 , respectively. To maintain uniform explant : medium ratios, three explants were grown on 45 ml of medium in GA7 vessels, two on 30 ml of medium in baby food jars, and one on 15 ml of medium in culture tubes. Explants were either oriented vertically or horizontally on the medium. Vessel closures were translucent polypropylene vessel caps manufactured specifically for the type of vessel (Magenta). Half of the replicate cultures were sealed with parafilm around the edge of the vessel and cap, the rest were not sealed with parafilm.

Fig. 1. Effect of vessel type on image density (fresh weight equivalent) of shoot cultures of five woody plant species. The vertical line above each bar represents +1.0 SE.

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