Larger Plants from Liquid-Based Micropropagation: A Case Study With *Hydrangea quercifolia* 'Sike's Dwarf'©

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INTRODUCTION

Liquid-based Micropropagation. Virtually all commercial micropropagation in the U.S.A. uses semi-solid "gelled" medium (agar or similar polymers) to support plantlets on medium surface. Gelling agents are the most expensive component of media. They slow laboratory operations, including medium preparation, dishwashing, subculture, and moving plants to greenhouse. Gels also slow plant growth by limiting the availability of water and nutrients. A variety of liquid bioreactor systems have been designed for micropropagation. The "motorized" function of these bioreactors is to provide oxygen to the propagules while their entire plant surface is wet with liquid medium. Cost and complexity are the largest barriers to commercial utility.

Our lab has shown a simple rocker system yielded more and larger plants than agar with herbaceous perennials including hosta (Adelberg, 2005), elephant ear (Adelberg and Toler, 2004), and daylily (Adelberg et al., 2005). Subsequent transfer to greenhouse and nursery yielded robust plants with high rates of survival. Woody plants, however, often become hyperhydric (waterlogged) if grown immersed in liquids. Hyperhydric shoots do not acclimatize to ex vitro greenhouse or nursery conditions. Temporary immersion systems (TIS) have been successful in avoiding hyperhydricity in large numbers of woody plant genera (Berthouly and Etienne, 2005). Our experience with woody plants on rocker (*Clematis, Hydrangea,* and *Nandina*) shows hyperhydricity occurred during partial submersion on the floor of the vessel.

Micropropagation of *Hydrangea quercifolia*. *Hydrangea quercifolia* is a four-season woody ornamental plant native to North America. Improved clones have been selected for compact stature, large flowers, brilliant fall color, and exfoliating bark. Commercial laboratories currently produce several cultivars of *H. quercifolia* on agar-based medium with some reports published on axillary shoot proliferation, rooting, and acclimatization (Sebastian and Heuser, 1987; Ledbetter and Preece, 2003).

Our objectives were to: (1) develop a liquid-based micropropagation system with *H. quercifolia* Bartr.'Sike's Dwarf' as a model plant; and (2) compare growth of plantlets from agar and liquid medium during greenhouse acclimatization.

MATERIALS AND METHODS

Explant Preparation. Shoots were collected in November from mature specimens at South Carolina Botanical Gardens. Large, dormant buds were cut from stems and stripped of pubescence with 70% ethanol. Several layers of bud scales were removed while immersed in 1 : 1 mix of commercial bleach to water. Buds were then rinsed in sterile-distilled water and individually planted in test tubes containing 5 ml of Murashige and Skoog (MS) medium, 1μM benzyladenine (BA), 30 $g \cdot L^{-1}$ sucrose and

Table 1. Size of *Hydrangea quercifolia* 'Sike's Dwarf' plantlets and shoots from two harvest periods, in Summer 2006.

		Plantlet length (lab) [cm]	Shoot length (greenhouse) [cm]	Large leaves (no.) [greenhouse]
Early summer	Agar	0.9 c	2.6 c	1.5 c
	Liquid	2.1 a	4.4 b	3.7 b
Late summer	Agar	1.7 b	5.0 b	3.9 b
	Liquid	2.2 a	6.0 a	5.1 a

Note: a, b, and c indicate treatment means within columns were different at p=0.05.

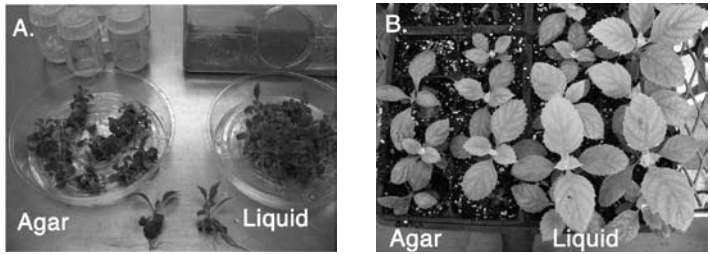

Figure 1. Plantlets from agar and liquid at planting (left) and after 3-weeks in mist (right).

solidified with 0.6% agar. Axillary divisions were replanted as single node cuttings every 6 to 8 weeks in 180 ml baby food jars containing 35 ml of medium with 3 to 6 nodes per jar. After about four transfers, 2.5 μM 2-iP was substituted as cytokine.

Liquid Systems. Liquid medium was same as above without agar. A rocker platform (Adelberg and Simpson, 2004) with an articulated shelf creating a 1-rpm swing every 15 min with the vessel remaining inclined for 14 min. Large rectangular rocker vessels (11 × 30 cm; Southern Sun Biosystems, Hodges, South Carolina) contained 210 ml of medium lined with germination paper (Anchor Paper Co., St. Paul, Minnesota) supported on a capillary mat (polyester fiber needle-punched to a density 150 $g \cdot m^{-2}$, Clemson University Non-woven Fabric Laboratory).

Comparison of Plants Produced on Agar and Liquid. Five nodes were placed in each of 35 jars containing agar gelled medium, as above. Thirty-five nodes were placed in each of five rocker vessels on germination paper supported by capillary mat. Shoots were grown for 6 weeks in the laboratory and used to establish a second repetition of the experiment. Plantlets not used for repetition were measured (shoot length and mass). Sugar level in residual medium was measured on a refractometer as % BRIX (BRIX = 1 g sucrose per 100 ml medium; Atago Instruments, Tokyo, Japan). Residual media was rinsed from the roots, and plantlets were placed in moist soilless mix (Fafard 3B, Fafard Inc., Anderson, South Carolina) in 1204 cells under intermittent mist (6 sec on every 6 min during day) for 3 weeks. Plants were moved to greenhouse for another 3 weeks and hand-water fertigated, as needed, with Peter's Peat-lite 20N–10P–20K (Scotts, Marysville, Ohio) at 100 ppm N. Plants from

Figure 2. Plants after 3 weeks in mist and 3 weeks in greenhouse range.

the first repetition (early summer) went to mist frame 10 June, and the second repetition followed on 25 July (late summer). Greenhouse plants were measured for stem length (cm) and number of large leaves (> 3 cm leaf blades) per plant. Plant size was graded by creating a numerical score (numbers of large leaves + stem length). Scores were sorted into five size categories by Statistica 7.1 (Statsoft, Tulsa, Oklahoma).

RESULTS AND DISCUSSION

Developing a Liquid System. 'Sike's Dwarf' shoots multiplied on agar-based medium in baby food jars. Agar could be replaced by floating a sheet of paper on top of the liquid medium on a sealed air raft (Osmotek Ltd, Rehovot, Israel). This produced a mixture of normal and hyperhydric shoots by allowing internode elongation to occur in gaseous headspace of vessel. We replaced the floating raft with a capillary mat infused with liquid medium that supported the paper. When placed on rocker shelf, a bead of liquid medium would intermittently wet the base of the shoots.

Comparison of Shoots Grown in Agar and Liquid. Both agar and liquid culture produced elongated shoots with fibrous roots that were ready for acclimatization to greenhouse (Fig. 1). Plantlets were not hyperhydric when grown in liquid on the paper/mat system. Shoots produced in liquid culture had longer stems than shoots produced in agar culture at both harvest dates (Table 1). There was a large amount of variation in shoot size from both treatments, and plantlets from the second harvest of agar were longer than plantlets from the first harvest on agar. Shoots from agar and liquid systems had similar fresh and dry weights when they left the lab (data not shown). There was a significantly higher concentration of sugar residual in agar medium than liquid medium at harvest (4% vs. 3% BRIX). This indicated that either less water was available to plants while growing in agar or plants in agar used less sugar.

Nearly 100% of plants survived acclimatization and subsequent transfer to greenhouse bench from both agar and liquid. The greatest difference between agar and liquid cultured plantlets was seen in the mist-bed and greenhouse nursery. Plants from liquid grew more quickly on the mist bed (Fig. 1b). Plants from agar were noticeably smaller than plants from liquid, and there was a large variation in plant sizes among treatments (Fig. 2). Plants from liquid had longer stems and more large

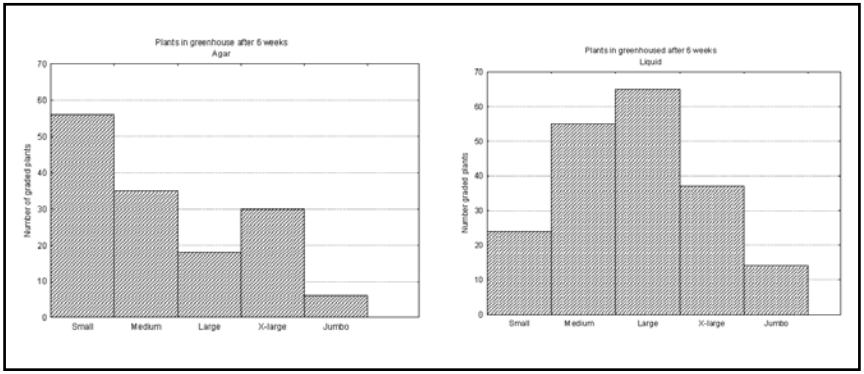

Figure 3. Five size grades (stem length + large leaves) were assigned to 336 plants.

leaves per plant (Table 1). Shoot and root fresh and dry weights of plants in greenhouse were also greater from prior liquid medium treatment (data not shown).

Variation in plant size is a common occurrence in commercial micropropagation. Industry labs usually sort liner materials in the greenhouse to ensure delivery of a uniform product. We conducted a scoring procedure to sort plants from agar and liquid by adding plant height to the numbers of large leaves. Plants from agar were represented in the five categories. The greatest numbers of plants from agar were in the "small" category and the population was skewed (Fig. 3). Plants from liquid produced a normally distributed population with the greatest number of plants being "large." There were more and larger plants produced from liquid than agar.

Hydrangea plants from liquid had greater greenhouse growth than plants from agar. We can speculate as to several possible reasons. Compared to agar, plants on liquid had greater access to water and sugar in the lab thus they possessed more stored carbohydrate available for new growth in greenhouse. Alternatively, there may be less root damage when rinsing liquid media than removing agar residues, during planting. Lastly, the taller shoots from liquid may have a morphological advantage for photosynthetic growth in greenhouse. We conclude that other woody species would benefit from liquid micropropagation systems, when hyperhydricity is controlled. These benefits become apparent during increased subsequent growth in greenhouse.

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