Physical Environment in vitro Affects Laboratory and Nursery Growth of Micropropagated Hostas

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ADDITIONAL INDEX WORDS. **acclimatization, liquid media, ventilation**

SUMMARY. *Hosta* × *hybrid* Tratt. 'Blue Cadet' and *Hosta tokudama* Tratt. 'Newberry Gold' were micropropagated in shaken liquid culture and on agar media, in conventional vessels and vessels modified for ventilation in vitro. Acclimatization under intermittent mist and growth in an outdoor nursery during the late spring and summer were monitored by dry weight analysis of sample plants every 4 days for a 60-day period (ex vitro growth). Results for 'Newberry Gold' were 1) in vitro shoot growth was greater in liquid than agar culture, regardless of vessel; 2) shoots from agar or liquid culture grew at similar rates ex vitro; 3) ex vitro root growth was greater for liquid than agar cultured plants, regardless of vessel type. Results for 'Blue Cadet' were 1) in vitro and ex vitro shoot growth was greater in liquid than agar culture regardless of vessel type and 2) ex vitro root growth was greatest for liquid cultured plants from conventional vessels. Ventilated vessels were generally beneficial for agar but not liquid culture. Benefits of liquid

culture for micropropagation of *Hosta* found in vitro are at least maintained and sometimes enhanced during ex vitro growth in the mist bed and nursery.

Although Hostas are relatively free of disease in the garden (Schmid, 1991), intensive division in conventional nursery systems has caused fungal, viral, and nematode pests to become serious production problems (S.N. Jeffers, personal communication). Micropropagation produces clean nursery stock, and in the United States, the demand for micropropagated *Hosta* is rapidly increasing (Zimmerman, 1996). While a select group of new cultivars currently are commanding high prices, most popular cultivars are produced as lower priced commodity items. Current methods of micropropagation need to be streamlined to maintain profitability, as stock plants for conventional divisions of *hosta* become abundant in the trade.

Since the micropropagation industry has functioned on tight profit margins over the past 15 years, little (<1% of gross) investment has gone into research (Kitto, 1997). The most expensive part of the micropropagation process is labor (Chu, 1995) and the most expensive media component is agar. Use of agar increases the labor cost component (media preparation, dishwashing, removing gel from roots), slows in vitro plant growth and reduces availability of certain growth regulators (Smith and Spomer, 1995). Exclusion of agar from the in vitro production process could reduce labor costs and improve productivity. However, hyperhydric plants often result

from the use of liquid media. Ventilation is the simplest method to remedy hyperhydric tissue. Filter-ventilated culture vessels act as an in vitro acclimatization process (Ziv, 1995). For example, carnation (*Dianthus carophyllus* L.) cultures on liquid medium in vitro, had greater dry matter accumulation in filter-ventilated vessels, and this increased the survival of plantlets ex vitro (Majada et al., 1997). The objectives of this experiment were to compare the growth of *hosta* cultures maintained on liquid or agar media in vitro in ventilated versus conventional vessels, and following acclimatization in the nursery, determine whether ex vitro growth was affected.

Materials and methods

Buds from commercially prepared stage II cultures of *Hosta tokudama* 'Newberry Gold' and *Hosta* × *hybrid* Tratt. 'Blue Cadet' that had been maintained in our laboratory by division every 6 to 8 weeks on agar solidified media were subdivided into individual buds and trimmed to about 3 mm (1/8 inch) length. Four uniform buds [0.79 g (0.028 oz) ± 0.1 g or 0.68 g (0.024 oz) ± 0.1 g fresh weight] of 'Newberry Gold' or 'Blue Cadet', respectively, were put into each 180-mL culture vessel (6-fl oz baby food jar) with 30 mL (1 fl oz) of medium. Murashige and Skoog (1962) medium was modified by the addition of 4.5 mM of NaH₂PO₄, 0.5 mM adenine hemisulfate and 1.0 mL·L⁻¹ (ppm) of MS vitamin solution (M3900, Sigma Chemical, St. Louis Mo.), 10 µM Kinetin, 10 µM IAA (indole acetic acid), 30 g·L⁻¹ (1 oz/qt) sucrose with or without 6 g·L⁻¹ (0.2 oz/qt) agar (A1296, Sigma). Vessels were capped with Magenta B-caps (Magenta Corp.,

Table 1. Dry weight of hosta shoots following 6 weeks of in vitro culture in agar and liquid media, using ventilated and conventional vessels. Cultivars were tested in tandem experiments (1.0 g = 0.035 oz).

Treatment	Shoot dry wt (g)	
	Newberry Gold	Blue Cadet
Agar, conventional	0.35	0.20
Agar, vent	0.36	0.25
Liquid, conventional	0.45	0.27
Liquid, vent	0.62	0.32
SE	0.03	0.02
ANOVA	<i>P</i>	
Agar vs. liquid (A)	0.0000	0.0030
Vents vs. conventional (V)	0.0045	0.0273
A × V	0.0086	0.9653

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Table 2. Results of hypothesis tests using the extra sum of squares principle to compare ex vitro shoot and root growth curves for two hosta cultivars following in vitro culture using agar (A) or liquid (L) media and conventional (C) or ventilated (V) containers.

Hypothesis	Newberry Gold						Blue Cadet					
	df	MSH ²	P	df	MSH	P	df	MSH	P	df	MSH	P
AC = AV	2	0.0165	0.2025	2	0.0195	0.0013	3	0.1134	0.0001			
LC = LV	2	0.0039	0.9621	3	0.0123	0.0048	3	0.0087	0.4383	3	0.0505	0.0001
A = L	2	0.4697	0.0001									
AV = L							3	0.786	0.0001			
AV = LV										3	0.0033	0.5688
MSE ³	56	0.0102		32	0.0024		48	0.0094		33	0.0048	

²Mean square for hypothesis that response curves for specific treatment conditions are equivalent.

³Mean square for error from unconditional model with separate curves fitted for each treatment.

Chicago Ill.), with or without vents. Twenty jars per cultivar were prepared for each of four treatments: 1) agar medium/conventional vessel, 2) agar medium/ventilated vessel, 3) liquid medium/conventional vessel, and 4) liquid medium/ventilated vessel. Agar cultures were maintained at $22 \pm 2^\circ\text{C}$ ($71 \pm 3.6^\circ\text{F}$) under $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux (PPF) light supplied by cool white fluorescent lamps. Liquid cultures were placed on a gyratory shaker rotating at 100 rpm under the same temperature and light conditions.

Following 5 weeks of in vitro culture, fresh and dry weights were determined for five randomly selected jars of each treatment and provided results for Day 0 ex vitro. Tissues were dried for 3 d at 60°C (140°F). Plantlets from the remaining 15 jars (60 plantlets) of each treatment were planted in 250 cm^3 (15.2 inch³) thermoformed pots (Landmark Plastics Corp., Akron Ohio) filled with Metro-Mix 360 (Grace-Sierra Inc., Greenville, S.C.) and were placed under intermittent mist (12 h daylight period with 8 s pulse every 8 min) in a greenhouse during May ('Blue Cadet') and July ('Newberry Gold') at Clemson, S.C. Every 4 d, fresh and dry weights were determined for the four plantlets from a single jar of each treatment. Allocation of jars to sampling dates was randomly determined for each treatment prior to ex vitro growth. When roots were about 5 mm (0.2 inches) in length, flats were moved outdoors under a 60% shade cloth. Plants were watered daily (when necessary) and fertilized weekly with water soluble 20N-8.7P-16.6K (Peters, W.R. Grace, Foglesville, Pa.) Roots and shoots were then measured sepa-

rately for fresh and dry weight.

The two cultivars were evaluated in separate experiments using completely randomized designs. Analyses of variance were performed on shoot dry weights following in vitro culture, and ex vitro shoot and root growth curves were obtained by using the method of least squares to fit linear or exponential models to dry weights of dissected plantlets sampled every 4 d for a 60-d period. The extra sum of squares principle (Draper and Smith, 1966) was employed to determine which model (linear or exponential) was adequate for characterizing growth responses for each treatment. The extra sum of squares principle also was used to compare growth curves and provide information for specific treatment comparisons. An unconditional sum of squares error (SSE) and associated degrees of freedom (df) were obtained by fitting separate growth curves for each treatment. Then a specific hypothesis was examined by fitting a common growth curve for certain treatments to provide a conditional sum of square error (CSSE) and associated df. The differences in error sum of squares, CSSE-SSE, and df for common and separate curves provides the extra sum of squares for which a mean square (MSH) is derived for testing the hypothesis that growth responses for the specified treatments are equivalent. When MSH is large relative to the MSE for the unconditional model, growth responses are declared different and separate curves

should be used. The 5% probability was used for all hypothesis testing.

Results

Hyperhydric shoots were not apparent in vitro in any of the treatments and grew as plantlets after transfer to ex vitro conditions. At the end of the 5-week in vitro culture period, 'Newberry Gold' in liquid culture had greater shoot dry weight than shoots cultured on agar (Table 1). Shoots from ventilated vessels of liquid media had greater dry weight than those from conventional vessels of liquid media. Shoots from agar cultures, both ventilated and nonventilated, had less dry weight than the liquid-cultured shoots.

Ex vitro shoot and root growth were characterized for the four treatment groups, and results of hypothesis

Fig. 1. Dry weight of *Hosta tokudama* 'Newberry Gold' shoots grown in a mist bed (days 0 to 25) and outdoor container nursery (days 26 to 63) with fitted response curves ($P \leq 0.05$); 1 g = 0.035 oz. In vitro culture used agar (A) or liquid (L) media.

Fig. 2. Dry weight of *Hosta tokudama* 'Newberry Gold' roots from plants produced in vitro in agar medium, grown in a mist bed (days 20 to 25) and outdoor container nursery (days 26 to 63) with fitted response curves ($P \leq 0.05$); 1 g = 0.035 oz. In vitro culture in agar media used ventilated (AV) or conventional (AC) containers.

tests performed for each cultivar are presented in Table 2. These tests provide meaningful comparisons among the growth curves and examine effects of medium type and culture vessel.

With 'Newberry Gold', shoots from liquid culture were significantly larger during the 60 d of growth in ex

Fig. 4. Dry weight of *Hosta* × 'Blue Cadet' shoots grown in a mist bed (days 0 to 24) and outdoor container nursery (days 25 to 63) with fitted response curves ($P \leq 0.05$); 1.0 g = 0.035 oz. In vitro culture used liquid (L) or agar (A) media in conventional (AC) or ventilated (AV) containers.

Fig. 3. Dry weight of *Hosta tokudama* 'Newberry Gold' roots from plants produced in vitro in liquid medium, when grown in a mist bed (days 20 to 25) and outdoor container nursery (days 26 to 63) with fitted response curves ($P \leq 0.05$); 1.0 g = 0.035 oz. In vitro culture in liquid media used conventional (LC) or ventilated (LV) containers.

plants cultured on agar, roots from plants cultured in liquid entered an exponential growth phase and had much larger dry weights at the end of the test period (Fig. 3). Plants from ventilated vessels with liquid media had less root growth than plants from

vitro conditions, and ventilation of the culture vessels during in vitro culture had no lasting effect on shoot growth during ex vitro growth (Table 2). This suggested lines for ventilated and non-ventilated vessels should be pooled, while lines for liquid and agar cultured shoots remain discrete. Shoots of 'Newberry Gold' plants that had been cultured on agar, or liquid media grew linearly ex vitro without a discernible acclimatization lag phase during the 60-d sample period (Fig. 1). Growth rate was similar for shoots from agar or liquid. Therefore, the larger shoots from in vitro liquid culture had remained larger during the ex vitro growth.

After about 20 d in the mist bed, plants from all treatments showed significant root formation. At that time, plants were moved from the mist frame to the outdoor nursery. Roots of agar-cultured plants of 'Newberry Gold' grew slowly at a linear rate and plants from ventilated vessels had improved root growth compared with conventional vessels (Fig. 2). Unlike roots of

Fig. 5. Dry weight of *Hosta* × 'Blue Cadet' roots when grown in a mist bed (days 15 to 24) and outdoor container nursery (days 25 to 63) with fitted response curves ($P \leq 0.05$); 1.0 g = 0.035 oz. In vitro culture used liquid or agar media in conventional (LC and AC, respectively) or ventilated (AV) containers.

nonventilated vessels. However, the general trend for greater root growth from liquid medium versus agar medium should be emphasized. Extrapolation of exponential and linear growth curves suggests differences in root biomass will continue to increase in the nursery past the 60-d sample period used for this investigation. Liquid culture in vitro resulted in larger plants of 'Newberry Gold' with a more vigorous root system in the nursery.

Shoots of *Hosta* 'Blue Cadet' produced in vitro had greater dry weight from liquid culture than agar culture (Table 1). Ventilated vessels produced shoots with greater dry weight than conventional vessels. During the 60-d of ex vitro growth, shoots from liquid culture were larger than agar produced shoots, shoots from ventilated vessels in agar culture were larger than shoots produced in conventional vessels, but from liquid culture the effects of ventilation on ex vitro growth were transient (Table 2). All shoots of 'Blue Cadet' grew exponentially over the 60-d ex vitro sample period (Fig. 4). However, shoots of plants cultured on agar in conventional vessels exhibited a lag phase of at least three weeks prior to exponential growth. Shoots from agar-cultured plants in ventilated vessels grew without a lag. Both groups of agar produced shoots were outperformed by shoots of plants from liquid culture during ex vitro growth.

Roots of 'Blue Cadet' were initiated after about 15 d ex vitro. Rooting of plants from agar culture in conventional vessels proceeded at a relatively slow, linear rate (Fig 5.). Roots of liquid cultured plants, and plants from ventilated agar vessels entered exponential growth without a discernible lag phase. To generalize growth of 'Blue Cadet', the larger liquid cultured plants maintained similar or greater dry weights than agar cultured plants over the entire period of ex vitro growth period.

The use of liquid culture, for both 'Newberry Gold' and 'Blue Cadet', resulted in plants with greater dry weight in vitro than plants cultured on agar. Nursery growth of liquid-cultured plants is at least as good as plants from agar. Ventilation apparently improves acclimatization of plants from agar. Hyperhydric tissues were not observed in any of the treatments for these two

cultivars, and we found no apparent benefits of ventilation for ex vitro growth of liquid cultured plantlets of these cultivars. Survival of plantlets ex vitro was excellent for all treatments.

Since effective acclimatization was evident by active growth ex vitro from liquid medium without a lag phase, we concluded that *Hosta* from liquid culture did not suffer upon transfer from in vitro conditions. Plants from agar were more sensitive. Liquid culture systems for *Hosta* are envisioned to be less costly during micropropagation and deliver plants with greater productivity for the nurserymen, a classic win-win situation.

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