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In Vitro Propagation of *Lythrum virgatum*

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Abstract. **Tissue culture propagation of *Lythrum virgatum* L. cv. Morden Pink was achieved from shoot tips using a modified Murashige and Skoog (MS) high salts medium. 6-Benzylamino purine (BA) at 3.0 mg/liter and naphthaleneacetic acid (NAA) at 0.1 mg/liter were optimal for shoot production. Proliferated shoots rooted and established 96% in a soilless medium under high humidity.**

Lythrum virgatum, wand loosestrife, is an ornamental herbaceous perennial grown for its long period of bloom between June and the end of September. Several selections varying in flower color and plant height have been introduced. Traditionally loosestrife is asexually propagated either by division or by shoot tip cuttings (3). While both methods are successful, *in vitro* propagation would reduce propagation stock requirements and remove the constraint of seasonality for commercial propagation.

Shoot tips obtained from actively growing plants of 'Morden Pink' loosestrife were surface-sterilized using 10% Clorox (0.5% sodium hypochlorite) for 15 min with constant agitation and then rinsed in 2 changes of sterile deionized water. Shoot tips 0.5–1.0 cm long were transferred to a stage I medium (1). The basal medium used for all cultures contained Murashige and Skoog salts (2) and the following, in mg/liter: myo-inositol, 100; nicotinic acid, 0.5; pyridoxine·HCl, 0.5; thiamine·HCl, 0.1; casein hydrolysate, 200; sucrose, 30,000; and Difco Bacto-agar, 7000. The pH was adjusted to 5.6 ± 0.1 and media were distributed into 25 × 95 mm glass culture tubes, 10 ml per tube. Media were autoclaved for 15 min at 121°C. Cultures were maintained at 26° under 14 μE $m^{-2}s^{-1}$ (Cool-White fluorescent lamps, F48T12·CW·HO) for 24 hr daily.

In the multiplication stage, explants were tested on media containing 0.1 or 1.0 mg/liter NAA and 0.3, 1.0, 3.0, or 10.0 mg/liter BA in all 8 combinations. Cultures on medium containing BA at 3.0 mg/liter and NAA at 0.1 mg/liter were grown at 1.5, 5.0, or 15 μE $m^{-2}s^{-1}$ to study the effect of irradiance on shoot number.

Shoot explants grew readily when cultured on a growth regulator free medium and these shoots served as a source of material for all subsequent studies. Increasing BA from 0.3 to 10.0 mg/liter increased axillary bud development at both auxin concentrations (Table 1). Up to 92 shoots developed per explant within 40 days. Increasing the NAA concentration from 0.1 to 1.0 mg/liter reduced shoot number at all BA levels by about one half. BA, when increased from 0.3 to 10.0 mg/liter, decreased axillary shoot length at both NAA concentrations (Table 1). BA at 3.0 mg/liter and NAA at 0.1 mg/liter were considered optimum because a higher concentration of BA resulted in very short shoots, while the higher auxin concentration decreased shoot number.

Table 1. The effect of BA and NAA on axillary shoot number and length of *Lythrum virgatum* cv. Morden Pink.

BA (mg/liter)	NAA (mg/liter) 0.1	1.0
	No. axillary shoots ± SE	
0.3	4.2 ± 1.1[z]	2.0 ± 1.1
1.0	12.1 ± 3.6	6.7 ± 1.1
3.0	42.3 ± 11.9	17.0 ± 4.3
10.0	91.6 ± 19.7	37.5 ± 7.5
	Axillary shoot length (mm ± SE)	
0.3	5.3 ± 0.9	8.1 ± 2.9
1.0	1.8 ± 0.4	2.9 ± 0.5
3.0	0.9 ± 0.1	0.9 ± 0.1
10.0	0.3 ± 0.1	0.4 ± 0.1

[z]Each value represents the average of 10 tubes per replication.

No differences were observed in axillary shoot number among the 3 irradiance levels: 39.0 ± 9.8 at 15 μE $m^{-2}s^{-1}$; 42.7 ± 9.9 at 6 μE $m^{-2}s^{-1}$; and 37.3 ± 7.0 at 1.5 μE $m^{-2}s^{-1}$ (each value represents the average of 20 tubes per replication).

Individual shoots 0.5 cm long rooted and established (96%) in a soilless mixture (1 sphagnum peatmoss : 1 vermiculite; v/v) without auxin treatment when placed in a high humidity chamber. The individual plants averaged 13.3 cm in height 2 months after sticking.

Results indicate that tissue culture has potential as a means for rapid propagation of 'Morden Pink' loosestrife. The proliferated shoots are rooted easily in a soilless medium under high humidity and this avoids the need for rooting under sterile conditions.

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In Vitro Propagation of *Nandina domestica*

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Abstract. **Lateral and terminal buds from *Nandina domestica* Thunb. 'Purpurea' (dwarf nandina) developed into shoots on a basal medium (Gamborg's B5 modified salts, sucrose, activated charcoal, and agar) supplemented with 0.1 mg/liter naphthaleneacetic acid (NAA) and 1 mg/liter 6-benzylamino purine (BA). Shoot multiplication rates of 4–5 per 6–8 weeks could be obtained by transfer to the same medium without charcoal. Shoots were rooted by transfer onto a 1/3-strength Murashige and Skoog medium containing activated charcoal. Rooted plants were successfully established in a sphagnum peatmoss–perlite mix.**

Dwarf shrubs of *Nandina domestica* are propagated commercially by cuttings. Because these shrubs are small and slow-growing, few rooted liners can be obtained from a single stock plant. Matsuyama (2) reported propagation of *Nandina domestica* 'Royal Princess' through tissue culture of lateral buds. Shoot tips contain 7 to 15 axillary buds, providing good explants for clonal propagation (3). A tissue-culture procedure for propagation of dwarf nandina using a different inorganic salt formulation and growth regulators is described.

Explant preparation. New terminal shoots of *N. domestica* 'Purpurea' (1.0–4.5 cm, with the outermost leaves removed) were used for isolation of lateral buds. The clasping basal portion of the petiole was left on for an initial wash in warm, soapy water and surface disinfestation in a solution of commercial laundry bleach (diluted to contain 1% NaClO) plus 0.1% Tween 20 emulsifier for 20 min. Shoot tips were rinsed in sterile distilled water.

Lateral buds were excised with the aid of a steroidissecting microscope and were placed immediately on a piece of water-moistened filter paper in a Petri dish to avoid desiccation. The clasping petiole was removed following a basal incision to expose the axillary bud. Axillary buds were removed by making a shallow incision into the stem basal to the point of attachment. Excess stem tissue was removed and the axillary bud was cultured.

Cultures were maintained under 24 μE $m^{-2}s^{-1}$ light intensity (Sylvania Grow Lux wide spectrum) for 16-hr daily illumination at 26 ± 3°C. All experiments included at a minimum 10 replications of each treatment and were repeated one to 14 times over a 5-yr period. The basal salt media examined were Murashige and Skoog (MS) (4) and modified Gamborg's B5 (1) using the MS inorganic iron source. Organic constituents per liter were 0.4 mg thiamine·HCl, 100 mg i-inositol, and 30 g sucrose.

Shoot development from explants. The effect of activated charcoal at 0, 1.0, 1.5, 2.0, 3.0, and 6.0 g/liter on shoot development of excised lateral and terminal buds was first determined (Table 1). MS and Gamborg's B5 salt formulations were compared with and without growth promoters (80 mg/liter adenine sulfate and 17 mg/liter $NaH_2PO_4 \cdot H_2O$) both with NAA and BA, and charcoal at 1.5 g/liter (Table 2).

Results (Table 1) indicate that without activated charcoal shoot development did not occur; 1.5 g/liter appeared most favorable and was retained in subsequent experiments. Later experiments using B5 salt formulation with 0.1 mg/liter NAA and 1.0 mg/liter BA with and without 1.5 g/liter charcoal also confirmed the charcoal enhancement of shoot development. Shoot tips averaged 29 mm and yielded 8 explants each. The shoot promoters in both the B5 and MS salts reduced the percentage of developed shoots (Table 2). Shoot development without promoters was higher in B5 than in MS media. On B5 inorganic salts both with and without charcoal the leaf blade did not expand and petiole elongation did not occur. The stem, however, was thickened and more elongated than on the MS inorganic salts. The MS medium with 2iP, which had been reported to support shoot development in standard nandina (2), resulted in 52% shoot development out of 112 cultures.

Shoot proliferation. Individual shoots were subcultured onto a shoot multiplication medium containing modified B5 salts and (per liter) 0.4 mg thiamine·HCl, 100 mg i-inositol, 1.0 mg BA, 0.1 mg NAA, 30 g sucrose, and 8 g agar at pH 5.7 (Fig. 1). Shoot multiplication rates after 6–8 weeks varied from 3.9 to 5.4 and averaged 4.6. Highest rates of multiplication are possible only if the shoot tip of the explant is removed;

Table 1. Effect of activated charcoal on *in vitro* shoot formation in *Nandina domestica* 'Purpurea' (MS basal medium with 0.1 mg/liter NAA and 1.0 mg/liter BA).

Charcoal (g/liter)	No. of cultures	Shoot development (%)
0	30	0
1.0	10	40 (6–74)[z]
1.5	30	53 (34–72)
2.0	10	20 (8–48)
3.0	30	20 (5–35)
6.0	20	10 (4–24)

[z]95% confidence limits in parenthesis.

Fig. 1. Shoot proliferation of *Nandina domestica* 'Purpurea' *in vitro*.

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apparently there is an inhibitory effect due to apical dominance.

Rooting. Rooting of shoots was accomplished on a 1/3 strength MS inorganic salt medium containing (per liter) 0.4 mg thiamine·HCl, 100 mg i-inositol, 30 g sucrose, and 1.5 g activated charcoal; 61% of the cultures formed roots on this medium. Culturing the shoot tips on full strength MS inorganic salts plus 3 mg/liter NAA or indolebutyric acid (IBA) for one week prior to transfer to 1/3 MS inorganic salts resulted in 58% rooting. Plants were established in a sphagnum peatmoss–perlite mix after a 3-week hardening-off period under high humidity.

Table 2. Comparison of inorganic salt formulation and growth promoters (16 mg/liter $Na\text{-}H_2PO_4\cdot H_2O$ and 80 mg/liter adenine sulfate) on shoot development of *Nandina domestica* 'Purpurea' (both media contained 0.1 mg/liter NAA, 1.0 mg/liter BA, and 1.5 g/liter activated charcoal).

Salt formulation	Growth promoters	No. of cultures	Shoot development (%)
MS	–	99	54 (44–64)[z]
	+	140	49 (41–57)
B5	–	64	80 (70–90)
	+	38	32 (17–47)

[z]95% confidence limits in parenthesis.

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Regeneration of Plants from Cell Suspensions of *Lactuca saligna*, *Lactuca sativa*, and *Lactuca serriola*

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Abstract. **'Black Seeded Simpson', 'Buttercrunch', and 'New York 12' lettuce (*Lactuca sativa* L.), Plant Introductions (PI) 261653 of *Lactuca saligna* L., and Acc. No. 500–4 of *L. serriola* L. regenerated plants from cell suspensions originated from leaf callus. Liquid cultures of these *Lactuca* spp. in either B5 or Murashige and Skoog (MS) basic medium amended with alpha-naphthaleneacetic acid (NAA) and 6-benzylamino purine (BA) developed roots, shoots, and complete plants when transferred to agar plates of B5 with several concentrations of BA under 16 hr of fluorescent illumination. Shoots were induced to produce a root system when cultured in B5 agar medium amended with NAA. Variation in response between cultivars and between species was observed, with *L. serriola* and 'Black Seeded Simpson' responding best to treatments.**

Two close relatives of lettuce (*Lactuca sativa*)—*L. saligna* and *L. serriola*—are potential sources of disease and insect resistance for commercial cultivars. Resistance to turnip mosaic virus, cucumber mosaic virus, and the cabbage looper (*Trichoplusia ni*) has been reported in PI 261653 of *L. saligna* (12, 15). Other representatives of this species may be sources of resistance to new races of the downy mildew pathogen (*Bremia lactucae*) that have caused a rapid breakdown of the resistance in lettuce that derived from *L. serriola* (11). Conversely, *L. serriola* may offer resistance where *L. saligna* fails, as in lettuce drop caused by *Sclerotinia minor* (1) and to strain CMV-LsS of cucumber mosaic virus (R. Provvidenti, personal communication), and also to *T. ni* (7).

The use of cell and tissue-culture methods and plant regeneration of *Lactuca* has been limited in the past to cultivars of *L. sativa* (2, 3, 5, 8, 13, 14). This report discusses the response of 'Black Seeded Simpson', 'Buttercrunch', and 'New York 12' lettuce (*L. sativa*), *L. saligna* PI 261653, and *L. serriola* Acc. 500–4 to culture *in vitro* as callus cell suspensions and the subsequent regeneration of plants. The purpose of these studies was to find efficient methods of *in vitro* culture of *Lactuca* spp. and subsequently to evaluate possible somaclonal variations for their usefulness in the development of disease resistant lines.

Commercial seed sources were used for the lettuce cultivars. Seeds of *L. saligna* and *L. serriola* were obtained from R. Provvidenti of the New York State Agricultural Experiment Station. To induce callus for cell suspensions, young expanded leaves were harvested from one- to 2-month-old seedlings maintained in a greenhouse at 24–30°C under sunlight conditions supplemented in winter by 2 additional hours of fluorescent lighting in the morning. The leaves were surface-sterilized by immersion in 40% ethyl alcohol for a few seconds and 10 min in 20% Clorox (1% NaOCl), and then washed 3 times in sterile distilled water. The leaves were cut aseptically into 2 cm^2 pieces, plated onto 0.8% Difco Bacto agar media in 100 × 15 mm disposable Petri dishes, and incubated in the dark for one month in the laboratory at 24–27°C. Callus that developed from the leaf pieces was transferred monthly to fresh agar plates for increase and maintenance. Callus cell suspensions were prepared by transferring 0.5 g of friable callus from the plates into 250-ml Erlenmeyer flasks with 50 ml of liquid medium and shaken at 40–60 rpm on Junior Orbit shakers. Transfers to fresh liquid medium were made weekly, separating the suspension roughly according to size in 2 categories: very fine suspensions with single cells to a few cell aggregates; and large aggregates that passed easily through large-bore pipettes. Twenty-five ml of the cell suspensions were added to 25 ml of fresh liquid medium and again placed on the shakers. After 4 weekly transfers, the cell suspensions were pipetted directly onto dishes with agar medium or centrifuged for 10 min at 100 g, washed twice in liquid basic medium, and plated at 3 ml per dish. The cultures were incubated in the dark, or with either 16 hr or 24 hr of fluorescent light (2.5 klx) for periods of up to 2 months. Regenerated shoots were transferred to root-inducing medium and one week later to 20-ml glass vials with Cornell mix (4). The plants were usually ready for transfer to the greenhouse one week later for further observation.

Soft, white-to-cream color, friable callus developed from the leaf pieces of most test plants in either B5 (6) or MS (10) basic media supplemented with 0.1 mg/liter of Picloram (4-amino-3,5,6 trichloropicolinic acid) or 1–2 mg/liter of NAA and 0.5–1.0 mg/liter kinetin, or BA. *L. saligna* calli were usually slower to develop and more compact than the rest, but satisfactory cell suspensions were also prepared from them. The callus pieces could be separated easily into small cell

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