PhytoTechnology
LABORATORIES, L.L.C.

TERRESTRIAL ORCHID SEED SOWING KIT

Product No. O788

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Table of Contents

Kit Components

Product Number	Product Description	Quantity
	Box	1
	Instruction Manual	1
C093/C215-10ea	Culture Container	1
F951-1ea	Forceps, 8”	1
S963-1ea	Scalpel Handle, No. 3	1
S971	Scalpel Blades	2
S088-1ea	Spatula/Scoop	1
P334-1 Roll	pH Strips, 4.5 - 7.5	1
D940-10ea	Petri Dishes	1
V886	Vinegar (15 mL)	1
S803-25g	Baking Soda	1
P068	Pipet, Plastic Transfer	2
K425-1L	Knudson Modified Plus Replate Medium	2
T849-1L	Terrestrial Orchid Medium	2
T839-1L	Terrestrial Orchid Medium	2
B141-1L	BM-1 Orchid medium	2
B142-1L	BM-2 Orchid Replate Medium	2
M551-1L	Malmgren’s Modified Orchid Medium	2

Materials Required But Not Provided

1. Beakers/containers: e.g., 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. 150 ml of 95% ethanol
6. 70% isopropyl alcohol
7. Bunsen or alcohol burner (Product No. B966 or B876, respectively)
8. Other items as required for individual applications

Introduction

*Phyto*Technology Laboratories' Terrestrial Orchid Seed Sowing Kit is designed to allow the user the flexibility of sowing a variety of terrestrial orchid species and hybrids. Included are seed sowing media (B141, M551, T839, T849) replate media (B141, M551, K425, T839, T849), and a multiplication/replate medium (B142) which enables the user to evaluate different media with the goal of determining the best ones for their species or hybrid of interest.

Because of the variety of orchids cultured, media are frequently supplemented with different additives (e.g., fruit extracts, organic compounds, and inorganic salts) to optimize it for specific species. These additives can alter the pH of the medium. Typically, orchid media is adjusted to a pH of 5.0 to 5.6. This kit contains pH indicator strips to evaluate the pH of the media, and baking soda (sodium bicarbonate) and vinegar to raise or lower the pH, respectively, to the desired value.

Seed Sterilization

Orchid seeds are very small and contain very little or no food reserves. A single seed capsule may contain 1,500 to 3,000,000 seeds. Sowing the seed in vitro makes it possible to germinate immature seed (green pods). It is much easier to sterilize the green capsule than individual seed after the capsule has split open. Lucke (1971) indicated that orchid seed can be sterilized when the capsule is about two-thirds ripe. Listed below is the estimated normal ripening times of capsules for various orchid species (Lucke, 1971).

ORCHID GERNERA	TIME TO MATURITY (MONTHS)	ORCHID GENERA	TIME TO MATURITY (MONTHS)
Bulbophyllum	3	Laelia	9
Calanthe	4	Masdevallia	3.5
Cattleya	11	Miltonia	9
Coelogyne	13	Odontoglossum	7
Cymbidium	10	Paphiopedilum	10
Cppripedium	3.5	Phalaenopsis	6
Dendrobium	12	Stanhopea	7
Epidendrum	3.5	Vanda	20

Green Capsule Sterilization

1. Soak the green seed capsule in 100% bleach solution for 30 minutes.
2. Dip the capsule in isopropyl alcohol or ethanol for 5-10 seconds. Remove the capsule from the alcohol and carefully flame off the excess alcohol.
3. Under aseptic conditions, using a sterile knife or scalpel, open the capsule and scrape out the seeds.
4. Carefully layer the seeds over the surface of the seed sowing medium (Product No. B141, M551, T839, T849). Seal the Petri plates or containers.

Dry Seed Sterilization

1. Collect seed and place in a small flask, bottle or a shortened pipet, which has one end sealed with cotton. Seal the other end of the pipet with cotton once the seeds have been inserted.
2. Prepare a solution containing 5-10% commercial bleach containing a few drops of Tween 20 (Product No. P720)
3. Add the bleach solution to the flask or draw up the solution into the pipet. Swirl the flask containing the seeds and bleach or repeatedly draw and aspirate the bleach solution in and out of the pipet.
4. Sterilize the seeds in the manner described in Step 3 for 5-10 minutes.
5. Remove the bleach solution and rinse the seeds with sterile tissue culture grade water.
6. Transfer the seeds to sterile seed sowing medium (Product No. B141, M551, T839, T849). Seal the Petri plates or containers.

Replating Seedlings

1. It may take anywhere from 1 to 9 months for the seed to begin to germinate. Approximately 30 to 60 days after germination begins, it will be necessary to transfer the seedlings to fresh media for continued growth.
2. Prepare orchid maintenance/multiplication/replate medium as desired (Product No. B141, B142, M551, T839, T849).
3. Under aseptic conditions, transfer the seedling from the Petri dishes to the containers containing the fresh replate medium. Seedlings should be spaced about ¼" apart on the medium.

4. Allow the seedlings to continue to grow and develop. Root formation generally begins when the plants have 2-3 leaves. Continue to transfer the seedlings to fresh media every 30-60 days, increasing the spacing between the plants with each transfer. When the container is ready for transfer to a community pot in the greenhouse, most containers will have 15 to 25 plants depending upon the species.
5. Transfer the plants into a community pot using a finely ground orchid mix.

Preparing Orchid Medium

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. Media containing charcoal, fruit extracts and/or agar will not completely dissolve
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 desired heat stable supplements. The media provided in this kit are complete and typically do not require supplements.
5. Add additional tissue culture grade water to bring the medium to the final volume.
6. 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 5.2 to 5.6 is typically recommended for most orchid media. 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.
7. Heat the solution to nearly boiling to melt the gelling agent in the medium.
8. 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. C093/C215) are autoclavable. Media should be dispensed in these vessels prior to sterilization in an autoclave or pressure cooker. The lids of culture vessels C093/C215 should not be tightly sealed during sterilization to allow for proper steam and pressure penetration.
9. Sterilize the medium in an 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.

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. 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

Media Composition

COMPONENTS (mg/L)	BM-1 Orchid Medium B141	BM-2 Orchid Medium B142	Knudson C Modified Plus Orchid Medium K425	Terrestrial (Cypripedium) Orchid Medium w/out Ammonium Nitrate T839	Terrestrial (Cypripedium) Orchid Medium III T842	Malmgren's Mod. Terrestrial Orchid Medium M551
Ammonium Citrate			Proprietary Formulation	19.0	19.0	
Boric Acid	10.0	10.0		0.5	0.5	
Calcium Nitrate				400.0	600.0	
Calcium Phosphate, Tribasic						75.0
Cobalt Chloride·$6H_2O$	0.025	0.025				
Cupric Sulfate·$5H_2O$	0.025	0.025		0.025	0.025	
Na2 EDTA	37.25	37.25				37.26
Ferric Ammonium Citrate				25.0	25.0	
Ferrous Sulfate·$7H_2O$	27.85	27.85				27.8
Magnesium Sulfate	100.0	100.0		97.69	97.69	97.69
Manganese Sulfate·H_2O	25.0	25.0		1.54	1.54	1.54
Molybdic Acid, Sodium Salt·$2H_2O$	0.25	0.25		0.02	0.02	
Potassium Chloride				100.0	100.0	
Potassium Iodide				0.1	0.1	
Potassium Nitrate				200.0	200.0	
Potassium Phosphate, Monobasic	300.0	300.0		200.0	200.0	75.0
Zinc Sulfate·$7H_2O$	10.0	10.0		0.5	0.5	
Activated Charcoal						1,000
Agar	5,000	6,000		6,000	6,000	7,000
6-Benzylaminopurine		0.2				
D-Biotin	0.05	0.05				0.05
Casein, Enzymatic Hydrolysate	500.0	500.0		400.0	200.0	400.0
Folic Acid	0.5	0.5				0.5
D-Glucose				20,000	20,000	
L-Glutamine	100.0	100.0				
Glycine (Free Base)	2.0	2.0				2.0
myo-Inositol	100.0	100				100.0
Nicotinic Acid	5.0	5.0				5.0
Pineapple Powder						20,000
Pyridoxine·HCl	0.5	0.5				5.0
Sucrose	20,000	20000				
Thiamine·HCl	0.5	0.5				10.0
Grams of powder to prepare 1 liter	26.22	27.22	79.11	27.44	27.44	28.84
pH±0.5 at RT	5.5	5.5	ND	5.5	5.5	4.25

Notes:

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