
In Vitro Propagation of Saintpaulia ionantha Wendl.¹

Norah D. Start and Bruce G. Cumming
Department of Biology, The University of New Brunswick, Fredericton, N.B., Canada

Additional index words. African violet, tissue culture

Abstract. Small leaf-sections of African violet proliferated rudimentary shoots when explanted on a modified Murashige and Skoog's (MS) high salt medium, containing 5.4 μM naphthalene-acetic acid (NAA), 22 μM 6-benzylamino purine (BA), and 308 μM adenine sulfate. The sub-divided rudimentary shoots grew and rooted after subculturing onto a modified Morel and Muller (MM) mineral salt medium, lacking growth regulators and having a reduced sucrose concentration. Rooted plants reached the flowering stage after 4 months of greenhouse culture. A single leaf can produce approximately 500 plants, having the desirable habit of a flat-leaved rosette and the capacity to flower within about 7 months.

African violet, generally propagated vegetatively from leaf cuttings, is grown on a large scale both commercially and by amateurs. When more than one shoot is allowed to develop on a cutting, restrictions imposed by the multiplicity of plantlets in a limited growing space generally result in unsymmetrical plants with elongated sideways-displaced petioles. We describe a tissue culture method of propagation which overcomes the disadvantage of multiple poorly-formed plants while providing more well-formed plants from a given amount of leaf tissue (Fig. 1).

General methods. Healthy mature leaf cuttings, consisting of the lamina with a petiole of 3–4 cm length, were taken from 15 African violet cultivars (‘Chanticlear’, ‘Christmas Holly’, ‘Double Black Cherry’, ‘Jean Talon’, ‘Lisa’, ‘Poodletop’, ‘Red Sparkler’, ‘Red Topper’, ‘Red Roses’, ‘Royalaire’, ‘Sultry Rose’, ‘Vermilion Lake’, ‘White Peacock’, and ‘Wind n’sea’). Each leaf was washed with soap and water before submerging it for 15 min in a solution of 2% calcium hypochlorite plus 1% Tween 20. Following a thorough rinse in sterile water, leaves were briefly immersed in 70% ethyl alcohol before being transferred to a 10% “Javex” (active ingredient, sodium hypochlorite) plus 1% ‘weed solution, for 20 min. Finally, the leaves were rinsed 4 times in sterile water. Survival rate of disinfected leaves was 100%, and contamination in explants was about 1%.

Following disinfection, the damaged base of each petiole was removed, as were the outside edges and tips of the leaves. Previous studies have shown that these areas are marginally productive for root or shoot generation (3, 8). Each

1Received for publication November 20, 1975. This work was supported by a grant-in-aid of research to B. G. Cumming from the National Research Council of Canada.

petiole was cut with a small triangular section of lamina attached to it, so that the total length of the lamina tissue was about 12 mm, while the base of the lamina portion was 15–20 mm. The remainder of each leaf was cut into 5 or 6 pieces (12 x 12 mm), ensuring that each carried a section of vein (Fig. 2). Each segment of leaf and petiole-lamina portion was placed upright in the culture jar with one-quarter of its length embedded in the agar.

Two media were used for the cultural process (Table 1). Shoot initiation was stimulated from leaf-tissue explants on a modified MS medium (7). Shoots formed on the explants were subcultured onto a modified MM medium (4) to stimulate rooting. The pH of both media was adjusted to 5.8 before autoclaving.

The leaf explants were cultured in 60 or 120 ml glass jars containing 20 or 30 ml of medium and subcultured into 120 or 240 ml glass jars containing 30 or 50 ml of medium. The glass jars were covered by metal caps screwed on loosely to allow some air exchange and tilted to allow maximum light reception. The cultures were maintained at 23°C in a 12-hr daily photoperiod of 3.2 klx. The experiments were repeated at least 4 different times using a minimum of 20 culture flasks of each cultivar in each experiment.

Induction of shoot formation. Tissues of explants in MS media enlarged and thickened as described by Kukulczanka and Suczyńska (3). Shoot formation appeared first at the base of the veins touching the medium and, within 3 weeks, was visible on the entire adaxial area of the leaf. Although all explants were placed upright in the medium, only a few shoots were formed on the abaxial surface of the lamina, and these were primarily located around the main veins.

Maximum shoot induction was obtained in a modified MS medium containing 5.4 μM (0.1 mg/liter) NAA, 22 μM (5.0 mg/liter) BA, and 308 μM (125 mg/liter) adenine sulfate (Table 2). A higher concn resulted in callus. More than 22 μM BA produced fewer

Fig. 1. Typical examples of 5 month old African violet plants: A. A poorly-formed plant grown from an in vivo leaf cutting. B. A well-formed plant with a flat rosette of leaves grown from tissue culture.

Fig. 2. Scheme for the propagation of African violet plants from a section of leaf.
shoots with dark-green curled and twisted leaves; less BA resulted in some callus formation with progressively fewer shoots. In previous work with other plants, adenine sulfate has been found to act synergistically with kinetin and zeatin to enhance shoot formation (9, 10). In the present work, the addition of adenine sulfate to a medium containing BA also resulted in an increase of shoot initiation and enhanced vigor. However, in a basal medium with adenine sulfate alone (without growth regulators), shoot formation was delayed and preceded by the formation of roots.

In modified MS the growth rates of shoots > 5 mm were made after 6 weeks from 5 randomly selected cultures of each cultivar (Table 2). The most effective medium was 5.4 μM NAA, 22 μM BA and 308 μM adenine sulfate. On this formula the explants produced uniform unrooted shoots with leaf sizes 3–8 mm in length that could be subcultured in clumps of 7–13 onto the MM medium (Fig. 3). If the shoots were not subcultured from the explant after 6–7 weeks, 5 to 10 shoots became dominant and suppressed the growth of the others. Adventitious roots also developed on the petioles of plant leaves, although the base of each plantlet had no roots.

Table 2. Shoot formation after 6 weeks on MS medium with various ratios of NAA and BAP; with and without 308 μM (125 mg/liter) adenine sulfate.

<table>
<thead>
<tr>
<th>NAA + BAP (μM)</th>
<th>Ratio NAA:BAP</th>
<th>No. of shoots per explant²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No adenine sulfate</td>
</tr>
<tr>
<td>0.02 + 40.0</td>
<td>1:2000.0</td>
<td>1</td>
</tr>
<tr>
<td>0.02 + 30.0</td>
<td>1:1500.0</td>
<td>5</td>
</tr>
<tr>
<td>0.02 + 20.0</td>
<td>1:1000.0</td>
<td>15</td>
</tr>
<tr>
<td>0.02 + 10.0</td>
<td>1:500.0</td>
<td>22</td>
</tr>
<tr>
<td>0.02 + 8.0</td>
<td>1:400.0</td>
<td>20</td>
</tr>
<tr>
<td>0.02 + 4.0</td>
<td>1:200.0</td>
<td>10</td>
</tr>
<tr>
<td>5.4 + 31.0</td>
<td>1:1.5</td>
<td>40</td>
</tr>
<tr>
<td>5.4 + 26.0</td>
<td>1:4.8</td>
<td>50</td>
</tr>
<tr>
<td>5.4 + 22.0</td>
<td>1:4.0</td>
<td>60</td>
</tr>
<tr>
<td>5.4 + 17.0</td>
<td>1:3.1</td>
<td>40</td>
</tr>
<tr>
<td>5.4 + 13.0</td>
<td>1:2.4</td>
<td>30</td>
</tr>
<tr>
<td>5.4 + 4.0</td>
<td>1:0.74</td>
<td>20</td>
</tr>
</tbody>
</table>

²Overall average calculated from all cultures.

Stimulation of growth and induction of rooting. Shoots were subcultured to a modified MM medium when they developed into unrooted plantlets with a leaf height of about 5 mm; they were cut away in clumps of 7–13 from the explant tissue. Remaining attached together, they were placed flat on the MM medium.

Rooting of subcultured shoots on the MM medium, modified with 16 g/liter sucrose, did not occur until the plantlets had formed leaves 10 mm long × 7 mm wide. This took about 4 weeks. Tran Thanh Van et al. (11) found that low concn of sucrose gave better results for de novo root development in Nicotiana tabacum L. Results of our investigation showed that with 0–5 g/liter sucrose, all shoots died within 14 days; with 12–20 g/liter, normal development occurred; with 20–30 g/liter rooting was inhibited. The addition of growth regulators to the medium produced adverse effects: the auxins NAA and IBA stimulated adventitious root formation on the petioles and lamina of plants; the cytokinin, kinetin, stimulated the development of axillary shoots. Plant growth was also positively correlated with the volume of container used for the subculture medium (ratios of shoot number to jar volume were the same in different size jars but plants in the 240 ml jars grew much faster than those in the 120 ml size). After 6 weeks the rooted plants separated from the subcultured clumps and could easily be transferred to a soil mixture. Shoot initiation and growth response were similar in all the cultivars.

As the growing plantlets became mature, the shoots grew taller, and a new flush of leaves emerged from the petioles. The petioles became wider and the leaves became larger. This took about 4 weeks. Tran and co-workers (11) used callus cultures in their studies on Nicotiana tabacum L. Results of our investigation showed that with 0–5 g/liter sucrose, all shoots died within 14 days; with 12–20 g/liter, normal development occurred; with 20–30 g/liter rooting was inhibited.
crowded, those with leaves under 10 mm high were placed on fresh medium. Planted with leaves 10 mm high were placed in a damp artificial soil mix, then transferred to the greenhouse.

**Greenhouse techniques.** In the greenhouse, plants were rooted in 5.8 cm pots using 1 perlite:6 peat:4 sand, containing Mg(NH₄)PO₄ ("Magamp"), Fe²⁺ (fritted trace elements of boron, copper, iron, manganese, molybdenum and zinc), and Dolomite limestone. The potted plants were placed in containers of damp vermiculite under plastic tents. The shaded greenhouse was kept at 25°C on a 16-hr photoperiod that reached a maximum of 13 klx on the brightest days. At the end of 2 months, the well-rooted and rapidly growing plants were removed from the plastic tents and exposed to a light intensity of 18.4 klx to induce early flowering.

**Further enhancement of rate of propagation.** In an attempt to improve the rate of propagation, individual plants grown in the MM medium (after MS) were subcultured back onto a modified MS medium containing a ratio of 1.0 NAA:0.74 BA and lacking adenine sulfate. These plants developed into a compact spherical mass of shoots in 6 weeks. Theoretically, this method could produce a millionfold increase of plants per annum but variation in the age and size of the shoots made it difficult to separate them without damaging the entangled leaves and elongated brittle petioles. Separated individually, these shoots rooted when subcultured onto the MM medium but did not have the flat-leafed rosette form of growth. However, after 7-8 months of greenhouse cultivation, a gradual improvement could be observed as new young leaves flattened into a rosette.

**Conclusion.** Enhancement of clonal multiplication within a minimum space is an economically-important advantage of tissue culture techniques (6). For example, gerbera (5), gloxinia (2), and strawberry (1), can be increased a millionfold per annum by the induction of lateral and axillary shoot formation from shoot-tip explants. We have found that while providing a millionfold increase of African violet plants, the use of this shoot-tip method of propagation (described in the foregoing section), does not provide well-developed plants for the mutual constriction of developing shoots. The esthetically and commercially desirable form of the African violet is a flat rosette whorl of leaves on short petioles. The main method described here does provide the desirable growth form and a much greater rate of propagation than is obtained from conventional leaf cuttings. Against the requirements for a Master of Science degree in Horticulture.

---

**HortScience 11(3):206–208. 1976.**

**Opening of Immature Chrysanthemums with Sucrose and 8-Hydroxyquinoline Citrate**

Richard J. Gladon and George L. Staby

The Ohio Agricultural Research and Development Center, Wooster, OH 44691

Additional index words. Chrysanthemum morifolium, postharvest physiology, cut chrysanthemums, bud-opening solutions, flower opening

**Abstract.** Once-over harvested bud-cut chrysanthemums, *Chrysanthemum morifolium* Ramat., were opened in solutions of 8-hydroxyquinoline citrate (8-HQC) and/or sucrose. Flowers opened in solutions containing 2 or 4% sucrose and 200 ppm 8-HQC were comparable to or better than control flowers which matured in the greenhouse. Flower shape was influenced by the length of time in solution and solution constituents. Flowers harvested at a minimum of 50 mm diameter developed to 125 mm in 5 to 7 days while the greatest increase in inflorescence height took place between days 7 and 14. Flowers opened in solution did not have a longer vase life than control flowers allowed to develop in the greenhouse.

Harvesting flowers as buds and subsequently opening them has been reported for carnations (6) and was later extended to standard chrysanthemums (3, 8, 10). Harvesting of entire bunches at one time (once-over harvest) in the bud stage has also been shown feasible with standard chrysanthemums (1, 11). The solution (soil) used for opening bud-cut flowers generally contains sucrose, a carbohydrate source, and 8-HQC, an anti-microbial agent, with optimal concn ranging from 1 to 5% and 100 to 300 ppm, respectively (7, 10, 11, 12). Marouksy (12), Kofranek and Halevy (3), and Kofranek et al. (5) present conditions for harvesting and opening bud-cut chrysanthemums.

The advantages of once-over harvesting of bud-cut flowers, as opposed to present practices, are: 1) reduced total crop time, 2) no need for second and third harvests, 3) increased postharvest storage life, 4) fewer postharvest injuries, and 5) reduced no. of shipping containers (9). This research was designed to investigate the feasibility of harvesting entire bunches of standard chrysanthemums as buds on a year-round basis and subsequently opening them to an acceptable size and quality.

Four crops of "Southern Comfort" and 3 crops of "May Shoeshmith" were grown as single-stem standards according to recommended cultural practices for year-round production. Entire bunches of flowers were harvested (except for control flowers) when 30% of a random sample of 50 flowers in the bunch were 50 mm or greater in diam. Inflorescence diam and ht were measured with a vernier caliper. Immediately after harvest, stems were cut to 75 cm in length, the lower 37 cm of foliage removed, fresh wt recorded, and stems placed in opening soln. Flowers developed at temp of 19 to 32°C with 20 to 1.6 klx of continuous fluorescent light, depending on the experiment. Deionized water was used for all soln. Opening soln were held in glass jars to

---

1Received for publication August 25, 1975. Approved for publication as Journal Article No. 72-75 of The Ohio Agricultural Research and Development Center, Wooster, Ohio. Research supported in part by Yoder Brothers, Inc., Barberton, Ohio, and taken from a thesis by the senior author in partial fulfillment of the requirements for a Master of Science degree in Horticulture.

2Mailing Address: Department of Horticulture, The Ohio State University, 200 Fyffe Court, Columbus, Ohio 43210.