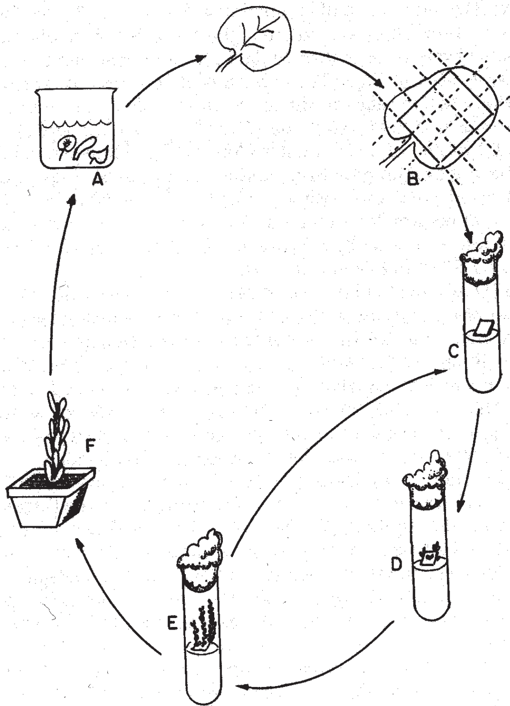
AFRICAN VIOLET MULTIPLICATION KIT

Product No. A137



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Kit Components

Product Number	Product Description	1 EA
	Box	1
	Instruction Manual	1
C913/C215-10ea	Culture Container	1
F951-1ea	Forceps, 8”	2
S963-1ea	Scalpel Handle, No. 3	1
S971	Scalpel Blades	2
P334-1 Roll	pH Strips, 4.5-7.5	1
D940-10ea	Petri Dishes	1
V886	Vinegar (100 mL)	1
S803-100g	Sodium Bicarbonate (Baking Soda)	1
P068	Pipet Plastic Transfer	2
M401-1L	Murashige & Skoog Modified Basal Medium (w/ BA)	6
M508-1L	Murashige Fern Multiplication Basal Medium	6
S391-500g	Sucrose	1
A296-9g	Agar	12

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Materials Required But Not Provided

1. Beakers/containers: three 500-ml and one 250-ml
2. Media preparation container.
3. 10% chlorine bleach solution supplemented with a few drops of Tween-20 (Product No. 720)
4. 1000 ml of sterile distilled water (Product No. W783)
5. 150 ml of 95% ethanol
6. 70% Isopropyl alcohol
7. Bunsen or alcohol burner (Product No. B966 or B876, respectively)
8. African violet leaves

Introduction

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 asymmetrical 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 small amount of leaf tissue.

Bilkey *et al*. (1978) 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.

This kit provides the necessary materials to initiate cultures from the petioles of African violet leaves.

Media Preparation

Powdered media are extremely hygroscopic and must be protected from atmospheric moisture. If possible the entire contents of each package should be used immediately after opening. Media stored at 2-6° C and tightly sealed should last 2-3 years. Preparing the medium in a concentrated form is not recommended as some salts in the medium may affect shelf life and storage conditions. The basic steps for preparing the culture medium are listed below:

1. Measure out approximately 90% of the desired final volume of tissue culture grade water, e.g. 900 ml for a final volume of 1000 ml. Select a container twice the size of the final volume.
2. While stirring the water add the powdered medium and stir until completely dissolved.
3. Rinse the container that the medium was packaged in with a small volume of tissue culture grade water to remove traces of the powder. Add to the solution in Step 2.
4. Add agar while stirring; It will not dissolve but should disperse into a uniform suspension.
5. Add 6-9 g/L of agar to all media. Add 30 g/L sucrose to M508; it is already contained in M401. Add any additionally desired heat stable supplements, such as PPM (Product No. P820) at 2 mL/L of media. Aside from these supplements, the media provided in this kit are complete and typically do not require other supplements.
6. Add additional tissue culture grade water to bring the medium to the final volume.
7. While stirring, determine the pH using the pH Strips (Product No. P334). If necessary, adjust the medium to the desired pH using the baking soda to raise the pH or vinegar to lower the pH. A pH of 5.6 to 5.8 is typically recommended for most plants, including African violets. Alternatively, the pH can be adjusted by using dilute potassium hydroxide or sodium hydroxide solution to raise the pH and dilute hydrochloric (muratic) acid to lower the pH of the medium.
8. While stirring, heat the solution to nearly boiling to melt the agar in the medium.
9. Dispense the medium into the culture vessels before or after autoclaving as indicated below:
   The Petri dishes (Product No. D940) included in this kit are sterile and <u>cannot</u> be autoclaved. They will melt if heated in an autoclave (or pressure cooker). Medium to be dispensed in Petri dishes must be sterilized and partially cooled before pouring it in the dishes.
   The culture vessels (Product No. C913/C215) are autoclavable. Media should be dispensed in these vessels prior to sterilization in an autoclave or pressure cooker. The lids of these culture vessels C093/C215 should not be tightly sealed during sterilization to allow for proper steam and pressure penetration.
10. Sterilize the medium in a validated autoclave or pressure cooker at 1 kg/cm^2, 121° C (15 psi, 250° F), for the time period described under "Sterilization of Media" below.
11. Allow medium to cool prior to use.

Sterilization of Media

Plant tissue culture media are generally sterilized by autoclaving at 121°C and 1.05 kg/cm^2 (15 psi). This high temperature not only kills bacteria and fungi, but also their heat-resistant spores. Media can be sterilized in either an autoclave or pressure cooker with similar results. Recently, the use of the microwave has also been shown to be successful at sterilizing media. The time required for sterilization depends upon the volume of medium in the vessel. The minimum times required for sterilization of different media volumes are listed below. It is advisable to dispense medium in small aliquots whenever possible as many media components are broken down by prolonged exposure to heat. Times for sterilizing in a microwave are based on using a 1000-watt microwave with a turntable for more even distribution of heat. The times required for sterilization may vary depending upon the model of the microwave, power wattage, and the number of vessels in the microwave.

Media Sterilization Time

Volume of Medium per Vessel (mL)	Minimum Autoclaving[a] Time (min.)	Minimum Microwaving[b] Time (min.)
25	15-20	4-6
50	25	6-8
100	28	8-10
250	31	10-12
1000	40	NR
2000	48	NR
4000	63	NR

[a] Minimum Autoclaving Time includes the time required for the liquid volume to reach the sterilizing temperature (121° C) and remain at this temperature for 15 minutes (Burger, 1988). Times may vary due to differences in autoclaves. Validation with your autoclave or pressure cooker is recommended.
[b] Minimum Microwaving Time includes the time required for the liquid volume to reach a temperature of 121° C and remain at this temperature for a period of 3-4 minutes. Media used in this study contained 1.0 mL/L of PPM. Validation with your microwave is recommended. NR = Not Recommended

Culture Procedure

1. Wipe down all surfaces of the transfer hood or work area with 70% isopropyl alcohol. If using a hood, allow it to run for 15 min before beginning transfer operations. Place all the materials listed in the previous sections the hood/work area. Place scalpels and forceps in a 250-ml beaker containing about 150 ml of 95% ethanol.
2. Select 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-Tween solution over the leaves, making certain all leaf surfaces are properly 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 in the hood/work area.
4. All tools which now contact the tissue should be sterilized in alcohol and then flamed to remove any alcohol.
5. Transfer each sterilized leaf to a separate sterile Petri dish and remove the petiole with a scalpel. Next, remove the outer edge of each leaf. Section the remaining leaf area and petiole into pieces about 6-12 mm (1/4”-1/2”) wide. After sectioning, transfer one to four leaf or petiole sections to each culture vessel so that the abaxial (underside) side or the leaf or cut surface of the petiole touches the medium. Once all cultures have been completed, place them in low light (e.g., fluorescent light) at 25º C.
6. Once shoots have developed they can be subcultured (individually transferred) onto fresh medium for continued multiplication or removed and planted in potting soil.

Schedule

Event	Timing
Isolation of fresh explants	Day 0
First appearance of shoots (organogenesis)	Day 14 (approximate)
Noticeable shoot formation	Day 30 (approximate)
First subculture	Day 60 (approximate)
Transfer to soil	Day 60+ (When plantlets are large enough to handle)

Literature Cited

Bilkey, PC, McCown, BM, and Hildebrandt, AC. 1978. Micropropagation of African violet from petiole cross-sections. HortScience 13(1): 37-38.

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rev. 6/08