# MICROPROPAGATION OF CHIMERAL AFRICAN VIOLETS

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#### **ABSTRACT**

The pinwheel flowering African violets are periclinal chimeras. Plantlets produced from tissue cultured leaf explants do not flower true-to-type. When intact inflorescences were cultured in vitro, plantlets arose in the axils of small bracts on the peduncles. These plantlets flowered between 80% and 95% true-to-type depending on the cultivar under consideration. It is hypothesized that these plantlets result from the growth of dormant axillary buds in the inflorescence. This hypothesis would account for the ability to propagate the periclinal chimeras in a true-to-type fashion since the apical organization of axillary buds is identical to that of the apical meristem.

### INTRODUCTION

African violets which have bicolor flowers with a banded arrangement of the colors are termed "pinwheel flowering". The lateral edge of each corolla segment is a different color than the central portion, giving the whole flower a "spoked" appearance, with the "spokes" being one color and the "spaces between the spokes" a different color.



Figure 1. The pinwheel-flowering African violet cultivar 'Valencia' is characterized by corolla segments with methyl violet margins and a white center stripe.

This patterned arrangement of the flower is not maintained by plants propagated by leaf cuttings, but can be maintained if the terminal portion of the crown is removed and the resulting "suckers" are separated and rooted (1). This technique of propagation gives rise to few propagules per plant, necessitates using large, well-established plants for crown removal, and exposes the stock plants to potential disease problems. The cost of these chimeral plants is therefore very high compared to other African violet types which can be propagated by leaf cuttings.

During the course of experiments designed to separate the component genotypes of several cultivars of pinwheel flowering African violets, it was noted that some plants produced from inflorescence explants produced pinwheeling flowering plants (2). The procedure reported herein is a refinement of this technique suitable for the high fidelity production of chimeral African violets through tissue culture.

### MATERIALS AND METHODS

Whole inflorescences of the African violet cultivars 'Valencia", 'Dardevil', 'Desert Dawn', and 'Mauna Loa' served as tissue explants for these studies. Inflorescences were harvested several days prior to the opening of the first flower. Explants were washed in 0.1% Alconox for 5 to 10 min., disinfested in 0.5% sodium hypochlorite for 15 min., and rinsed twice in sterile distilled water. The peduncle was cut 5 to 10 mm below the attachment of the lowest flower buds and the whole inflorescence was placed in 25 x 150 mm test tubes containing 12.5 ml of tissue culture medium. The medium used contained the Murashige and Skoog salt formulation and organics (3), with 100 mg/l myo-inositol, 200 mg/l casein hydrolysate, 3% sucrose, 1 mg/l naphthaleneacetic acid, 1 mg/l benzyladenine, and 0.6% Difco Bacto agar (pH 5.7). Cultures were grown in a culture room providing 16 hr. per day of cool white fluorescent light (40 $\mu$  Einsteins/m2/sec).

The small plantlets which had formed by 5 weeks were removed from the peduncle and placed in plastic covered foil tins containing moistened Reddi Earth soilless medium (W.R. Grace Co., Cambridge, MA 02140) for rooting. Plantlets were well rooted within 3 to 4 weeks, at which time the plastic lids were loosened to allow the plants to acclimate to lower relative himidities. After approximately 2 to 3 weeks of acclimation, plants were potted into 8 cm plastic pots containing Metromix 350 soilless medium (W.R. Grace Co., Cambridge, MA 02140), placed on a capillary mat watering system in a shaded greenhouse (70% shade), and grown to flowering according to standard African violet culture. Plants were observed through at least one full flowering cycle to ascertain trueness-to-type.

### RESULTS AND DISCUSSION

Plants produced through in vitro culture of leaf tissue displayed a wide variety of flowering patterns, none of which was the characteristic pinwheel flower (Fig. 2A, compare to Figs. 2B-2L).

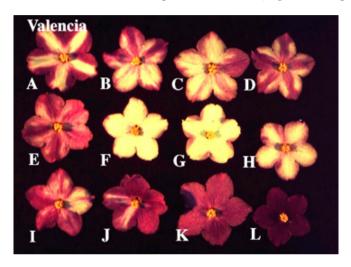


Figure 2. Various flower patterns produced on tissue cultured 'Valencia' African violets. A. 'Valencia', true flower type. B - J. Various unstable off-type flower patterns. K, L. Monochromatic flowers of the same color as the corolla segment margin.

Similar variation was observed in plants produced from 'Dardevil' leaf tissue (Table 1). Only one type of variant was produced by leaf culture of 'Desert Dawn' (Table 1). In general, the plants produced through culture of leaf tissue most often displayed monochromatic (solid color) flowers of the same color as the margin of the corolla segments. Some bicolor, irregular combinations of both colors were produced, but in these studies pinwheel flowering plants were never obtained from leaf tissue (Table 1).

Table 1. Flowering Pattern of Plants Produced by In Vitro Culture of Leaf Explants of Three Cultivars of Pinwheel Flowering African Violets.

		Plants with Stated Flowering Pattern				
Cultivar	Number of Plants Observed	Margin Color	Stripe Color	Bicolor	Pinwheel	
'Valencia'	82	67%	0	33%	0	
'Dardevil'	49	43%	35%	22%	0	
'Desert Dawn'	36	100%	0	0	0	

When whole inflorescences were placed in culture, plantlets grew from the axils of the bracts in a short time period (Fig. 3).

Figure 3. Plantlets produced in the bract axil of 'Valencia' after 5 weeks in vitro.

These plantlets were large enough to be removed for rooting at the end of 5 weeks.



Adventitious shoots which differentiated on leaf or peduncle tissue were just barely visible to the naked eye by 5 weeks, suggesting that these shoots arose from dormant vegetative buds in the inflorescence structure. Further evidence in support of this hypothesis was obtained when small plantlets were observed growing in the inflorescence of an intact 'Valencia" plant in the greenhouse.



Figure 4. Expanded vegetative plantlets produced on a flowering plant of 'Valencia' in the greenhouse.

The occasional production of true-to-type flowering plants from rooted inflorescences also has been reported (1).

Plants produced through short term culture of inflorescence tissue exhibit a high frequency of true-to-type flowering (Table 2). All of the 'Mauna Loa' plants regenerated through tissue culture were pinwheel flowering, while about 80% of the 'Dardevil" and 'Desert Dawn' plants flowered true-to-type. The multiplication rate varied with cultivar, with 'Valencia' achieving the highest multiplication rate (Table 2).

Table 2. Flowering Pattern of Plants Produced by Short Term Culture of Inflorescence Tissue.

		Plants with Stated Flowering Pattern					
Cultivar	Average No. of Plants per Explant After 5 Weeks	No. of Plants Observed	Same Color as Segment Margin	Bicolor	Pinwheel		
'Valencia'	9.0	236	1.5%	3%	95.5%		
'Dardevil'	3.2	62	8%	0	82%		
'Desert Dawn'	3.7	65	20%	0	79%		
'Mauna Loa'	2.3	42	0	0	100%		

These rates of multiplication appear low for a tissue culture system, but they are quite acceptable since: 1) the system has high fidelity, 2) the explant source (i.e., inflorescence) is produced in abundance on a mature plant, and 3) the taking of explants does not reduce the vigor of the stock plant.

It should be emphasized that the period of in vitro culture should not extend beyond 5 or 6 weeks. Adventitious shoots are produced on the peduncle in the vicinity of the plants believed to be produced from the axillary buds and these adventitious shoots would not be pinwheel flowering types. This phenomenon likely accounts for the observed variation in fidelity of the plants produced by the different cultivars. For example, the 'Desert Dawn' cultures may have been "contaminated" by adventitious shoots to a greater degree than the cultures of 'Valencia'.

The inflorescence culture technique should allow true-to-type propagation of other African violet cultivars which are periclinal chimeras. Plants are produced rapidly on the explants and these plants show excellent rooting and survival. Care must be taken, however, to determine the extent of variation in the tissue cultured plants, since trueness-to-type was cultivar dependent and varied between 80% and 100%.

### LITERATURE CITED

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