



***In Vitro* Propagation of African Violets**

The African violet, *Saintpaulia ionantha*, is propagated vegetatively from leaf cuttings and is grown on a large scale commercially and on a small scale by many home gardeners. When one or more shoots are allowed to develop on a cutting during vegetative propagation, constrictions are imposed by the multiplicity of plantlets in a limited growing space, resulting in unsymmetrical plants with elongated sideways-displaced petioles. Propagation by tissue culture overcomes this problem and results in a large number of well-formed, single-stemmed plants from a given amount of leaf tissue.

Bilkey *et al.* demonstrated the high regeneration capacity of African violets petiole tissue when optimal levels of growth hormones, particularly cytokinins, are present. They observed swelling of the petiole cross-section, especially around the circumference, and death of the central portion after the first week. Plantlet regeneration is usually noticeable within 6 weeks. Nearly 5000 commercially usable plants can be regenerated from a single petiole in 3-4 months.

Materials Required

1. 10 sterile Petri dishes, either plastic or glass (100 mm in diameter) (e.g., Prod. No. D940, D965, respectively)
2. 3 500-ml beakers and one 250-ml beaker
3. 3 pairs of forceps and 2 scalpels
4. Waterproof marking pen and labels
5. Glass Bead Sterilizer (Prod. No. S636)
6. 1000 ml of 10% chlorine bleach solution supplemented with a few drops of Tween-20 (Prod. No. P720)
7. 1000 ml of sterile distilled water
8. 150 ml of 70% ethanol
9. 70% isopropyl alcohol (rubbing alcohol)
10. Murashige African Violet/Gloxinia Multiplication Medium (Prod. No. M517) or MS (e.g., Prod. No. M519) medium with 0.1 mg/L NAA and 0.1—1.0 mg/L BA.
11. Medium-sized African violet leaves

Procedures

1. Wipe down all surfaces of the transfer hood 70% alcohol. Allow the hood to run for 15 min before beginning transfer operations. Place all the materials listed in the previous section under the hood. Place scalpels and forceps in a 250-ml beaker containing about 100 ml of 70% ethanol.
2. Select 12-15 medium-sized healthy leaves and cut the petiole near the point where it attaches to the stem. Rinse the leaves under running water then transfer the leaves to the 500-ml beakers. Place the beaker under the hood and pour the 10% bleach solution over the leaves, making certain all leaf surfaces are properly

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covered. Leave the leaves in the sterilization solution for 10 min and then pour off the solution. Rinse the leaves three times in sterile distilled water with each rinse lasting approximately 1 min.

3. Place the culture vessels containing the media under the hood. Label each rack with treatment, start date of experiment, and cultivar.
4. Transfer each sterilized leaf to a separate sterile petri dish and remove petiole with scalpel. Next, remove the outer edges of each leaf; section the remaining leaf blade. Once a leaf has been sectioned, weigh each isolated explant and then transfer one leaf section to each culture vessel so that the abaxial (underside) side touches the medium. The petiole can also be cut into 3-5 mm sections and cultured. When all culture tubes have been inoculated, place them in racks and incubate in low light at 25°C.

Scheduling :

Event

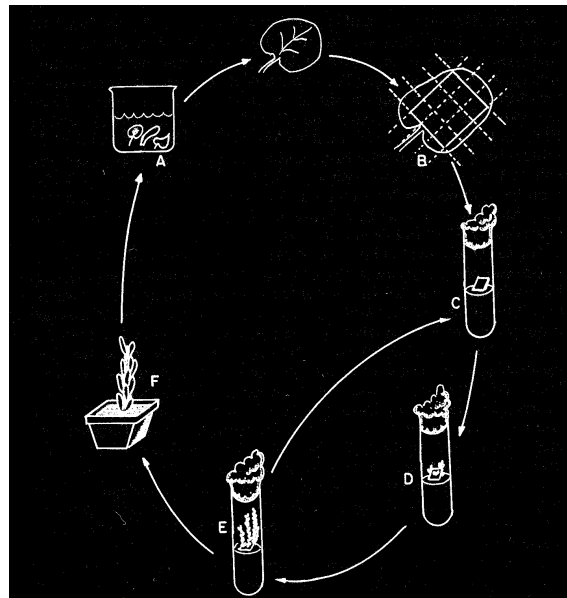
Isolation of fresh explants
First appearance of organogenesis
Noticeable shoot formation
First subculture

Timing

Day 0
ca. Day 14
ca. Day 30
ca. Day 60

Recording Results :

1. Record all details of setting up the experiment
2. Make visual observations at 14-day intervals
3. Determine fresh weight and shoot number after ca. 60 days.



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