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TECHNIQUES FOR PRODUCTION OF QUALITY PLANTING MATERIALS IN ORCHIDS

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ABSTRACT

Among orchids, *Cymbidium, Dendrobium, Vanda, Phalaenopsis, Aerides, Mokara* and *Paphiopedilum* and their related hybrids are highly valued for long lasting cut flowers. The six main techniques used for orchid propagation are division, backbulbs, serial cuttings, kiekies, micro-propagation and seed culture. Conventionally, sympodial orchids like *Cattleya, Dendrobium, Paphiopedilum* and *Cymbidium* are multiplied through divisions whereas monopodials viz. *Vanda, Aerides, Arachnis, Mokara* etc. through cuttings. Tissue culture using meristem and shoot tips as explants used for production of disease free quality planting materials for all types of orchids in large scale. Seed culture is an important field in orchid culture with many hybrids and intergeneric crosses being bred to exhibit new and different physical characteristics.

INTRODUCTION

Orchids are prized for their incredible diversity in the size, shape and colour and attractiveness of their flowers and high keeping qualities even up to 10 weeks. Among 30,000-35,000 species, the Cymbidium is one of the top ten cut flower of the world market whereas Dendrobium is most widely adopted tropical orchid grown for export purposes. In India, it comprises 158 genera and 1330 species which grow upto an elevation of 5000m. Indian terrestrials are located in humus rich moist earth under tree shades in North Western India. Western Ghatsharbour the small flowered orchids. Epiphytic orchids are common in North eastern India which grow upto an elevation of 2000m from sea level. The Cymbidium is mainly grown in NEH Region, Sikkim, A.P. and Assam. Tropical orchids are cultivated in Kerala and some parts of Tamil Nadu. Both monopodial (Single stemmed growth) and sympodial (Multistemmed growth) are preferred for commercial cultivation. In India, some of native genera and elite hybrids of *Cymbidium, Paphiopedilum, Vanda, Arachnis* and *Dendrobium* are cultivated on a large scale for cut flower production (De, 2014). Lack of quality planting materials has become a major factor for limiting commercial cultivation of various orchids. There are six main techniques used for orchid propagation: division, back bulbs, aerial cuttings, keiki, micropropagation and seed culture. Other techniques used in propagation are aerial shoots and tubers (Bhattacharjee and De, 2010).

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Division

This is the easiest method of propagation used for sympodial orchids. In this case, the rhizomes are cut between pseudobulbs and potted the pieces separately so that each part have at least three healthy pseudobulbs and one dormant bud for producing new growth. The best time for division of orchids is early spring. Division of an orchid encourages the plant to produce more vigorous shoots of a better quality. *Brassavola, Calanthe, Laelia, Miltonia, Odontoglossum, Oncidium*, *Cattleya*, *Dendrobium*, *Paphiopedilum* and *Cymbidium* can be multiplied through division.

Back Bulbs

These are previously flowered or unflowered back pseudobulbs. In this case, it my take upto three years to obtain a flowering size plant.

Divisions in *Cymbidium*

Backbulbs in *Cymbidium*

Divisions in *Cattleya*

Backbulbs in *Oncidium*

A backbulb having roots are pulled or rhizomes are cut just beyond it and are inserted at one side of a pot filled with orchid compost or sharp sand or grit keeping the cut surface of the bulb nearest the edge of the pot. The bulbs emerge shoots within two or three months which can be potted in orchid compost. *Cymbidium, Cattleya* and *Coelogynes* are propagated through this means. In *Cymbidium*, it has been found that both saw dust and cocopeat are effective media for generation of plants through backbulbs. Prior to planting, treatment the backbulbs of *Cymbidium* orchids with BA 200 ppm or coconut water (1: 5 or 1: 10) is effective to enhance per cent of germination. By this method, both the media take 90-99 days in spring season and 42-48 days in summer season to generate new plants (Table 1).

Table 1. Generation of planting materials of Cymbidium through backbulbs

Name of hybrid	Type of Media	Season	Duration
Cym. 'H.C. Aurora"	Cocopeat	Spring	90 days
	Cocopeat	Summer	42 days
	Saw dust	Spring	93 days
	Saw dust	Summer	48 days
Cym. 'W.W.W.'	Cocopeat	Spring	99 days
	Cocopeat	Summer	42 days
	Saw dust	Spring	93 days
	Saw dust	Summer	48 days

Cuttings

In monopodial orchids like *Vanda, Aerides, Arachnis, Mokara*, the upper most parts of the stem are cut off just under the aerial roots and the removed part is planted in porous media for producing an individual plant and it is called as top cuttings. In sympodial orchids like in Dendrobium cuttings of 10-15 cm having 4-5 segments are taken from canes during spring or rainy season and planted in cocopeat or saw dust for rooting. Flower stalk cuttings are useful in genera like *Phalaenopsis, Phaius, Calanthe* and *Thunia*. Cutting are usually potted in propagation beds or directly in pots after treating the cut ends with fungicides like bavistin @ 3 g/litre. Cutting of genera, like *Aerides, Arachnis, Vanda* etc., are very hardy and are directly potted in pots, whereas those of *Dendrobium* and *Phalaenopsis* need special care to root and should be rooted in propagation beds. In *Phalaenopsis*, offshoots are produced by cutting out or mutilate the growing point, removing small leaves and treating the injured portion with fungicides.

Air layering

In this method, a cut is made through the stem 20 to 30 cm below the apex and most sphagnum moss is wrapped around the cut portion. The rooting media is kept moist and once the

roots are formed, the layer is detached from the mother plant and potted in small-sized pots. *Vanda* and other monopodial orchids are easily multiplied by air-layering or marcotage.

Keiki

A keiki is a small plant which generally grows from one of the nodes along the stem instead of branch. They occur through the accumulation of growth hormones at a specified point. Kekis grow in two forms a regular and a basal keiki. The regular keiki is a small plant growing from one node along the flower stem, instead of a branch. This is induced by the accumulation of growth hormones at that point, either naturally or by the application of keiki paste, a cytokinin hormone which induces growth in the node of an orchid inflorescence. The basal keiki is a baby plant growing from the base of the mother plant. Sometimes keikis bloom while still attached to the mother plant. Keikies are used as propagules in *Dendrobium, Ascocendra, Phalaenopsis* and *Epidendrum*.

Keikis in *Dendrobium*

Keikis in *Epidendrum*

Aerial Shoots

Most of the Dendrobiums give aerial shoots or bulbs on old back bulbs devoid of leaves. They usually develop on the upper part of the back bulbs and grow out slowly. These aerial shoots take 90-120 days to develop roots. At this stage, they are detached along with the portion of back bulb and potted as independent plant in orchid compost. In genera like *Goodyera*, the rhizome gives off special lateral branches which turn up and produce aerial shoots (Abraham and Vatsala, 1981).

Tubers

In few genera, like *Peristylis* and *Nervillia*, the roots are produced from above the tubers, which are transformed in to tubercles. These small tubers produce new plants the year after.

Tissue Culture

Tissue culture is one of the most rapid methods of multiplying vegetative plant. It develops new plants in an artificial medium under aseptic conditions from very small parts of plants, such as shoots tip, root tip, pollen grain. Thousands or even millions of identical plants can be produced from a small tissue in a relatively short time. Among these, meristem and shoot tip culture are most popular for mass propagation of commercial species and hybrids. Axillary buds are good source of explants in monopodials. Both liquid and solid media are used for culture of orchid tissues. The widely used media are Knudson's C medium, Vacin and Went's medium, Murashige and Skoog's medium. Additives like coconut water (15%), banana pulp (10%) are found beneficial for promotion of shoots. The mineral salts, carbon source, vitamins, plant growth regulators are used in the media. Sucrose as carbon source promotes organogenesis at suboptimal concentrations and protocorm formation at supra-optimal concentrations. Among vitamins, thiamine and growth regulators, auxin, cytokinins are used for callus formation. Protocorms of Cymbidium 'Soul Hunt-1' were cultured on media incorporated with different levels of IBA & GA3 revealed that MS+AC+GA3 (0.5 mg/l) resulted faster plb proliferation (18 days for 5thplb stage). Combination effect of both hormones on plb proliferation was found best for MS+AC+IBA (0.5 mg/l)+GA3(1 mg/l). *In vitro* plants are hardened off in vitro only, before transferring to main field. Application of paclobutazol delays chlorophyll loss, reduces the activities of enzyme and delays senescence.

Shoot Tip Culture

In this method, shoot tips are extracted from the vegetative buds located on pseudobulbs. The necessary steps for propagation of micro propagated orchid planting materials are:

- Selection of healthy and disease free mother plant and establishment of mother blocks nursery,
- Indexing of viruses of mother plant in the nursery,
- Initiation of cultures,
- Proliferation of cultures,
- Primary hardening and rouging of undesirable plants,
- Secondary hardening and rouging of undesirable plants,
- Genetic fidelity testing and virus indexing at various stages of micro propagation.

Meristem –tip Culture

This method involves the use of apical dome or shoot tip with a few leaf primordial of the size less than 1 mm in length as explants.

Seed Culture

Orchid sexual propagation is practised through seed embryo culture. Orchid seed is so minute and do not have stored food for seed germination. However, during germination, fungi infect orchid seeds and help convert complex starch to simple sugars, which serve as energy source. That fungi and orchids have symbiotic relationship during germination. Seedling orchids are grown to with the objective of providing seedling plants and to breed new plants. This is an important field in orchid culture, where many hybrids and inter-generic crosses are being bred to exhibit new and different physical characteristics.

An F_1 Hybrid plant is produced from seed which is obtained from cross pollination between two different species or two different varieties of the same species. Hybrid seeds can be produced the following ways:

- By hand pollination: This is usually practiced to produce new varieties for testing purposes. (i.e: Most new varieties are developed by this way).
- Production in seed plantations: In this method, two different varieties are inter-planted or placed in an area and seeds are collected from those plants.
- Chance seedlings: In this case, seed is collected from plants i.e. the wild or elsewhere which are suspected to have cross pollinated with different varieties. The seedlings are grown on to flowering stage and then the best varieties are selected.
- Seed collected from F_1 Hybrid plants. The initial hybrid is produced by cross pollination (i.e: as above). This hybrid is grown until it produces seed, and the seed is then collected. This seed is second generation hybrid seed, can be called as F_2 seed. New plants are grown as seedlings from this seed.

Orchid seed is not generally bought. It can be bred it or collected and can be sown as soon as possible. As the pods mature they change into yellow colour and then start to show signs of splitting along the placenta. The plants must be inspected carefully. At this stage, and not before, the seeds are harvested into a polythene or paper bag. Off one off type plant may have as many as a million or so viable seed, or as few as a dozen. (e.g.: one pod of a *Cymbidium tracyanum* contains nearly 3 million seeds). Seeds should be shaken in Chlorinated water (usually 1 in 20 with water) for 10 minutes before planting to kill any disease. Seeds are sown using droppers or needles in a sterile environment, into flasks. The flasks are sealed and placed in high humidity and warm environment (20-28^{o}C). It normally takes 4 - 6 months to grow up into seedlings to the point where they can be transplanted. It can take 4 - 10 years for the seedlings to come into blooms. Under artificial or laboratory conditions, a sterile artificial medium with sugar and other nutrients is necessary. Through research, an excellent medium for growing seeds without fungi can be developed. Inside the bottle where orchid seedlings are grown is a miniature glasshouse which protect seedlings from unfavorable environmental conditions. Using artificial media it has become possible to grow nearly all orchid seeds into mature plants.

Flasking and Reflasking of Protocorms

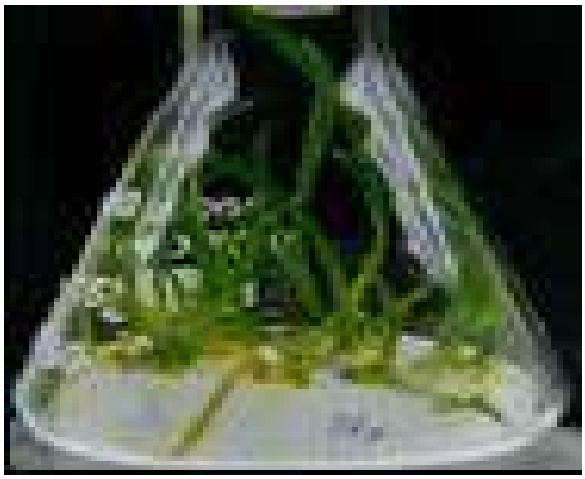
When orchid seed or embryo is planted in a culture bottle, numerous seedlings germinate in a very limited space with little available food. The first sign of successful germination is found when orchid seeds start to swell and turn green. As growth continues, the embryo becomes bigger and assumes a flattened top shape called 'protocorm'. A small amount of seed sown can produce hundreds of tiny photocorms growing in limited space. At this stage, they are transplanted into fresh medium and kept for further development and rapid growth.

Composting and Repotting Seedlings

Orchid seedlings become ready for transplanting from culture bottles when roots and leaves are fully developed. Dendrobiums are potted after 4 to 6 months. Vandas, Phalaenopsis and Cattleyas in 6 to 8 months after reflasking before seedlings are ready for transplanting in pots. Seedlings should be potted only in sterile potting medium and pots to avoid damping-off diseases. Potting medium may consists of sterilized leaf mould, charcoal and chopped tree fern. After removing seedlings from bottles, all agar are washed out from seedlings and are treated in fungicide suspension. Excess moisture is drained out and seedlings are sorted out according to size. Small seedlings are transferred in community pots, while the bigger ones are potted individually in small pots.

Production of disease free planting materials through micro-propagation

The necessary steps needed for producing virus, pathogen and insect free planting materials of orchids are as follows (Rampal, 2014):



Micropropagation in *Cymbidium*

- Virus diagnosis using visual and molecular detection techniques applied at mother plant selection, 2nd and 3rd subcultures and primary and secondary hardening stages.
- Elimination of viruses using molecular techniques like ELISA, PCR, RT-PCR etc. and other techniques like chemotherapy using anti-viral substances such as acyclic adenosine analogue, thermotherapy using heat treatment either 'in vivo' or 'in vitro' and cryo-therapy means

storage of samples at ultra low temperature of liquid nitrogen (-196^{o}C).

- Management of other diseases during secondary hardening.

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