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In Vitro Propagation and Growth of Hydrangea

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Abstract: ***Hydrangea macrophylla*** **Thunb. 'Merveille' can be propagated by tissue culture and the resulting plants acclimated to a greenhouse environment. Utilizing rapidly replicating cultures for continued propagation and slow replicating cultures for producing plants in a greenhouse is an effective method of increasing the overall tissue culture propagation rate. Planting out in a greenhouse by 30 July at the latitude of Lexington, Ky., provided adequate time to develop hydrangea plants that produced 3 or more flowers per plant by 15 Apr.**

Fewer hydrangea are grown as a florist pot plant today than in past decades. Weiler (6) considers the long cropping time, difficulty of transporting when in flower, and the large postgreenhouse demand for water to be major factors for the production decline. Some growers in the North frequently save stock plants for propagation. With the current high cost of greenhouse space, carry-over crops often are eliminated to accommodate a revenue producing crop. In addition, hydrangeas frequently are found to have virus diseases, especially hydrangea ringspot virus (5). Carrying diseased stock plants from year to year perpetuates the problem. In Europe, hydrangeas have been cultured aseptically to obtain stock plants free of virus, and subsequent propagation was by cuttings from these stock plants (1, 2, 5). Jones (3) has reported successful *in vitro* propagation and preliminary virus indexing of 5 of 10 hydrangea cultivars attempted; 'Merveille' hydrangea was not one of those tested. Commercial laboratories also have produced hydrangeas by *in vitro* propagation, but no reports of their studies are available.

Tissue culture propagation has the potential of producing large numbers of plantlets. Once the starting material is disease free, it can be maintained readily in aseptic culture. In addition, very little space is required in a tissue culture growth room to carry propagation stock from one year to the next. This study was undertaken to determine 1) if 'Merveille' hydrangea would respond to tissue culture propagation techniques, 2) if individual shoot isolates vary significantly in propagule production, and 3) if market quality pot plants could be produced in less than one year from plants propagated *in vitro*.

Stems were harvested on 28 June from a single 'Merveille' hydrangea growing outdoors at Lexington, Ky. The leaves were removed and the stems were immediately placed in 0.5% sodium hypochlorite solution for 10 min. Buds were cut from the stems and placed in fresh 0.5% sodium hypochlorite solution for 5 min, rinsed twice with sterile distilled water, and placed individually on 10 ml of culture medium in 25 × 150 mm culture tubes.

The basal medium used for all studies was Murashige and Skoog (MS) mineral salts (4) supplemented with the following (mg/liter): NaH_2PO_4, 170; *myo*-inositol, 100; thiamine·HCl, 0.4; Difco Bacto agar, 7000; and sucrose, 20,000. The initial isolation medium consisted of a basal medium with 0.1 mg NAA and 1 mg BA per liter. All media were adjusted to pH 5.7–5.8 before autoclaving. To obtain cultures free of microbial contamination, shoots were grown singly on 10 ml of basal medium with 1 mg/liter BA in 25 × 150 mm culture tubes. To study shoot production response to growth regulators, cultures were grown on 15 ml of basal medium with 4 levels of BA (0.2, 0.5, 1.0, 5.0 mg/liter) without auxin and also with 0.2 mg/liter of NAA. Ten replications were used for each treatment. Shoots were harvested 3 times at 35-day intervals.

To study shoot production as a function of individual shoot isolates, 20 random cul-

Table 1 Total shoot production of 20 randomly selected shoots through 5 harvests at 28-day intervals.[z]

Line no.	Total shoots
7	227 a
20	153 b
8	114 bc
14	102 cd
3	93 cd
12	90 cd
10	89 cd
15	81 cd
13	76 cd
9	72 cd
11	70 cd
2	69 cd
17	68 cd
1	66 cd
6	66 cd
16	66 cd
18	66 cd
5	62 cd
4	61 cd
19	50 d

[z]Shoot numbers followed by a different letter are significant at the $P = 0.05$ level as determined by Duncan's multiple range test.

Table 2. Mean number of nonflowering, flowering, and total stems per plant produced by tissue cultures 'Merveille' hydrangeas transplanted to 13 cm pots 30 July and transplanted to 10 cm pots 20 Aug.

Pot Size	Mean number of stems per pot[z]		
	Nonflowering	Flowering	Total
10 cm	3.3 A	1.0 B	4.3 B
13 cm	2.1 B	3.7 A	5.8 A

[z]Means within a column followed by a different letter are significantly different at $P = 0.01$ as determined by a single degree of freedom F test.

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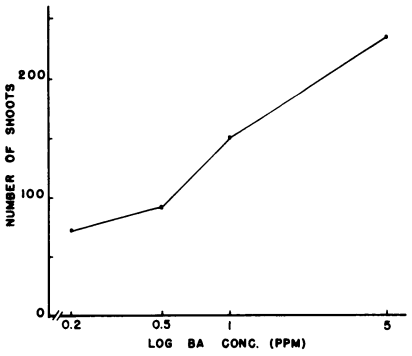

Fig. 1. Linear response of *in vitro* shoot production of 'Merveille' hydrangea to the log of the BA concentration in the culture medium.

tures, all grown previously on basal medium with 1 mg/liter BA, were carried through 5 harvests at 4-week intervals. Two high and 2 low shoot producing lines were carried through an additional 3 harvests. The term 'line' is used to mean all of the tissue and propagule production from one original shoot isolate. The tissues were grown on 35 ml of medium in 55 × 110 mm bottles. For *in vitro* rooting, shoots 2 to 3 cm in length, taken at random from cultures, were transferred to 25 × 150 mm culture tubes with 10 ml of medium consisting of ½MS mineral salts with 20 and 7 g/liter sucrose and agar, respectively. The 2 basal leaves of each shoot were removed prior to inserting the shoot 5 to 8 mm into the medium.

All cultures were maintained at 26°C under cool-white fluorescent lights (30–35 μ mol $m^{-2}s^{-1}$) for 16 hr daily and at 23° during the 8 hr scotophase. Extracts made from 10 random plantlets were inoculated to *Chenopodium amaranticolor* Coate. and Reyn., a local lesion indicator host for a wide range of viruses that infect hydrangea.

Rooted shoots were washed free of agar medium, dipped in aqueous Captan solution, planted into sterile growing medium in Cellpak units, and placed under intermittent mist (6 sec each 3 min) in an unshaded greenhouse. After 7 days, they were removed from the mist and placed on a greenhouse bench shaded with 70% plastic shade cloth. After

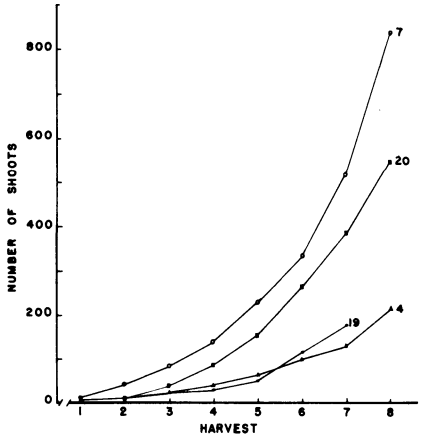

Fig. 2. Cumulative number of shoots produced by tissue cultured 'Merveille' hydrangea through 8 harvests for lines 7, 20 and 4 and 7 harvests for line 19.

14 days, the plants were moved to a greenhouse bench without shade, and fertilization with each watering was begun. One hundred plantlets were transferred to the greenhouse on 30 July, transplanted to 7.5 cm pots on 21 Aug., and 50 of these were transplanted to 13 cm pots on 10 Sept. An additional 100 plantlets were transferred to the greenhouse on 20 Aug., transplanted to 7.5 cm pots on 10 Sept., and 60 were transplanted to 10 cm pots on 8 Oct.

All plants were grown under natural photoperiod in a greenhouse maintained at 21°C at night until 9 Nov., at which time the plants were transferred to a cool house maintained at 10° at night. Dead and senescing leaves were removed from the plants periodically, and watering was done as needed until 9 Feb. At this time, the plants were transferred to a greenhouse maintained at 21° nights, and fertilization at each watering and routine greenhouse maintenance was begun. Because of bench space limitations, only 44 of the plants in 13 cm and 56 of those in 10 cm pots were forced. The plants were set on a bench with 4 replications of each pot size. Number of flowering and nonflowering shoots and plant height were measured on 20 Apr.

All buds initially isolated contained internal microbial contamination. The contamination was considered to be internal, since it appeared as a clouding of agar originating beneath the bud in the area of xylem tissue. To eliminate microbial contamination, small shoot tips, 0.1–0.5 cm, were collected from the tall shoots in the culture and transferred to fresh basal medium with 1 mg/liter BA. Transfer times varied from 14 to 45 days, depending upon shoot size and severity of microbial contamination. This procedure provided shoots which were consistently subcultured without evidence of contamination. Subsequent tests were not begun until the shoots had undergone at least 4 successive subcultures at 35-day intervals without evidence of contamination.

All media containing NAA resulted in callus growth. This growth was especially pronounced for shoots on the medium containing 5 mg/liter BA with 0.1 mg/liter NAA. The medium with 5 mg/liter BA without NAA also resulted in callus growth. Callus tissue was considered undesirable because of the possibility of shoots originating from the callus tissue with a subsequent change in clonal characteristics. Because there was no significant interaction of BA with NAA, only the shoot production on media with BA alone is presented. The cumulative number of shoots produced for 3 harvests at 35-day intervals was greatest for the medium containing 5 mg/liter BA. The total shoot production for the 3 harvests showed a linear increase to the log of the BA concentration that was significant at the 95% level, $r = 0.981$ (Fig. 1). Because callus tissue formed on propagules on the 5 mg/liter BA medium, the basal medium with 1 mg/liter BA was selected for subsequent propagation. The optimum BA level for maximum hydrangea shoot production without callus formation probably lies between 1 and 5 mg/liter.

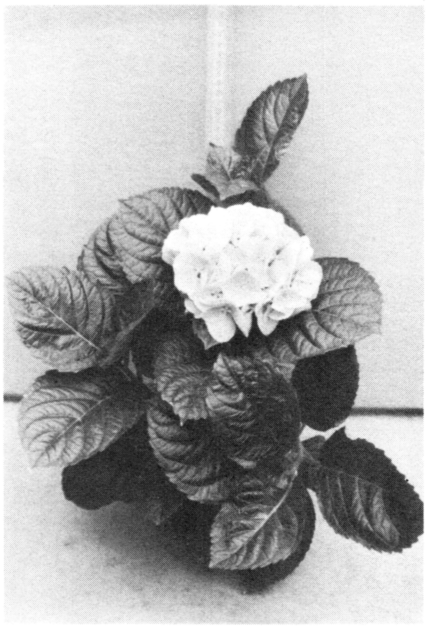

Fig. 3. Hydrangea 'Merveille' grown in 10 cm pot showing several vegetative shoots but only one flowering shoot.

Total shoot production from 20 random shoots through five 28-day harvest periods is shown in Table 1. Total shoots produced by the individual lines varied from 50 to 227 shoots with a mean of 87 shoots and a total of 1741 shoots harvested. Line 7, the most productive, yielded over 4 times the number of shoots as line 19, the least productive line. To evaluate the advantage of selecting lines that produce greater numbers of shoots further, the 2 least productive lines (4 and 19) and the 2 most productive lines (7 and 20) were carried through 3 additional harvests. The cumulative shoot production for these lines is shown in Fig. 2. After log transformation of the shoot number means, regression analysis indicated significant differences among lines in the rate of shoot production. Comparison of the slopes indicated that lines 4 and 19 were not significantly different from each other at the $P = 0.05$ level, but line 20 and line 7 were significantly different from each other and from lines 4 and 19 at the $P = 0.05$ level. Line 19 became contaminated between the 7th and 8th harvest, and there-

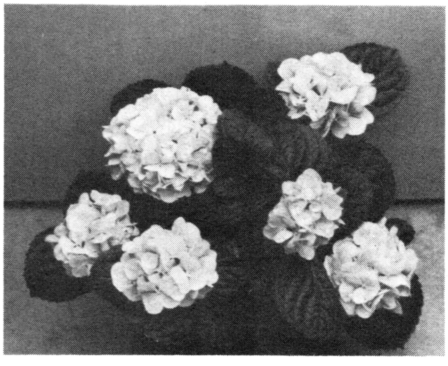

Fig. 4. Hydrangea 'Merveille' grown in 13 cm pot with 6 flower heads. The largest originated from the primary apical bud.

fore only 7 harvests are shown. Line 7 produced more shoots, a total of 835 for 8 harvests, than did line 20. Line 19, originally the least productive, surpassed the shoot production of line 4 after the 5th harvest. Line 7 maintained a 4-fold shoot production advantage over lines 4 and 19 (Fig. 2). Thus, an economic advantage can be obtained by using the rapidly producing lines for continued propagation and using the slow producing lines for greenhouse plant production. The increased cumulative shoot production by selected individual shoots is not considered to be a heritable change but rather the result of utilizing those shoots that have readily adapted to *in vitro* culture conditions.

Rooting occurred in 7 to 16 days. The rooted shoots were left under fluorescent lights in the laboratory for 30 days before transferring to the greenhouse. After 30 days the shoots were all well rooted. Extracts of 10 random plants were used to determine presence of viruses. No viral local lesion response was found from any extracts inoculated to indicator host plants. The viral status of the plant from which original buds were isolated was not assayed because the plant was destroyed shortly after the cultures were established.

Only one of the 100 shoots transplanted to sterile growing medium in the greenhouse on 30 July died; none of those transplanted on 21 Aug. were lost. 'Merveille' hydrangeas can be acclimated readily to the greenhouse environment by the system described. The small plants exhibited a natural tendency to form shoots from lateral buds near the base of the plant. The plants were grown without pinching since the original intent was to grow them as single stemmed flowering plants. The plants varied from 7 to 13 cm tall at the time they were transferred to the 10°C night greenhouse, and it was thought the small, thin lateral branches would not set flower buds. The lateral branches were left intact so that they might contribute toward a full attractive plant. The plants grown to flowering in 13 cm pots produced more total shoots and more flowering shoots per pot than those grown to flowering in 10 cm pots. The plants grown to flowering in 10 cm pots produced more vegetative shoots per pot (Table 2). The plants in 10 cm pots were small, 6–9 cm tall, at the time of transfer to 10° nights, and although lateral breaks were present, they were only 1 to 2 cm in length and about 2 mm in diameter.

The size of the infloresence was not measured, but the infloresences of plants in 10 cm pots generally were not as large as those produced from the primary apical bud of plants in 13 cm pots (compare Figs. 3 and 4). Most commonly, they compared in size to infloresences produced on secondary apical buds of plants in 13 cm pots.

Both groups of plants received 60 days of temperature below 15°C for flower bud formation and, when forcing was begun, only the primary apical bud appeared swollen and to include a flower bud. Either the plants in 10 cm pots had sufficient growing time and foliage to accumulate reserves for flower bud formation on just the primary apical bud, or the secondary apical buds were not mature enough to respond to the floral initiation stimulus. Plants in 13 cm pots were transplanted only 21 days earlier; they not only had set flower buds on the primary apical bud but on most of the secondary apical buds as well. Infloresences produced from lateral branch apical buds were smaller than those produced from the apical bud of the central stem (Fig. 4). Regardless of pot size the plants grew to an overall height of 35 to 40 cm. Height was not significantly different for the 2 pot sizes.

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