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Micropropagation and Field Establishment of *Tiarella cordifolia*¹

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Abstract

Tiarella cordifolia L. (foamflower) was proliferated on an MS-based nutrient medium. Proliferation was maximal on medium gelled with 0.4% Difco-Bacto agar containing 1.0 mg benzyladenine (BA)/l and 0 or 0.025 mg naphthaleneacetic acid (NAA)/l. Proliferation of *T. cordifolia* var. *collina* was maximal on medium gelled with Gelrite® containing 0.25, 0.5 or 1.0 mg BA/l plus NAA (0, 0.025, 0.05, 0.1, 0.5 mg/l). Greenhouse rooting and survival was directly related to an increase in microcutting length, whereas leaf number (two to six leaves per microcutting) had no effect. At least 48% of the microcuttings of *T. cordifolia* inserted directly in the field rooted and survived.

Index words: native plant, tissue culture, herbaceous perennial, Saxifragaceae

Significance to the Nursery Industry

Methods are described for rapid clonal micropropagation of the native herbaceous perennial, *Tiarella cordifolia* (foamflower). This research demonstrates that microcuttings of *T. cordifolia* are fairly resilient. Thus nurseries should be able to purchase less expensive Stage II microcuttings from commercial tissue culture companies and root them with little difficulty. One advantage of this tissue culture protocol is that for plants found to have desirable characteristics, potentially new cultivars, can be quickly multiplied and made available to the public.

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Introduction

As demand for native plants in the landscape increases, nurserymen must seek more economical means of propagation (9, 13) since collecting of plants in the wild is harmful to the environment and such plants are less likely to survive transplanting (7, 13). Propagation of selected natives using conventional methods such as division is a slow process compared to micropropagation which offers economical, year-round production in a disease-free environment. However, while in vitro proliferation is relatively inexpensive, the rooting/acclimatization phase is labor intensive and usually requires a greenhouse with a mist or high humidity system (5). Therefore, there is interest in developing protocols for direct field establishment of microcuttings both to reduce costs and to improve acclimatization success (2).

Tiarella cordifolia (foamflower) is a native perennial groundcover of rosette growth habit having evergreen leaves.

While preferring shade with moist, well-drained soil, it tolerates morning sun and is fairly drought tolerant. Flowers appear in early spring and last for about a month. The 0.6 cm (0.25 in) wide, white (may be tinged with pink) star-shaped flowers are born somewhat terminally on a raceme 15 to 30 cm (6 to 12 in) tall imparting a feathery appearance, hence the common name. Flowering first occurs at the base and proceeds upward. Leaves may be downy, are maple-like in shape, and are 5 to 7.5 cm (2 to 3 in) wide and 7.5 cm (3 in) long. Seedlings are variable in leaf color and morphology, with burgundy-red or silver coloration along the veins and sharply lobed leaves being highly desirable. In some plants, the red coloration is enhanced during winter months increasing garden interest. Two growth habits are recognized: *T. cordifolia*, which grows closer to the ground and spreads by stolons, and *Tiarella cordifolia* var. *collina* (commonly referred to as *T. Wherryi*), less commonly available, which grows as a clump. Therefore, the objective of this research was to develop micropropagation and field establishment protocols for this garden-worthy native.

Materials and Methods

Seeds of *T. cordifolia* and *T. cordifolia* var. *collina* (Brandywine Conservancy, Chadds Ford, PA) were sown in market packs containing a soilless mix (Redi-Earth [W.R. Grace, Fogelsville, PA]) and placed under intermittent mist (6 sec every 6 min, during daylight) until germinated and then were placed on a greenhouse bench and watered as needed. Greenhouse stock plants were grown in a soilless mix (2 parts Redi-Earth [W.R. Grace, Fogelsville, PA]: 1 part vermiculite; v/v) and fertilized with Peter's 20-10-20P (Grace-Sierra Horticultural Products Co., Milpitas, CA) twice a month.

Plants having desirable characteristics, e.g. deeply lobed leaves and red coloration, were selected for micropropagation. Shoots from greenhouse stock plants were defoliated and surface disinfested as follows: 0.5% sodium hypochlorite (10% commercial laundry bleach) plus 0.1% Tween 20 for 10 min followed by 3 rinses with sterile, distilled water. Basal medium consisted of Murashige-Skoog salts and vitamins (8) and the following addenda per liter: sucrose, 30 g; glycine, 2 mg; washed Difco-Bacto agar, 8 g; benzyladenine, 0.5 mg (2.2 μ M) and naphthaleneacetic acid, 0.1 mg (0.54 μ M). Medium components examined included gelling agent type and concentration [Difco-Bacto agar (Difco Laboratories, Detroit, MI); 0.4, 0.6, 0.8, 1.0, 1.2% vs. Gelrite® (Kelco, San Diego, CA); 0.1, 0.15, 0.2, 0.25, 0.3, 0.4%], sucrose concentration (1.5, 3.0, 4.5, 6.0%) and growth regulators [BA; 0.1, 0.25, 0.5, 1.0 mg/l (0.44, 1.1, 2.2, 4.4 μ M) and NAA; 0.025, 0.05, 0.1, 0.5 mg/l (0.14, 0.27, 0.54, 2.7 μ M)]. Agar had been washed with distilled water, dried and reground prior to use. Laboratory stock cultures were maintained on basal medium and subcultured every 4 weeks. One explant was inserted vertically into 25 ml of medium contained within a 50 × 70 mm jar, unless noted otherwise. In the container-environment experiment, medium was dispensed into 50 × 70 mm (12.5 or 25 ml [standard]) or 90 × 95 mm (42 or 85 ml) jars. Medium was autoclaved for 15 min at 121°C (250°F) and 124 kPa. Cultures were maintained at 25 ± 5°C (77 ± 5°F) with a 16 hr photoperiod (50 μ mol · s⁻¹ · m⁻² of photosynthetically active radiation) provided by cool-white fluorescent lamps. Microcuttings were inserted in soilless mix (2 parts

Redi-Earth [W.R. Grace, Fogelsville, PA]: 1 part vermiculite; v/v) and placed under intermittent mist (as described above) for 2 to 5 weeks when they were moved to a greenhouse bench.

Field study. Microcuttings (5½ weeks old) were inserted into soil outdoors (Matapeake silt loam) in a 91 cm² (3 ft²) plot containing 9 individual square-foot blocks each having 20 equally spaced holes on October 3, 1986. Microcuttings within each block were randomly assigned a cover treatment and were either left uncovered or covered with salt hay or a 140 ml (5 oz) paper cup. The plot was hand watered twice, just prior to and directly after inserting the microcuttings. The entire plot was mulched with salt hay on November 20, 1986 and the salt hay was secured in place with a wooden frame covered with shade cloth. The salt hay mulch was removed on April 22, 1987 and the experiment terminated June 26, 1987.

Experimental design and statistical analysis. Experiments were conducted as completely randomized designs and repeated at least once (except for the field and the cutting length studies). Data were subjected to analysis of variance procedures with means separated by Duncan's multiple range test. Where appropriate, contrasts were used to compare the leading treatment to each of the other treatment combinations. To correct for unbalanced data, contrasts were tested using the Satterthwaite approximate F-test (10).

Results and Discussion

Proliferation.

Growth regulators. Proliferation of foamflower variety was affected by growth regulator combination and concentration (Table 1). Microcutting proliferation of *T. cordifolia* was significantly greater when medium contained 1.0 mg BA/l and 0 or 0.025 mg NAA/l, while microcuttings of *T.*

Table 1. Shoot proliferation of microcuttings of *Tiarella* cultured on media containing BA and NAA.

BA (mg/liter)	Mean total no. shoots ^a				
	NAA (mg/liter)				
	0	0.025	0.05	0.1	0.5
<i>Tiarella cordifolia</i>					
0	1.8	1.4	2.1	1.7	0.9
0.1	5.8	5.5	4.9	5.6	6.8
0.25	8.5	8.9	6.5	7.4	4.9
0.5	11.4	9.0	7.3	7.9	7.6
1.0	13.8 a ^y	<u>15.4</u> a	10.8	10.1	5.9
<i>Tiarella cordifolia</i> var. <i>collina</i>					
0	2.1	2.4	1.8	2.8	3.0
0.1	7.2	7.0	6.8	9.2	8.9
0.25	12.4 a	15.8 a	13.1 a	10.8 a	9.9
0.5	14.1 a	<u>16.5</u> a	3.0	13.2 a	11.5 a
1.0	12.4 a	<u>12.1</u> a	14.4 a	16.2 a	13.6 a

^aAnalyzed using contrasts where the leading treatment was compared to each of the other treatments. To correct for unbalanced data, the contrasts were tested using the Satterthwaite approximate F-test (10).

^ya = not significantly different from the leader mean (underlined) within a variety.

Table 2. Effect of gelling agent type and concentration on shoot proliferation of *T. cordifolia*.

Gelling agent (%)	N ^y	Mean shoot number ^z		
		>1 cm	<1 cm	Total
Agar				
0.4	55	7.2 a ^x	6.2 a	13.4 a
0.8	56	3.7	3.3	7.0
1.2	57	2.2	2.9	5.1
Gelrite®				
0.1	48	5.6	4.4 a	10.1
0.2	57	5.6	3.1	8.7
0.4	56	4.0	3.5	7.5

^xAnalyzed using contrasts where the leading treatment was compared to each of the other treatments. To correct for unbalanced data, the contrasts were tested using the Satterthwaite approximate F-test (10).

^yN = number of microcuttings.

^aa = not significantly different from the leader mean (underlined) within a column.

cordifolia var. *collina* generally proliferated equally well over all NAA concentrations when medium contained 0.25, 0.5 or 1.0 mg BA/l.

Gelling agent. Gelling agent and gel concentration both affected shoot proliferation. Microcutting proliferation of *T. cordifolia* and *T. cordifolia* var. *collina* generally decreased as gelling agent concentration increased (Tables 2 & 3). Microcuttings of *T. cordifolia* cultured in medium containing 0.4% agar proliferated the greatest total number of shoots, the greatest number of shoots taller than 1 cm (Table 2) and the greatest number of axillary shoots per explant (data not presented). Microcuttings of *T. cordifolia* var. *collina* proliferated a significantly greater total number of shoots when cultured on Gelrite® (mean of 11.8 total shoots) compared to agar (mean of 8.4 total shoots) (Table 3).

Gelling agent and sucrose. Microcuttings of *T. cordifolia* were cultured in a 5 × 3 factorial experiment examining sucrose (0, 1.5, 3.0, 4.5, or 6.0%) and gelling agent (0.4% agar, 0.8% agar [control], 0.2% Gelrite®). Gelling agent

Table 3. Effect of gelling agent and concentration on proliferation of *T. cordifolia* var. *collina*.

Gelling agent (%)	N ^y	Mean shoot number ^z		
		>1 cm	<1 cm	Total
Agar				
0.4	20	6.6 abc ^x	5.2 a	11.8 abc
0.6	18	4.0 bc	3.8 a	7.8 cd
0.8	20	3.4 c	4.6 a	8.0 cd
1.0	20	3.2 c	4.6 a	7.8 cd
1.2	21	2.4 c	4.1 a	6.5 d
Gelrite®				
0.1	19	10.2 a	5.4 a	15.6 a
0.15	20	8.6 ab	5.6 a	14.2 ab
0.2	21	6.7 abc	3.8 a	10.5 bcd
0.25	18	3.2 c	5.7 a	8.9 cd
0.3	20	4.8 bc	4.8 a	9.6 cd

^xData analyzed using the General Linear Models procedure.

^yN = number of microcuttings.

^aMeans separated within columns for a gelling agent by Duncan's Multiple Range Test, 5% level.

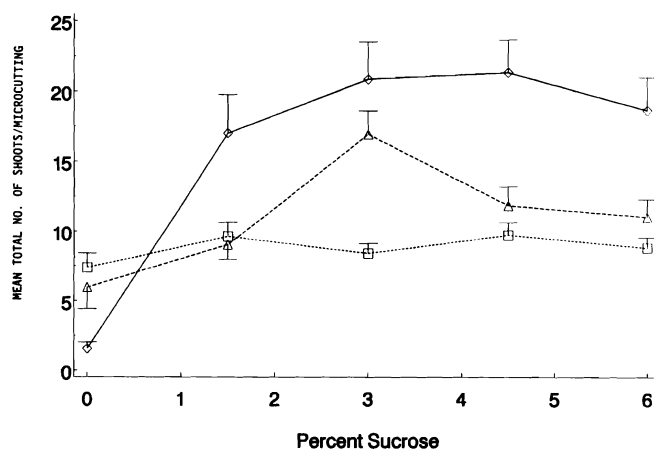


Fig. 1. Proliferation response (mean total number of shoots/microcutting) of *Tiarella cordifolia* to sucrose and gelling agent. (◇ = 0.4% agar, □ = 0.8% agar, △ = 0.2% Gelrite®; vertical bar = ± S.E.).

had a greater effect on proliferation than did sucrose concentration except when medium contained both 0.2% Gelrite® and 3% sucrose (Fig. 1). Microcuttings cultured in medium containing 0.4% agar proliferated the greatest total number of shoots (Fig. 1), the greatest number of shoots >1 cm in length and the greatest number of axillary shoots per explant (data not presented).

Container environment. Microcuttings of *T. cordifolia* proliferated equally well regardless of container size (50 × 70 mm; 90 × 95 mm), medium volume (12.5 or 25 ml per 50 × 70 mm jar; 42 or 85 ml per 90 × 95 mm jar) or microcutting density (one to four per 50 × 70 mm jar; two to seven per 90 × 95 mm jar) (data not presented).

Rooting.

Greenhouse. Microcuttings were inserted in soilless medium and placed under intermittent mist in a greenhouse. Microcuttings of *T. cordifolia* produced in agar- or Gelrite®-gelled media rooted and developed into plants equally well (data not presented).

A significantly larger percentage of longer microcuttings (>10 mm) of *T. cordifolia* rooted and survived compared to shorter microcuttings (<5 mm) (Table 4). Microcuttings of *T. cordifolia* var. *collina* ranging from 10 to 30 mm in length rooted and grew equally well (data not presented).

Microcutting leaf number (two to six leaves/cutting) did

Table 4. Effect of microcutting length of *T. cordifolia* on survival after rooting.

Microcutting length (mm)	N ^z	Survival (%) ^y
>15	5	83 a ^x
10 to 15	5	81 a
5 to 10	5	68 ab
<5	4	59 b

^zN = no. flats/treatment. Each flat contained 13–20 microcuttings.

^yPercentage of microcuttings to survive 5 weeks under mist plus 4 weeks in the greenhouse.

^xData were analyzed using the General Linear Models and means separated by Duncan's multiple range test, 5% level.

Table 5. Direct field establishment of microcuttings of *T. cordifolia*.

Cover	N ^y	Microcutting survival (%) ^z			
		Months			
		1½	5½	7½	9½
Uncovered	60	85 b ^x	77 a	70 a	55 a
Paper cup	60	100 a	90 a	87 a	73 a
Salt hay	60	90 ab	80 a	72 a	72 a

^zMicrocuttings were inserted October 2, 1986; 1½ months = survival prior to overwintering; 5½ months = survival after overwintering under a salt hay mulch, based on the number of microcuttings alive when the mulch was applied; 7½ months = survival after the mulch had been removed 2 months, based on the number alive when the mulch was removed; and 9½ months = survival based on the initial number of microcuttings.

^yN = number of microcuttings.

^xMeans separated within columns by Duncan's multiple range test, 5% level (an arsine transformation was done before analysis to stabilize variances).

not affect rooting, acclimatization or survival (99.2% for *T. cordifolia* and 100% for *T. cordifolia* var. *collina*) under greenhouse conditions (data not presented).

Field study. Microcuttings of *T. cordifolia* were inserted directly into a Matapeake silt loam soil in the field and either not covered or covered with salt hay or a paper cup. After 1½ months, significantly more microcuttings had survived when placed under paper cups compared to uncovered microcuttings (Table 5). All three microcutting treatments survived overwintering equally well under a salt hay mulch, although survival was generally reduced. Problems associated with field rooting and establishment included rain wash outs, earthworms and frost heaving.

While it was possible to determine optimum growth regulator and gelling agent types and concentrations for proliferation of *Tiarella*, no optimum sucrose concentration or container environment was determined. As reported for other Saxifragaceae (1, 4, 11, 12), proliferation of *Tiarella* was dependent on the presence of a cytokinin with little or no beneficial effect attributed to the addition of NAA. The cytokinin effect on proliferation of *Tiarella* was similar to that found in the related genera × *Heucherella* (4), *Deutzia* (3) and *Hydrangea* (12); when cytokinin concentration was increased, proliferation increased (Table 1). Proliferation of *Tiarella* was simplified compared to × *Heucherella* (4) or *Heuchera* (11) since adventitious shoot production was not a problem.

Gelling agent concentration was inversely related to average proliferation in *Tiarella* (Tables 2 & 3), × *Heucherella* (4) and *Artemisia dracunculoides* L. var. *sativa* (French tarragon) (6). Also, production of longer shoots (>1 cm) generally was more associated with increasing gelling agent concentration than production of shorter shoots (<1 cm).

Proliferation-medium gelling agent, agar or Gelrite®, had no effect on rooting success in *Tiarella*, as was reported for French tarragon (6).

As demonstrated previously for other species (3, 6, 11), microcutting length was critical to rooting success; longer microcuttings rooted and survived better than shorter microcuttings (Table 4). However, leaf number (two to six per microcutting) did not increase rooting and survival ability.

Microcuttings of *Tiarella* which were inserted directly into the field, rooted and grew over a period of months, albeit not as well as most placed under mist in the greenhouse. As with direct field establishment of *Solanum tuberosum* L. microcuttings (5), field establishment of *T. cordifolia* was improved when microcuttings were protected from drought. However, microcutting survival over 9½ months was not improved by a simple cover since 55% of the uncovered microcuttings also survived. It is worth noting that after 9½ months, field survival was as high as some greenhouse treatments (Table 4) especially considering the minimal care the field plot received.

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