Micropropagation of *Trillium grandiflorum* 'Flore Pleno'

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Plant material
On April 14, 2003, mulch was brushed to the side from *Trillium grandiflorum* 'Flore Pleno' plants in the Mt. Cuba Center, Inc. (accession 19950125) nursery and four stems approximately 2.5 cm long were excised as close to the rhizome as feasible. Stems were stored at 4ºC until April 23 at which time stems were surface disinfested and cultured.

Surface disinfestation
Half of the stems were heat treated at 40ºC for 10 min after which all stems were washed for 10 min in a solution of 10% (v/v) bleach + 0.1% (v/v) Tween 20 + 1 drop 95% ethanol. Individual stems were rinsed in 10 ml sterile water and shaken (100 rpm) for 10 min at each rinse (repeated 3x). Each was incubated in 10 ml liquid regeneration medium and shaken for 4 hr. Stems were washed in bleach solution, rinsed as described previously, and reincubated in regeneration medium. After 4 hr, stems were washed and rinsed once again and bleach damage trimmed. Stems were cut into 0.5 cm sections and cultured horizontally on gelled regeneration medium. Cultures were maintained under cool white fluorescent lamps (50 $\mu mol \cdot m^{-2} \cdot s^{-2}$ PAR) at 27ºC with a 16-hour photoperiod.

Media
Basal medium consisted of ½ Murashige-Skoog salts (1962) and (in mg l^{-1}), 100 *i*-inositol, 0.5 nicotinic acid, 0.5 pyridoxine•HCl, 0.4 thiamine•HCl, and 2 glycine. Regeneration medium consisted of basal medium supplemented with 3% (w/v) sucrose, 4.4 µM benzyl adenine (BA), and 9 µM 2,4 – dichlorophenoxyacetic acid (2,4-D). Proliferation medium consisted of basal medium supplemented with 1.5% (w/v) sucrose, 4.4 µM BA, and 1.4 µM 2,4-D. Rooting medium consisted of basal medium supplemented with 1.5 % (w/v) sucrose and 4.9 µM indolebutyric acid (IBA). The pH of all media was adjusted to 5.7-5.8, 7 g l^{-1} Phytagar was added, and the solution was autoclaved at 121°C and 124 kPa for 15 min. Medium was dispensed into Petri plates (60 x 15 mm) at 10 ml/plate or into jars (55 mm x 72 mm) at 25 ml/jar.

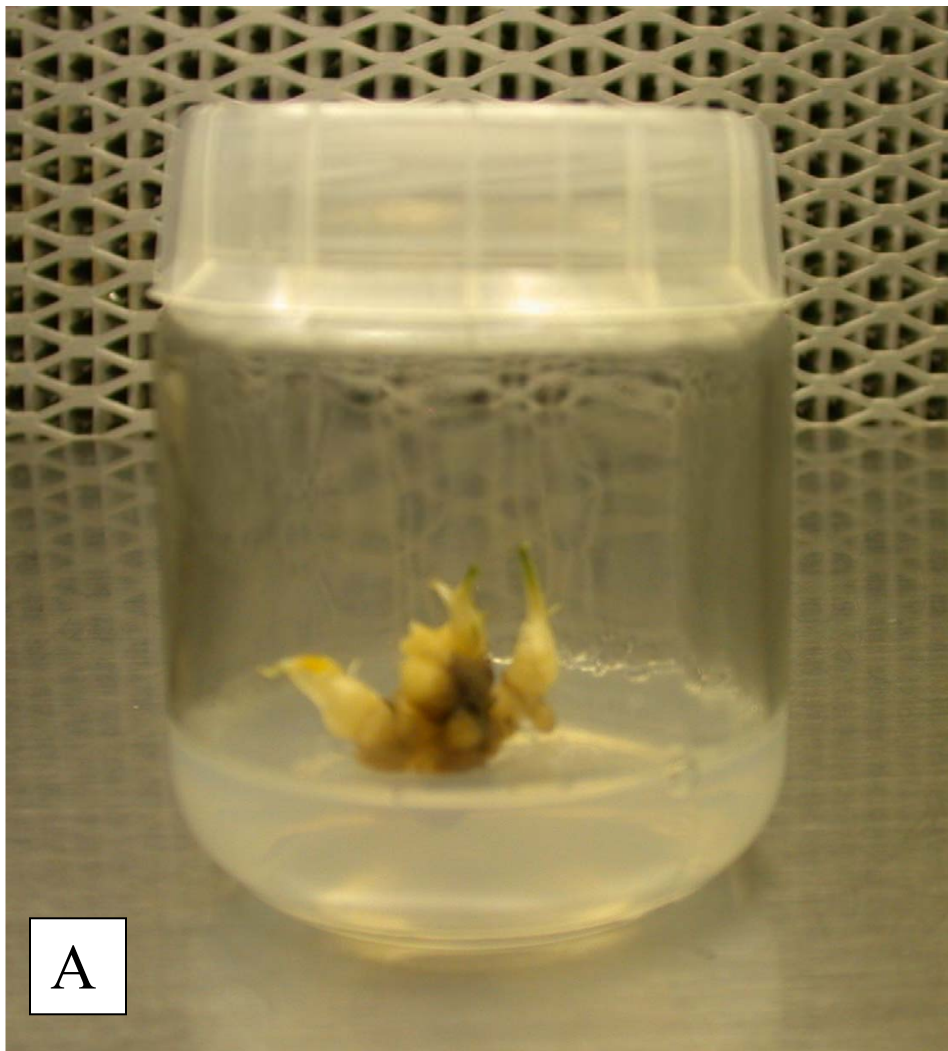
Regeneration
After 4 wk, one stem segment from each treatment (± heat) began to regenerate 'mini' rhizomes, which were transferred to proliferation medium for massing up. Plant material (A) was subcultured to fresh medium every 4 wk with experiments set up at this subculture time.

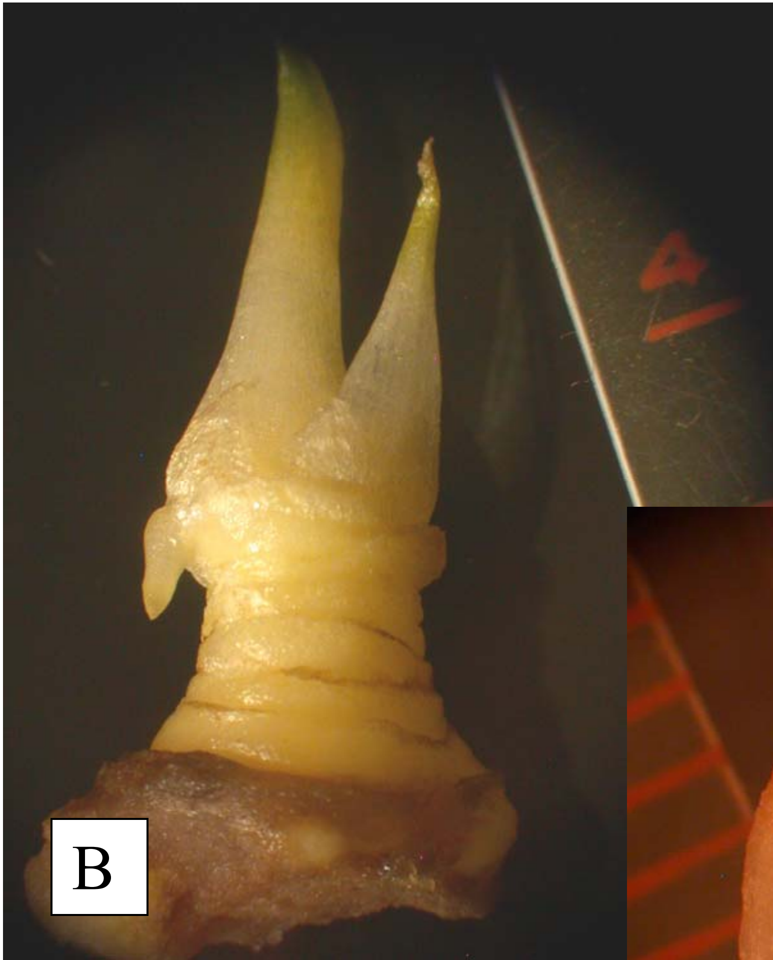
Rooting
On August 23, 2007, individual rhizomes were excised and cultured on basal medium for 4 wk, subcultured to rooting medium for 8 wk, and then placed in the dark at 4ºC for 4 wk. The rhizomes [16% (4/25) had rooted (B)] were then gently rinsed to wash off

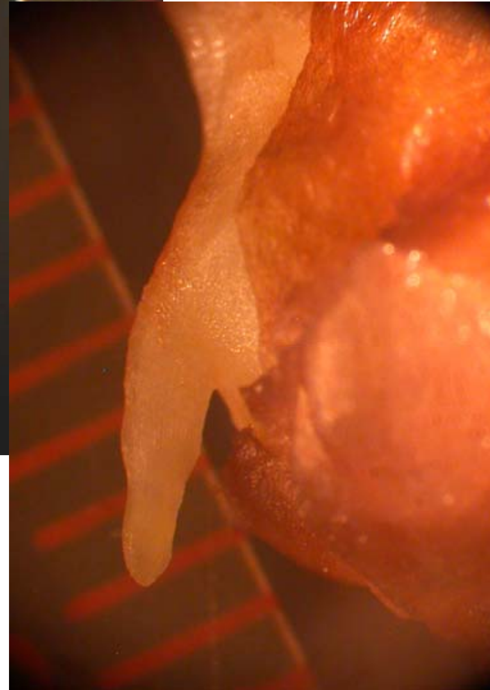
gelled medium, dipped for 3 sec in 5% (v/v) Dip-N-Gro® (Astoria-Pacific, Clackamas, OR) and dried for 5 min. Rhizomes were placed in market packs (4 - 6 rhizomes/pack) containing wetted Redi-earth (Sun Gro Horticulture Distribution, Inc., Bellevue, WA), and the flat was covered with a humidity dome before setting under mist (6 sec every 15 light-sun-accumulation units [1 unit = Watt·m^2] or every 6 min, whichever was sooner from 6:30 a.m. to 6:30 p.m.; however, the mist came on every 3 min if light was between 20-50 Watt·m^2). The flat was drenched with a fungicide solution of 79 µl l^{-1} Subdue and 182 mg l^{-1} Medallion (Syngenta, Greensboro, NC) to control fungal contamination. On February 9, 2008, the flat was placed in a cold frame until April 7 (temperature range: 0 – 20º C) when it was placed on a greenhouse bench under shade. Forty-four percent (11/25) of the rhizomes had rooted and 20% (5/25) had rotted.

Literature cited

Murashige, T.; Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.







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