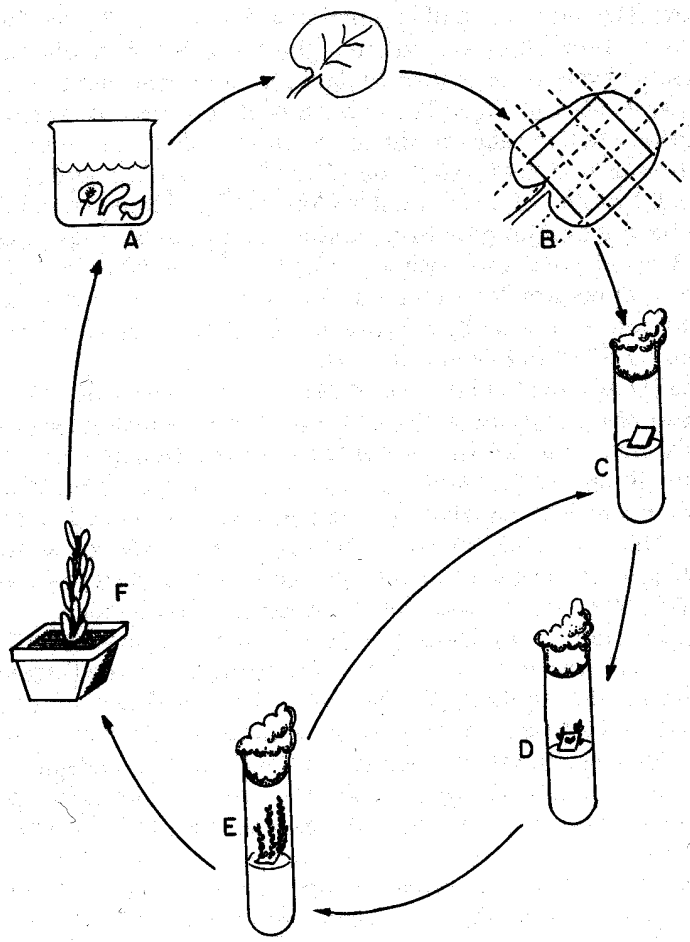
AFRICAN VIOLET MULTIPLICATION KIT

Product No. A137



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TABLES OF CONTENTS

KIT COMPONENTS

Product No.	Product Description	1 Each
	Box	1
	Instruction Manual	1
C215 – 10 ea	Culture Container	1
F951 – 1 ea	Forceps, 8"	2
S963 – 1 ea	Scalpel Handle, No. 3	1
S971	Scalpel Blades, No 11	2
P334 – 1 roll	pH Strips, 4.5 – 7.5	1
D940 – 20 ea	Petri Dishes	1
V886 – 15mL	Vinegar	1
S803 – 25 g	Sodium Bicarbonate (Baking Soda)	1
P068	Pipette Plastic Transfer	2
M401 – 1L	Murashige & Skoog Modified Basal Medium (w/ BA)	3
M508 – 1L	Murashige Modified Fern Multiplication Basal Medium	3
M517 – 1L	Murashige African Violet/Gloxinia Multiplication Medium	3
M518 – 1L	Murashige African Violet/Gloxinia Pretransplant Medium	3
S391-500g	Sucrose	1
A296-9g	Agar	12

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. P720)
4. 1000 mL of sterile distilled water (Product No. W783)
5. Tissue culture water (distilled or deionized)
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 plant growth regulators, 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 leaf in 3-4 months.

This kit provides the necessary materials to initiate cultures from the leaf blades and 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 precipitate. 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, M517 and M518; it is already contained in M401. Add any additionally desired heat stable supplements to media.
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. 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. Dispense the medium into the culture vessels before or after autoclaving as indicated below:
    a. The Petri dishes (Product No. D940) included in this kit are sterile and cannot 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.
    b. The culture vessels (Product No. C215) are autoclavable. Media should be dispensed in these vessels prior to sterilization in an autoclave or pressure cooker. The lids of culture vessels C215 should not be tightly sealed during sterilization to allow for proper steam and pressure penetration.
9. Sterilize the medium in a validated autoclave or pressure cooker at 1kg/cm2, 121 °C (15 psi, 250 °F), for the time period described under "Sterilization of Media" below.
10. 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. 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.

MEDIA STERILIZATION TIMES

Volume of Medium per Vessel (mL)	Minimum Autoclaving Time (min.)
25	15-20
50	25
100	28
250	31
1000	40
2000	48
4000	63

Please Note: Minimum Autoclaving Time includes the time required for the liquid volume to reach the sterilizing temperature (121 °C) and 15 minutes at 121 °C (Burger, 1988). Times may vary due to differences in autoclaves. Validation with your autoclave or pressure cooker is recommended.

CULTURE PROCEDURES

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 70% isopropyl alcohol.
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 25 ^{o}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.

APPROXIMATE 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.

Burger, D.W. 1988. Guidelines for autoclaving liquid media used in plant tissue culture. HortScience 23:1066-1068.

MEDIA FORMULATIONS

All components express in mg/L	Murashige & Skoog Modified Basal Medium (w/ BA)	Murashige Fern Multiplication Medium	Murashige African Violet/ Gloxina Multiplication Medium	Murashige African Violet/ Gloxina Pretransplant Medium
COMPONENT	**M401**	**M508**	**M517**	**M518**
Ammonium Nitrate	1650	1650	1650	1650
Boric Acid	6.2	6.2	6.2	6.2
Calcium Chloride, Anhydrous	332.2	333	333	333
Cobalt Chloride□6H_2o	0.025	0.025	0.025	0.025
Cupric Sulfate·5H_2O	0.025	0.025	0.025	0.025
Na2 EDTA	37.26			
Ferric Sodium EDTA		36.7	36.7	36.7
Ferrous Sulfate·7H_2O	27.8			
Magnesium Sulfate	180.7	181	181	181
Manganese Sulfate·H2O	16.9	16.9	16.9	16.9
Molybdic Acid (Sodium Salt)·2H2O	0.25	0.25	0.25	0.25
Potassium Iodide	0.83	0.83	0.83	0.83
Potassium Nitrate	1900	1900	1900	1900
Potassium Phosphate, Monobasic	170	170	170	170
Sodium Phosphate Monobasic		255	170	
Zinc Sulfate·7H2O	8.6	8.6	8.6	8.6
Adenine Hemisulfate			80	
6-Benzylaminopurine (BA)	1			
Indole-3-butyric Acid			2	
Indole-3-acetic Acid				1
Glycine (Free Base)	2			
MES (Free Acid)			4	
Kinetin		2		
myo-Inositol	100	100	100	100
a-Naphthaleneacetic Acid	0.1	0.1		
Nicotinic Acid (Free Acid)	0.5			
Pyridoxine·HCI	0.5			
Sucrose	30000			
Thiamine·HCI	0.4	0.4	0.4	0.4
Grams of powder to prepare 1 liter	34.44	4.66	4.66	4.40

STOCK SOLUTION AND MEDIA PREPARATION LOG

Product Number: _____ Medium: _____

Lot Number: _____ Prepared By/ Date: _____

Volume to Prepare: _____ Autoclave Sterilization Time: _____

pH Desired: _____ Actual Final pH: _____

Instructions: Complete the table with all components of the stock solution or medium to be prepared, including the product number, lot number, and grams/batch. As each component is weighed record the actual weight on the sheet. Check off each component after it is added to the solution/medium.

Component	Product Number	Lot Number	Grams/ Batch	Actual Weight	Added ☑
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐

Instructions/ Comments:

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_____

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Species/Tissue Cultured: _____

NOTES

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