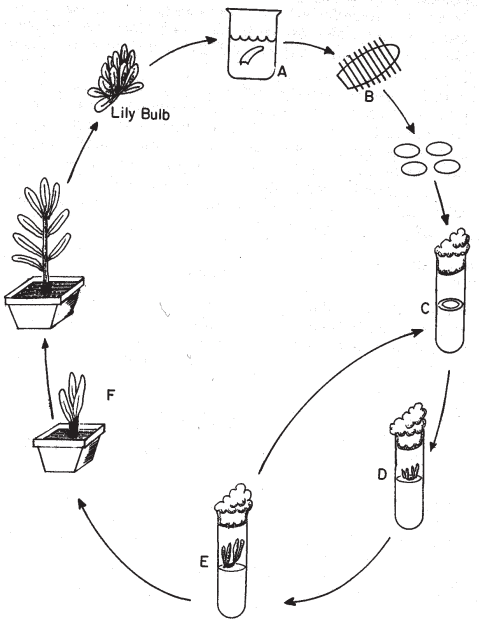
LILY MULTIPLICATION KIT

Product No. L577



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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)	4
M555-1L	Murashige & Skoog Modified Multiplication Medium (w/ Kinetin)	4
M508-1L	Murashige Fern Multiplication Medium	4
S391-500g	Sucrose	1
A296-9g	Agar	12
	Lily Bulb (NOT included in kits sent outside the continental USA)	1

Materials Required But Not Provided

1. Beakers/containers: three 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)

Introduction

Hussey studied the effect of the cytokinin 6-benzylaminopurine (BA) on the release of axillary buds of *Lilium pyrenacium Govan* and an Asiatic hybrid using adventitious buds from stems as the explant. Optimal production of buds (i.e., 1 to 5 laterals) was promoted by BA concentrations of 2.0-8.0 mg/liter on a Murashige and Skoog (MS) medium. Hussey later found that shoot proliferation in *L. longiflorum* and *L. pyrenacium* was promoted by the use of a cytokinin plus an auxin when pieces of leaves were used as the explant source. Stem sections cultured on a MS medium supplemented with 2.0 mg/liter Indole-3-acetic acid (IAA) and 0.5 mg/liter BA also produced approximately 10 bulblets per section.

Steinberg et al. obtained optimal bulblet formation from leaf blades of *L. longiflorum* 'Nellie White' when cultured on a MS medium supplemented with 10.0 mg/liter Naphthaleneacetic acid (NAA) and 1.0 mg/liter kinetin. This combination, however, was the least effective in promoting shoot formation. Initiation of one to three bulbs occurred at the base of each leaf.

The purpose of this kit is to demonstrate vegetative propagation and the effects of the position that an explant is taken from bulb and the role it may play in shoot multiplication. This kit will also examine the effects of vitamins on shoot proliferation.

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 of sucrose to M508; it is already contained in M401 and M555. Add any additionally desired heat stable supplements. 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. P959). 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 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.
   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. 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 95% ethanol.

2. Remove outer layer of damaged scales from the lily bulbs and discard. Under running water, begin removing the rest of the scales making certain to remove all soil; then place the scales in a 250-ml beaker containing tap water. Remove the scales down to the point where they become quite narrow and small, then discard the rest of the bulb. Place the nylon mesh over the beaker and place under running tap water so that the water gently agitates the scales. Adding a small amount of detergent may be helpful in the cleaning process. Allow the scales to rinse in this manner for 5-10 min. Once the scales have been washed thoroughly, pour off all water, remove the nylon mesh, and transfer the beaker of scales to the laminar flow hood.
3. Pour enough 95% ethanol over the scales to cover them, swirl for 3-4 min, and decant the ethanol. Next pour the bleach solution over the scales and sterilize for 15 min before decanting off the solution. Rinse the scales three times in sterile distilled water with each rinse lasting approximately 1 min.
4. Place the culture vessels containing the media in the hood/work area.
5. All tools which now contact the tissue should be sterilized in alcohol and then flamed to remove any alcohol.
6. Transfer several sterilized scales to a sterile Petri dish. Section the scales into pieces about 4 mm wide. Note whether each section comes from the base or tip of the scale. After sectioning, transfer several sections to each culture vessel. Note whether each section comes from the base or tip of the scale. Place sections from both the tip and base on the medium. Once all cultures have been completed, place them in low light (e.g., fluorescent light) at 25º C.
7. 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 nodules (organogenesis)	Day 14 (approx.)
Noticeable bulblet formation	Day 30 (approx.)
First subculture	Day 60-90 (approx.)
Transfer to soil	Day 90+ (When plantlets are large enough to handle)

Media Formulations

All components expressed in mg/L	Murashige & Skoog Modified Basal Medium (w/ BA)	Murashige & Skoog Modified Multiplication Medium (w/ Kinetin)	Murashige Fern Multiplication Medium
COMPONENT	**M401**	**M555**	**M508**
Ammonium Nitrate	1650	1650	1650
Boric Acid	6.2	6.2	6.2
Calcium Chloride, Anhydrous	332.2	332.2	333
Cobalt Chloride•6H_2o	0.025	0.025	0.025
Cupric Sulfate•5H_2O	0.025	0.025	0.025
Na2 EDTA	37.26	37.26	
Ferric Sodium EDTA			36.7
Ferrous Sulfate•7H_2O	27.8	27.6	
Magnesium Sulfate	180.7	180.7	181
Manganese Sulfate•H_2O	16.9	16.9	16.9
Molybdic Acid (Sodium Salt)•2H_2O	0.25	0.25	0.25
Potassium Iodide	0.83	0.83	0.83
Potassium Nitrate	1900	1900	1900
Potassium Phosphate, Monobasic	170	170	170
Sodium Phosphate Monobasic		148	255
Zinc Sulfate•7H_2O	8.6	8.6	8.6
Adenine Hemisulfate		80	
6-Benzylaminopurine (BA)	1		
Glycine (Free Base)	2		
Kinetin		1	2
myo-Inositol	100	100	100
α-Naphthaleneacetic Acid	0.1	0.1	0.1
Nicotinic Acid (Free Acid)	0.5		
Pyridoxine•HCl	0.5		
Sucrose	30000	30000	
Thiamine•HCl	0.4	0.4	0.4
Grams of powder to prepare 1 liter	34.44	34.66	4.66