# Chapter 22

# Micropropagation of African Violet (*Saintpaulia ionantha* Wendl.)

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## Abstract

Micropropagation is an important tool for rapid multiplication and the creation of genetic variability in African violets (*Saintpaulia ionantha* Wendl.). Successful in vitro propagation depends on the specific requirements and precise manipulation of various factors such as the type of explants used, physiological state of the mother plant, plant growth regulators in the culture medium, and growth conditions. Development of cost-effective protocols with a high rate of multiplication is a crucial requirement for commercial application of micropropagation. The current chapter describes an optimized protocol for micropropagation of African violets using leaf explants obtained from in vitro grown plants. In this process, plant regeneration occurs via both somatic embryogenesis and shoot organogenesis simultaneously in the explants induced with the growth regulator thidiazuron (TDZ; *N*-phenyl-*N*′-1,2,3-thidiazol-5-ylurea). The protocol is simple, rapid, and efficient for large-scale propagation of African violet and the dual routes of regeneration allow for multiple applications of the technology from simple clonal propagation to induction or selection of variants to the production of synthetic seeds.

**Key words:** African violet, *Saintpaulia ionantha*, Micropropagation, Somatic embryogenesis, Organogenesis, Regeneration, Root initiation, Thidiazuron

## 1. Introduction

African violet (*Saintpaulia ionantha* Wendl.; Gesneriaceae) is a commercially important indoor ornamental plant species highly valued in many parts of the world. Thousands of *Saintpaulia* cultivars have been selected for plant size, floral colors, leaf shapes and pattern, growth, uniform flowering, and better performance as a house plant. While these plants are propagated most commonly by vegetative leaf cuttings, in vitro methods are widely used both for large-scale production and the introduction of genetic variability for new cultivar development. Micropropagation of African violet

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from various types of explants, including leaf discs, petioles, petals, and anthers has been reported by various researchers (1–8). Regeneration of African violet has been achieved through direct differentiation of shoots from different explants (9–12) as well as an indirect mode of organogenesis with an intermediate callus phase (13, 14). African violet leaf and petiole tissues have been shown to regenerate via organogenesis and somatic embryogenesis (7) following induction with thidiazuron (TDZ). TDZ is a highly potent plant growth regulator (PGR) known to stimulate a number of different physiological responses in plants including de novo plant regeneration (15–22). The specific mode of action of TDZ in plant tissues remains undetermined and a dual role for the PGR as synthetic stimulator of both auxin and cytokinin metabolism continues to be proposed (8, 17, 23, 24).

In this chapter, we describe an efficient protocol for micropropagation of African violet based on the use of TDZ as an inductive signal of regeneration and axenic shoot cultures (ASC) as the source of the explants. Various stages of the development of the plantlets in this process include: (1) initiation of ASC, (2) micropropagation via simultaneous shoot organogenesis and somatic embryogenesis from the explants of ASC, (3) rooting and root growth of the regenerated shoots and somatic embryo-derived plantlets, and (4) acclimatization and greenhouse transplant of regenerated plantlets.

## 2. Materials

### *2.1. Surface Sterilization of Source Material*

1. Tap water.
2. Ethanol 70% (v:v).
3. Autoclaved distilled water; 250 mL aliquots in 500 mL screw capped bottles.
4. Commercial bleach solution (e.g., "CLOROX®" bleach; 5.5% (v/v) NaClO), diluted 2:10 (v:v) with autoclaved distilled water.
5. Tween 20 (Fisher BioReagents, USA).
6. Magnetic stirrer, magnetic bar, 600 mL beaker (autoclaved).
7. Instruments (scalpel, forceps, glass bead sterilizer), laminar flow bench.
8. Media preparation and tissue culture facilities, culture room.
9. Potted plants of African violet cv. Benjamin obtained from a commercial greenhouse (Harster Greenhouses Inc., Dundas, ON, Canada).

**Table 1**
**Murashige and Skoog (MS) basal medium with vitamins**

| Constituents | | Chemical formula | Concentration (mg/L) |
|---|---|---|---|
| Macroelements | Ammonium nitrate | $NH_4NO_3$ | 1,650 |
| | Potassium nitrate | $KNO_3$ | 1,900 |
| | Calcium chloride | $CaCl_2{\cdot}2H_2O$ | 332.2 |
| | Magnesium sulfate | $MgSO_4{\cdot}7H_2O$ | 180.7 |
| | Potassium phosphate | $KH_2PO_4$ | 170 |
| Microelements | Potassium iodide | KI | 0.83 |
| | Boric acid | $H_3BO_3$ | 6.2 |
| | Manganese sulfate | $MnSO_4{\cdot}4H_2O$ | 16.9 |
| | Zinc sulfate | $ZnSO_4{\cdot}7H_2O$ | 8.6 |
| | Molybdic acid (sodium salt) | $Na_2MoO_4{\cdot}2H_2O$ | 0.25 |
| | Cupric sulfate | $CuSO_4{\cdot}5H_2O$ | 0.025 |
| | Cobalt chloride | $CoCl_2{\cdot}6H_2O$ | 0.025 |
| Iron | Ferrous sulfate | $FeSO_4{\cdot}7H_2O$ | 27.80 |
| | $Na_2EDTA{\cdot}2H_2O$ | | 37.26 |
| Vitamins | Myo inositol | | 100 |
| | Glysine | | 2.0 |
| | Nicotinic acid | | 0.5 |
| | Pyridoxine-HCl | | 0.5 |
| | Thiamine-HCl | | 0.1 |

### *2.2. Culture Media*

1. Culture media contained salts and vitamins according to Murashige and Skoog (25) and PGRs viz. BA, NAA, and TDZ (Sigma-Aldrich Co., St Louis, MO, Difco, Detroit, MI and Phytotechnology, KS, USA).
2. The media formulation are listed in Tables 1 and 2 for
   (a) Establishment of in vitro culture from leaf and petiole explants.
   (b) Regeneration from ASC.
   (c) Root induction on regenerated shoots.
3. Petri dishes (100 × 15 mm; Phytotechnology, KS, USA).
4. Magenta boxes (3 × 3 × 4″; Phytotechnology, KS, USA).
5. Other glassware (Fisher Scientific, USA).

### *2.3. Acclimatization of Regenerated Plants in Greenhouse*

1. Tap water.
2. Plastic pots (6 in.; ITML Horticultural Products, Middlefield, OH, USA).

**Table 2**
**Preparation and storage of different plant growth regulators (PGR) used for in vitro propagation of African violet**

| Growth regulator | Molecular weight | Preparation and storage | | |
|---|---|---|---|---|
| | | Solvent | Diluents | Storage (°C) |
| BAP | 225.3 | 1 N NaOH | Water | 0–5 |
| NAA | 186.2 | 1 N NaOH | Water | 0–5 |
| TDZ | 220.2 | DMSO | Water | 0–5 |

3. Plastic trays with cover (72 cells; ITML Horticultural Products, Middlefield, OH, USA).
4. Soil-less mix prepared by combining ProMix™ and Perlite (Therm-O-Rock East, Inc., New Eagle, PA, USA) (1:1 by volume).
5. Sunshine® professional growing mix (Sun Gro Horticulture, Vancouver, BC, Canada).
6. Plant growth chamber.
7. Greenhouse.

## 3. Methods

### *3.1. Preparation of Culture Media*

The composition of basal medium containing the mineral components of MS medium supplemented with vitamins (25) is provided in Table 1. PGRs and the conditions of their storage are listed in Table 2. The medium is solidified with 2.5 g/L Gelrite (Sigma, USA) and the pH of the media adjusted to 5.7 before autoclaving at 121°C and 1.1 kg/cm$^2$ for 20 min. The volumes of the culture medium dispensed in each Petri dish and Magenta box are 15 mL and 50 mL, respectively.

### *3.2. Establishment and Maintenance of Source Material*

1. Maintain the plants of *S. ionantha* Wendl. cv. Benjamin in 6 in. pots filled with an artificial soil mix (Sunshine® professional growing mix) in the greenhouse. The temperature in the greenhouse should be within a range of 20–24°C with a 16/8 h photoperiod (day/night). The light intensity should be at 80–85 μmol/s/m$^2$ and the relative humidity at 55–60%. Select the healthy plants with fully expanded leaves for initiating micropropagation. Use the leaf disc or petiole explants from the green house-grown plants to generate ASC.
2. Prepare the disinfectant bleach solution by diluting the commercial bleach (20 mL of 5.4% sodium hypochlorite), with

80 mL sterile distilled water, and adding three drops of the wetting agent "Tween 20." Disinfect leaf tissue by placing 2–4 leaves in a 250 mL sterile beaker and rinse in 70% ethanol for 1 min. Decant ethanol and gently pour the diluted bleach solution on the leaves to submerge completely for surface sterilization. Stir the contents gently for 15–20 min. Rinse the leaves five times with sterile de-ionized distilled water to remove the bleach. Each rinse should be for a period of 3–4 min.

3. For preparing the leaf explants, cut the mature leaves (4–6 cm long) into rectangular discs, approximately 1.5 × 1 cm in size, using a scalpel. Remove the petiole explants and section them transversely (<0.25 mm thick) with a sharp scalpel. Keep the explants in sterile water prior to culture.
4. Culture the leaf disc and petiole explants on MS medium containing MS salts, vitamins, 3% sucrose and a growth regulator combination of 1 μM benzyladenin (BA), and 1 μM naphthalene acetic acid (NAA) or 5 μM TDZ (*N*-phenyl-*N*′-1,2,3-thidiazol-5-ylurea). Culture the leaf disc explants in test tubes (5 × 15 cm) or magenta boxes whereas the petiole explants in Petri dishes. Place the leaf explant with the abaxial surface in contact with the medium and ensure that the orientation of the petiole explants is such that the surface farthest from the leaf blade is exposed to the culture medium. Maintain the cultures in a growth room at 24°C under a 16 h photo period (50 μmol/s/$m^2$) provided by cool white fluorescent lamps.
5. De novo shoots and somatic embryos will develop within 3–4 weeks (Fig. 1a–d) and well-formed shoots after 6 weeks (Fig. 1e). Somatic embryos develop from the epidermal cells surrounding the petiole slices and are loosely attached to the maternal tissues by a suspensor of transparent cells and are easily removed with forceps (Fig. 1b, c). The explants cultured with cut surface closest to the leaf blade exposed to the medium may not form somatic embryos or produce a few embryos infrequently and with an arrested development.
6. Remove the shoots (Fig. 1d) with the help of scalpel and forceps and subculture in Magenta box containing MS basal medium (50 mL) with 3% sucrose. These ASC are maintained by subculturing on basal medium at 4-weeks interval (Fig. 1f).

### 3.3. Micropropagation

1. Regeneration of African violet via shoot organogenesis or somatic embryogenesis has been reported from various explants. The protocol described below uses the ASC as the primary source of explants for micropropagation. Remove fully expanded leaves from ASC and place in sterile Petri dish containing a few drops of sterile water to prevent desiccation. Cut the leaves into approximately 1 cm segments using a sharp scalpel. Culture excised leaves on the induction medium containing the


Fig. 1. Micropropagation of African violet. (**a**) Regeneration from leaf explants of greenhouse grown plants. (**b**) Development of somatic embryos from epidermal region of petiole explants. (**c**) A somatic embryo loosely attached to the explant. (**d**) Shoot and somatic embryos at various stages of growth and elongation. (**e**) Regenerating shoots from leaf disc explants. (**f**) An axenic shoot culture (ASC) with well-formed leaves. (**g**) A mature in vitro grown plant with well-formed flowers. (**h**) Greenhouse transplant of regenerated plantlets. (**i**) A variegated chimera of African violet originating from regenerated plantlets.

ingredients of MS medium (Table 1) and various concentrations of TDZ (Table 3).

2. Culture five explants in each Petri dish containing 15 mL medium. Ensure that the abaxial surface of the leaf is in contact with the culture medium. Seal the Petri dishes with Parafilm and place the cultures under the same growth conditions as described earlier for ASC.
3. Frequency of regeneration is affected by the type and duration of exposure of explants to the induction medium. Both the concentration of TDZ and the duration of exposure must be optimized for each cultivar or genotype. Select the optimum

**Table 3**
**Effect of the concentration of TDZ and the duration of exposure (3 or 9 days) on average number of regenerants per explant**

| | Number of regenerants per explant | |
|---|---|---|
| TDZ concentration (μM) | 3 Days | 9 Days |
| 0.0 | 0 | 0 |
| 1.0 | 6–10 | 8–12 |
| 2.0 | 19–26 | 20–23 |
| 5.0 | 16–21 | 15–18 |
| 10.0 | 15–21 | 10–18 |

concentration of TDZ for inducing de novo development of shoots and somatic embryos (Table 3). For the cv. Benjamin, the optimum concentration of TDZ is 1–5 μM with an exposure period of 9 days to produce a significantly higher number of regenerants (shoots and embryo) compared to other concentrations (Table 3). Concentrations of TDZ higher than 5 μM increases the number of somatic embryos compared to shoots although the total number of regeneration remains similar.

4. Transfer the explants induced on TDZ for 9 days onto the MS basal medium without growth regulators in Magenta boxes each containing 50 mL medium for 2–3 regenerating explants.
5. Multiple shoots and somatic embryos develop after 3–5 weeks on the explants induced with 5 μM TDZ in a similar manner observed for the greenhouse-grown petioles (Fig. 1a–e). The somatic embryos are loosely attached to the source tissue and the shoot can also be easily excised for further development into plantlets. Carefully remove regenerating shoots and germinated embryos and transfer on to MS basal medium for further expansion and growth into well-formed shoots in about 4–5 weeks. The stages of shoot regeneration and somatic embryo development resemble those shown in Fig. 1a–e. Various steps of African violet micropropagation are shown in Fig. 2.

### 3.4. Rooting and Plantlet Development

Separate the shoots and germinated somatic embryos with or without visible roots. Transfer these shoots (approximately 4.0 cm long) to MS medium supplemented with 0.60 μM NAA. A well-formed root system will develop after 1–2 weeks and these plantlets are easily grown to mature flowering plants (Fig. 1g) following transplant in the greenhouse.

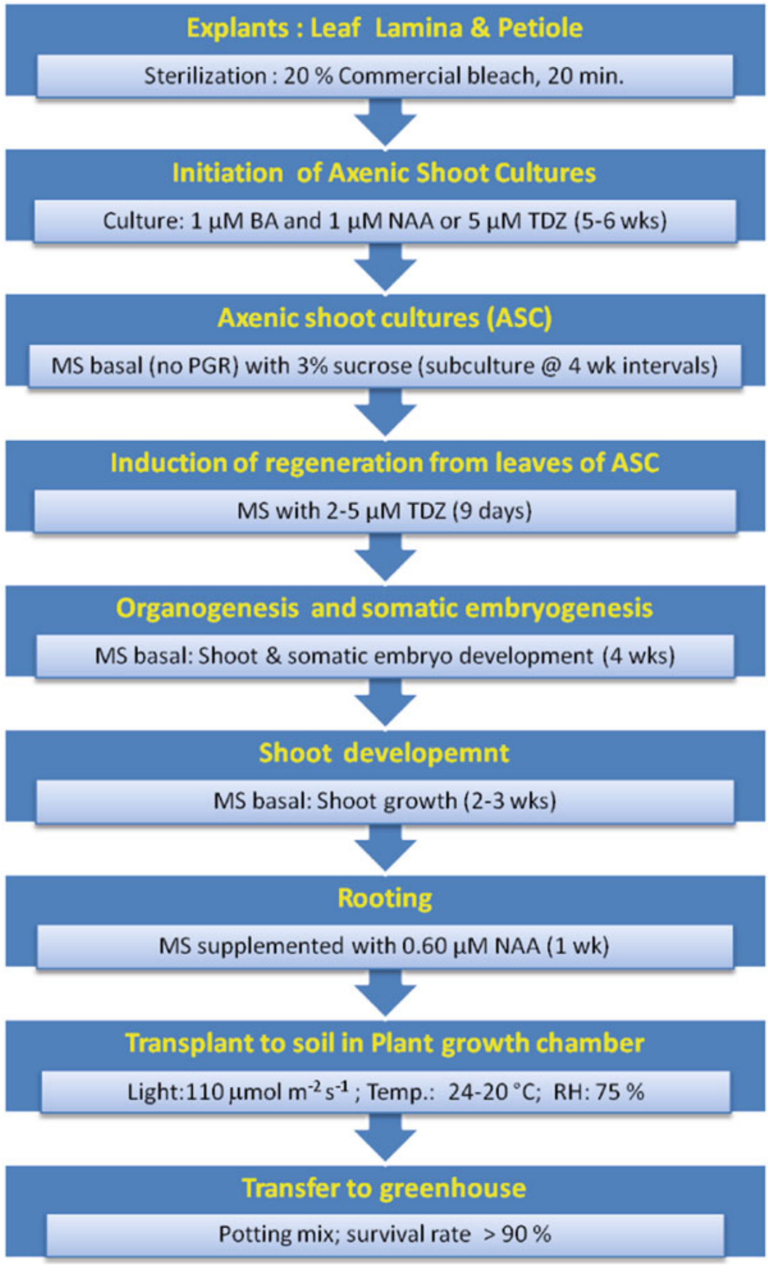


Fig. 2. Protocol for micropropagation of African violet (*Saintpaulia ionantha* Wendl.).

### *3.5. Acclimatization and Growth in the Greenhouse*

1. Acclimatize the regenerated, rooted plantlets before transfer to the greenhouse. Gently remove the plantlets from the culture medium and carefully wash with running tap water ensuring minimum damage to the tissue. Transplant the clean plants into 72 cell trays containing a soil-less mix prepared by combining ProMix™ and perlite in equal volumes. Cover the trays

with transparent plastic covers and place in the growth chambers set at 16 h light (24°C) and 8 h dark (20°C) cycle and 95% relative humidity. Reduce the relative humidity in the chambers every week by 5% for 3 weeks and thereafter maintain consistently around 80%. Remove the plastic covers at the end of the third week.

2 Transfer the flats to the greenhouse at the end of the fourth week and transplant into 6-in. standard pots filled with Sunshine® professional growing mix (Fig. 1h). Grow the potted plants under the typical greenhouse conditions to evaluate their growth performance and flowerings. Occasionally, spontaneous variants such as chimeras with variegated leaves (Fig. 1i) are seen in regenerated progeny, although at a low frequency.

## 4. Notes

1. For micropropagation of African violet, the most commonly used explants are leaf discs and petioles. The ASC are a better source of explants due to their consistent availability, physiological uniformity, and preconditioning in vitro. In addition, the use of ASC eliminates the need of surface sterilization. However, the process of establishing ASC can also be effectively used for direct micropropagation from the greenhouse-grown plants, and presumably from the plants grown in natural environments. Both the leaf discs as well as the petioles can be used as explants, but the frequency of regeneration would vary from one cultivar to another. Regeneration efficiency of leaf and petiole explants from in vitro and the greenhouse-grown plants also varies (7, 8) and requires optimization of PGR concentrations.
2. This micropropagation protocol of African violet involves the induction of shoot organogenesis and somatic embryogenesis on the same explant. Somatic embryos develop frequently at higher concentrations (5–10 μM) compared to shoots at low concentrations (1–2 μM) of TDZ. Somatic embryogenesis is unique characteristic of plant cells for obtaining a large number of genetically similar plants in a short time. The somatic embryo production in African violet is of commercial importance as this is a high-value crop with potential problems of somaclonal variation and chimeric plants. Additionally, regeneration via somatic embryogenesis is advantageous for genetic manipulation and propagation of this species due to the single cell origin and bipolar growth habit of the embryos. Somatic embryogenesis also facilitates encapsulation of embryos with synthetic gels to develop artificial seeds and cryopreservation

for long-term germplasm conservation. Regardless of the nature of regeneration, the micropropagation protocol described here can be further adapted for large-scale propagation using bioreactors.

3. TDZ is a unique and highly potent growth regulator which can be substituted for both the auxin and cytokinin requirements of organogenesis and somatic embryogenesis in several species (7, 8, 17, 26). The TDZ-induced somatic embryogenesis of African violet will also provide a system for the investigation of the biochemical and molecular factors controlling the development of somatic embryos including the transport of interacting endogenous and exogenous compounds.

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