My journey towards conservation: Propagating orchids from tissue culture/ flasks

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Agenda

Overview of types of orchid propagation

Micro propagation Tissue culture (from explants)

Process of propagation through culture flasks

Note/disclaimer/acknowledgements:
• I am no expert in the field. Have very little “qualifications” in botany or wildlife.
• Presentation based on first hand experiences of 7-8 yrs of raising orchids by this method.
• There may be other alternate materials and approaches.
• Happy to discuss, share and learn.
• Key is to grasp concepts and extrapolate, use imagination.
• Thanks to Dr Shashidhar for sharing materials and advice.
Propagation is a means of keeping the plants healthy and produce new stock.

Orchids can be propagated in several ways depending on their growth habit.

Propagation – a few ways – vegetative, seed and tissue culture.

Vegetative propagation is common, easy, popular and can be done with minimum facilities: The numbers of plantlets offsprings obtainable are minimal (1:1).

For massive multiplication: seed culture and tissue culture are best methods… eg resulting in cloning.

However, this entire artificial process to set up from scratch is expensive-

But we can take advantage of flasks being available in retail- to raise new plants in reasonable numbers: process is still long drawn out and taxing on the patience, but can be fun and great learning experience for the enthusiast…

Pains and Pleasure: Akin to raising a baby or a pet!
Different forms of vegetative propagation are:

i. Cuttings  Ex: Monopodials such as Vanda- top cuttings

ii. Back bulbs Ex: Cymbidiums, Ground orchids

iii. Plantlets from flower stems Ex: Phalaenopsis flower stalk

iv. From keikis

iv. Meristem culture
Den keikis

Bulb separation – ground orchids, cymbidiums

Stem cuttings eg arachnis, vanda, epidendrum
The second method is through seeds..

In nature, orchid seeds germinate through a process of symbiosis with mycorrhiza (type of growth accelerating fungus) because…

- Orchid seeds are non endospermic – without nutritive tissues
- Orchid seeds have only embryo
- Seeds need nutrition for its germination – achieved through mycorrhiza

Seed culture or propagation through seeds can be achieved by…..

i. Natural, symbiotic method (as it occurs in nature)
- can be done by sprinkling the seeds over the prepared surface of a mother plant – possibility of the association and chances of small quantity germinating
Natural germination of *Spathoglottis plicata*
ii. **Artificial or asymbiotic method (tissue culture)**

- Involves sowing the seed or “explant” (eg bud, root, stem, leaf) in a sterile, nutritive media (agar gel) to get clones

**Need for artificial micro-propagation and advantages**

- *Great demand for orchid plants*
- *Maintains true to type*
- *Plants will be free from diseases*
- *Method of conserving the orchids*
- *Pod culture helps in maintaining genetic diversity*

• However- this process in **whole** is quite **expensive** for the hobbyist
• Involves laboratory investment and complicated processes, far out of reach to the individual, and yields literally lakhs of **plantlets/seedlings (in flasks)**
• However because of the **volume nature** of the biz, it is possible to **buy flasks** containing **rare and quality species** at relatively **low cost/per plant**
• Great way for a hobbyist to experience nurturing and growing plants from the very beginning.
• Also excellent for conservation of natural species in wild…does away with need for poaching from the wild
Plant and flower of *Phaius tankerville* (nun orchid)
In the laboratory, the preparation involves

1. Washing
   - Washing the pods under running water for 1-2 hours
   - Then immerse the pods in 0.1 % liquid detergent Tween 80 shake for 7-8 minutes - removes dust from the pod surface
   - Wash it thoroughly to remove the detergent traces
   - Dip the pods in 0.25 % Bavistan for 5-6 mins – surface sterilisation and again wash in distilled water

2. Surface sterilisation
   - Surface sterilisation of pods are done in the Laminar flow under aseptic conditions
   - Use 70 % Ethanol for 1-1.5 mins followed by washing for 4-5 times
   - Surface sterilisation using 0.15 % mercuric chloride for 5-6 mins
   - Through washing 6-7 times with sterile distilled water
3. Inoculation

- Dry the pods in the laminar flow before dissecting
- Dissect the capsules longitudinally with a surgical blade in the laminar flow
- Scoop the seeds from the capsules and sow uniformly and thinly in the culture jars each containing 50-60ml of medium

These jars with cultures are kept under following conditions in the laboratory:
- Temperature of 25 C
- Light intensity of 2500 lux for 12 hours
Growth + Hardening

- After about 16-20 weeks of growth (varies from species to genus), seedlings at one leaf stage is further used for hardening and separated and transferred to independent jars for further growth.
- After sufficient shoot development, they are transferred to medium containing rooting base.
- After about 20 weeks of growth and proper root formation, seedlings are put for hardening.
Plantlets of phaius and Cattleya  Plantlets of Phalaenopsis hybrid
What does it take to take advantage of tissue culture flasks and to turn these into the flowering plants, we all so covet?
THE MATERIALS REQUIRED

*Sealed Flask containing tissue/seed culture plantlets in agar gel

*Potting materials:
1. Small sized netted pots (allows drainage/aeration)/ small regular plastic or earthen well drained pots
2. Clean Brick pieces (to retain moisture)
3. Charcoal pieces (to balance the humidity, provide support to roots)
4. (Optional) Moss / cocopeat/coco husk… maybe used at a later stage

*Bavistin (fungicide) (have used neem oil also!)
*A couple of buckets & running tap water
*A Hardening chamber/ greenhouse (I have improvised with a fish aquarium) which was obtained from a pet store.
*A transparent glass lid/cover for chamber
*A few bricks to form the bottom absorbent layer
PREPARATION:

Ensure that all materials being used are first sanitized/sterilized in boiling hot water
Allow these to cool to room temperature
Ensure that all operations are conducted in a cool, clean and dry surrounding

DEFLASKING & HARDENING PROCESSES

• Break open the jar carefully, separating the glass from the plants
• Plantlets with well developed shoots and roots to be taken out of the jars
• Wash the plants thoroughly under tap water to remove the agar gel completely
• Keep the plants dipped in a weak Bavistan soln /disinfectant
• They are planted in trays / individual pots with well drained media
• Media: brick and charcoal pieces in lower half of pot, plant inserted delicately on top, embedded within the coco peat or coconut husk pieces or even moss
• Place the plants so potted, in the hardening chamber & cover it up
• Ensure that the chamber doesn’t get direct sun light or heat
• Spray water frequently, ensuring that there is no sogginess around the rots
• Esp in case of vanda and phalenopsis, make sure that water doesn’t stay on the “crown” …risking rot
• DO NOT spray any fertilizer at this stage
Seedling mixture and tray with netted pots

Bavistin treatment

Different Genera Seedlings at the time of washing

Bulbophyllum seedlings in hardening chamber (very fine roots)
• Trays kept in greenhouse with temperature of around 26 C and humidity of 95 percent with for a few months.
• Gradually, the humidity is brought down in a phased manner to the ambient conditions.
• During this period, spraying of water is resorted to maintain humidity.
• Simultaneously, the greenhouse is opened to atmosphere— with duration lengthening over time.
• After this, the seedlings are brought out and repotted.
• Care should be taken to ensure that ambient conditions are favourable when exposing the plant.
• Esp in Bangalore, using moss at this stage would help in maintaining humidity.
Trays kept in hardening unit under controlled conditions
Various Post-Hardening stages (6 mos- 2 years)

Doritis Pulcherrima

Vanda sanderiana
Cattleya (BLC) specimen (~7 yrs), started flowering 3-4 yrs back

Currently in bloom (Feb ‘13)
Cattleya (BLC) specimen (~5 yrs), started flowering 2-3 yrs back
Vanda coerulea x vanda sanderiana

7 year specimen flowering annually for last 3 + years
Vanda sanderiana (flowered twice this year in space of 6 mos)

Started flowering 5 yrs after deflasking
Rhynchostylis gigantea (Pink var)
My Orchid Species Pictures
Many of these have been grown from tissue culture flasks

For viewing full pictures…please go to below link
https://plus.google.com/photos/112490990837812780961/albums?banner=pwa&gsrc=pwrd1#photos/112490990837812780961/albums/5537451413120561473
Questions?

Thank you