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

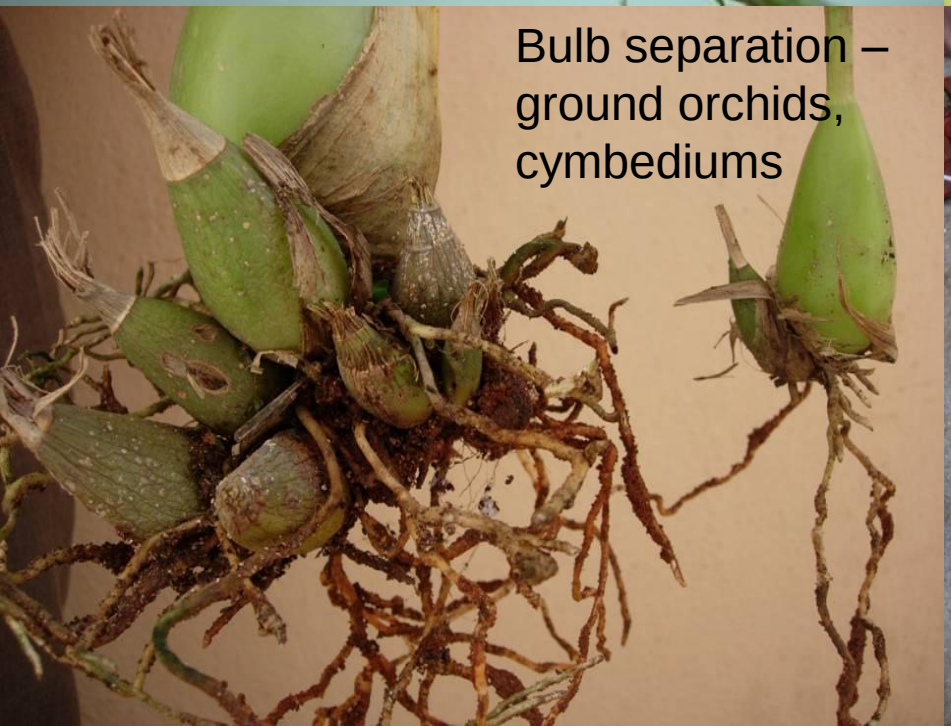
iii. Plantlets from flower stems Ex: Phalaenopsis flower stalk

iv. From keiki

iv. Meristem culture

You know these things….


Den keikis


Bulb separation – ground orchids, cymbediums


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
- 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 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

SKIM THRU THIS SLIDE/ OUT OF SCOPE

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

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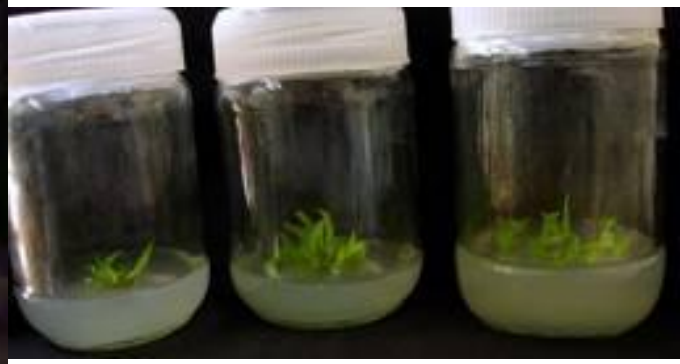

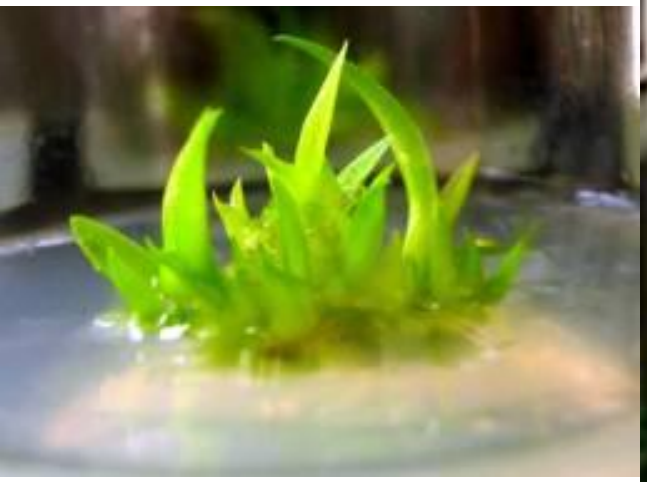

Growth + Hardening

- After about 16-20 weeks of growth (varies from species to genus), seedlings at one leaf stage is further used for culturing and separated and transferred to independent pots 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.

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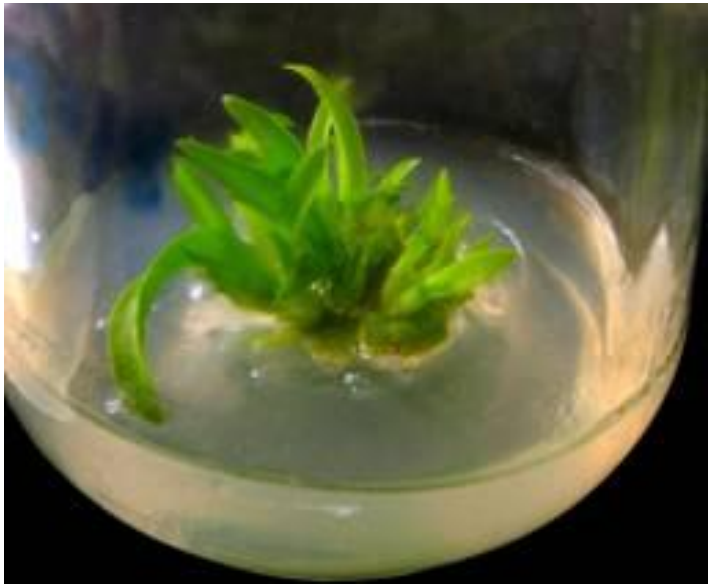


Plantlets of phaius and Cattleya

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

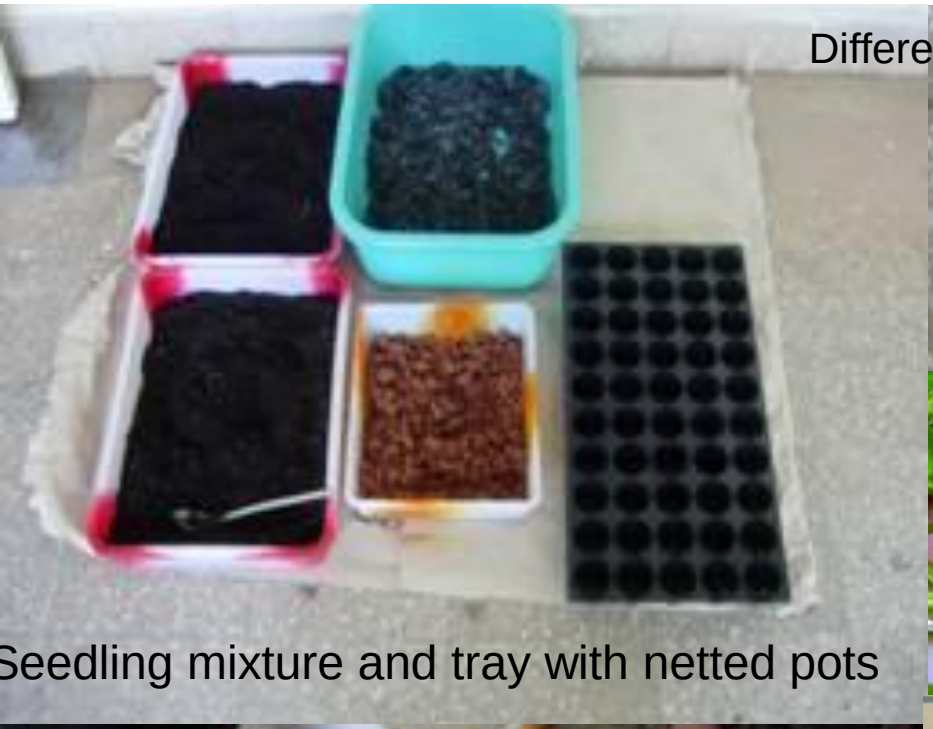
*Bavistin (fungicide) (have used neem oil also!)
*A couple of buckets & running tap water
*A Hardening chamber/ greenhouse (I have improvised with a fish aquarium) which was obtained from a pet store.
*A transparent glass lid/cover for chamber
*A few bricks to form the bottom absorbent layer

PREPARATION :

Ensure that all materials being used are first sanitized/sterlized 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

Different Genera Seedlings at the time of washing

Bavistin treatment

Bulbophyllum seedlings in hardening chamber (very fine roots)

TRANSPARENT GLASS AQUARIUM ACTING AS A GREENHOUSE/ HOT CHAMBER

• Trays kept in green house 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)

Currently in bloom (Feb ‘13)

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

Cattleya (BLC) specimen (~5 yrs), started flowering 2-3 yrs back

7 year specimen flowering annually for last 3 + years

Vanda coerulea x vanda sanderiana

Vanda sanderiana (flowered twice this year in space of 6 mos)

Started flowering 5 yrs after deflasking

Rhynchostylis Gigantea

Rhynchostylis gigantea (Pink var)

Brassavola Nodosa


SPATHOGLOTTIS FROM WYNAD, KERALA


SPATHOGLOTTIS


Den primilinium


Dendrobium aggregatum


Den primilinium and Den aggregatum


Aerides odorata


vanda velthuis


Eria flava


Eria flava


Paphiopedilium spiceranum: Geographic Distribution::Bhutan,

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&gpsrc=pwrd1#photos/112490990837812780961/albums/5537451413120561473

Rhychostylis Gigantea spotted

Paphiopedilium exul

Paphiopedilium exul

Pomatocalpa spicata

Epidendrum

Haemaria discolor is native to Indonesia and Burma. Jewel

Jewel orchid: note the incricate design and colours of the

Den candidum: this scented flower appears almost uncannily

Pomatocalpa spicata

Pomatocalpa spicata

Dendrobium pierardii

Vanda coerulens

Dendrobium pierardii, shorn of its leaves exhibiting the flower

D secundum : Flower Size to 1/2" [to 1.25 cm] This semi-pend

Paphiopedilium exul

Bulbophyllum medusae- Scented flower, small sized, warm to

Brassavola nodusa spikes closeup- growing on the bottlebrush

variant of vanda sanderiana


Rhynchostylis coelistis


Dendrobium primilinum


Rhynchostylis retusa


Rhynchostylis retusa


cymbedium species


Rhynchostylis coelestis


Rhynchostylis retusa closeup


Aerides odorata closeup shots


Rhynchostylus gigantea (pink var)


Dendrobium secundum


Phaius tankervilleae -Nun Orchid


Dendrobium pierardii /cucullatum/aphyllum


Dendrobium fimbriatum species


Coelogyne pandurata


Coelogyne pandurata


Rhynchostylis gigantea (var with pink spots)


Den species (from Tripura)


IONOPSYS SPECIES


Epidendrum species (from Baguio, Philippines)


RHYNCHOSTYLIS GIGANTEA SPECIES (MAGENTA/PINK)

ulbophyllum careyanum var. crassipes (Hook.f.) Pradhan 1979.

Dendrobium fimbriatum growing in Srini's garden Bangalore,

Genus: Eria, species: flava or lasiopetela ; Pic from Srini's

Aerides maculosum Or is it Rhynchostylis gigantea/retusa? Pic

Dendrobium Chlorostylum -from Srini's garden, habitat SE Asia

At left is Dendrobium chlorostylum- habitat SE Asia to North

Miltonia spectabilis var mooreliana grown in Srini's garden-Ban

Miltonia spectabilis var moreliana from Srini's garden. nat

Peristeria elata: Dove Orchid- terrestrial orchid -Common Name

Paphiopedilum spicerianum in Srini's garden. Flower Size to

he beautiful Nun Orchid or Phaius tankervilliae: P. tankervill

Vanda coeruleans mounted on ashoka tree, exposed to direct

Large dendrobium (kannayo) blooms on top of the bottlebrush

Dense dendrobium blooms perched atop bottlebrush tree

Brassavola nodusa atop the bottlebrush tree

Paphiopedilum spiceranium Flower Size to 3" [6 to 8 cm] This

Dendrobium candidum..on Xmas tree in Srini's garden,

Dendrobium candidum - plant mounted on Xmas tree..Found in

Vanda sanderiana in Srinis Bangalore garden, mounted here

Vanda rothschildiana ...one of the natural hybrids between V

Vanda coerulea natural cross variety variant, mounted on bottle

Rhynchostylis gigantea (alba-white).. growing next to palms in

Dendrobium anosmum (white var Dearie)...large breathtakingly

Den Anosmum alba ...large size brilliant white flowers on a 4-5

Den Anosmum alba...very large sized, pendulous, cane orchid

Rhynchostylis gigantea (purple and white combo)..This species

Arachnis hookeriana (or labrosa or annamensis) perched on

Variant of Vanda coerulea perched on /near the dead curacao

Vanda rothschildiana (a naturally occuring hybrid of V. coerulea

Dendrobium moschatum...Flower Size 3 1/2" [8.75 cm] Found

Den pierardii aka D aphyllum/cucullatum...Flower Size 2" [5

Closeup of Dendrobium aphyllum/pierardii/cucullatum..1 to

Cymbedium from western ghats-Sirsi... yellow and maroon

Closeup view of Cymbedium aloifolium (Cym bicolour) from

Bulbophyllum careyanum var. crassipes (Hook.f.) Pradhan 1979.

Questions?

Thank you