



POPULAR SCIENCE ARTICLE

Production of quality planting materials in orchids

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Introduction

Orchids are prized for their incredible diversity in the size, shape and colour and attractiveness of their flowers and high keeping qualities even upto 10 weeks. Among 30,000-35,000 species, the *Cymbidium* is one of the top ten cut flowers of the world market whereas *Dendrobium* is most widely adopted tropical orchid grown for export purposes. In India, it comprises 155 genera and 1256 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 Ghats harbours 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 (Bhattacharjee and De, 2010; 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

Abstract

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. In India, it comprises 155 genera and 1256 species which grow up to an elevation of 5000m. 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. 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.

Keywords: Propagation, backbulb, cut flower, tissue culture, potting

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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. *Brassovola*, *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 may take up to three years to obtain a flowering size plant. 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 (De et al., 2015) (Table1).

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
Cym. 'W.W.W.'	Saw dust	Summer	48 days
	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*. Cuttings 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 moist 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. Keikis 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. Keiki's are used as propagules in *Dendrobium*, *Ascocendra*, *Phalaenopsis* and *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.

Tubers: In few genera, like *Peristylis* and *Nervillia*, the roots are produced from above the tubers, which are transformed into 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 (Thorpe, 2007). 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 5th plb 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. Explants are

taken from shoots of 5 to 7.5 mm long. Growing with small shoot tip explants (0.3 to 0.5mm) are reported to be the best plant material for the propagation of endangered and floriculturally important epiphytic orchid, *Dendrobium primulinum* Lindl. (Pant and Thapa, 2012). In *Dendrobium*, axillary and terminal shoot tip as explants give better protocorm than stem internode as explants.

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 shoots tip with a few leaf primordial of the size less than 1 mm in length as explants.

Flower stalk culture

The Flower stalks are to be sectioned into 2-3 cm segments containing flower nodes, internodes, and the meristem, then surface sterilized with a 10% sodium hypochlorite solution, followed by 70% ethanol and Tween-20. After rinsing, chemically exposed parts were excised, and bracts covering nodes and buds to be removed. Sterilized explants will be transferred to half-strength MS media with 2% sucrose and varying concentrations of plant growth regulators and incubated in darkness at 22 ± 2 °C and 65–70% humidity. Regenerated plantlets can be rooted in different IBA concentrations, acclimatized, and hardened.

The stalks are to be immediately immersed in distilled water and then sectioned into 2-3 cm segments containing flower nodes, internodes, and the flower meristem. Sterilized explants are to be transferred to half-strength Gamborg B5 medium supplemented with 2% sucrose, 2g/L banana powder, and varying concentrations of plant growth regulators (Table 2). Cultures are to be maintained in darkness at 22 ± 2 °C and

65–70% humidity, with subculturing every 30 days in fresh medium under the same conditions.

Seed Culture: Orchid sexual propagation is practiced 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 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.

PLBs proliferation and regeneration of plantlets through seed culture: Seed capsules obtained from various selfing and crossing of different genera of orchids are to be surface sterilized by dipping in 10% sodium hypochlorite solution, 70% ethanol, and a drop of Tween-20. After that the capsules are to be rinsed five times with sterile distilled water. The sterile seed capsule can be vertically cut open with a sterile scalpel, and the seeds will be transferred into different basal medium supplemented with various growth regulators. The cultures are then to be incubated at a controlled temperature of 22 ± 2 °C and a relative humidity of 65–70% in a culture room. For induction of PLBs from *in-vitro* plantlets, leaves are to be segmented into 1 cm pieces and placed abaxial side down on half-strength MS or Gamborg media with 2% sucrose and BAP/TDZ/IBA / NAA combinations. Subculturing can be done every 30 days in fresh media under dark conditions.

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 number of seed sown can produce hundreds of tiny protocorms growing in limited space. At this stage, they are transplanted into fresh medium and kept for further development and rapid growth (Rampal, 2014).

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 consist of sterilized leaf mould, charcoal and chopped tree fern. After removing seedlings from bottles, all agars 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.

Table 2. Protocols for mass propagation of orchids through tissue culture techniques

Sl. No.	Genus	Explant	Media	PGR's
1.	Phalaenopsis	i) Flower bud stalk, node, leaves, in vitro leaves ii) Flower bud stalk iii) Leaves, internode, root tip, flower bud iv) Leaves, internode, root tip, flower bud	i) Gamborg ii) MS media iii) MS media iv) Gamborg	i) BAP (1-2 mg/l) + NAA (0.5-1.0 mg/l) ii) BAP (2 mg/l) + NAA (0.5 mg/l) iii) NAA (0.5 mg/l) + BAP (0.2 mg/l) + coconut water (20 ml/l) iv) NAA (0.5 mg/l) + BAP (0.5 mg/l)
2.	Dendrobium	i) Side shoot, leaves ii) Flower bud stalk iii) Flower bud stalk iv) Seed v) Seed vi) Seed	i) MS media ii) MS media iii) MS media iv) MS media v) Gamborg vi) Nitsch	i) BAP (1.0 mg/l) + TDZ (0.2-1.0 mg/l) + 2,4-D (2.0-2.5 mg/l) ii) BAP (1.0 mg/l) + NAA (1.0 mg/l) iii) NAA (1.0 mg/l) + TDZ (0.5 mg/l) iv) BAP (0.2 mg/l) v) Nil vi) Nil
3.	Paphiopedilum	i) Leaves, side shoot ii) Seed iii) Leaves	i) MS media ii) MS media iii) MS media	i) BAP (2.0-2.5 mg/l) + TDZ (0.5 mg/l) ii) BAP (0.2 mg/l) iii) 2,4-D (0.2 mg/l) + coconut water (20 ml/l)
4.	Aranda	Flower bud stalk	MS media	BAP (1.0 mg/l) + NAA (0.5-1.0 mg/l)
5.	Phaius	Side shoot	MS media	NAA (1.0 mg/l) + BAP (1.0 mg/l) + TDZ (0.3 mg/l) + coconut water (0.3 ml/l)
6.	Cymbidium	i) Seed ii) Seed iii) Leaves iv) Flower stalk	i) MS media ii) MS media iii) MS media iv) MS media	i) IBA (0.5 mg/l) + NAA (0.2 mg/l) + BAP (0.5 mg/l) ii) NAA (0.2 mg/l) + BAP (0.5 mg/l) iii) NAA (0.5 mg/l) + BAP (0.8 mg/l) + coconut water (20 ml/l) iv) NAA (0.5 mg/l) + BAP (0.8 mg/l) + coconut water (20 ml/l)
7.	Vanda	i) Leaves, flower bud stalk, root ii) Flower bud stalk iii) Seed	i) MS media ii) MS media iii) MS media	i) BAP (2.0 mg/l) + TDZ (1.0 mg/l) ii) NAA (0.5 mg/l) + BAP (0.5 mg/l) + TDZ (0.5 mg/l) iii) NAA (0.2 mg/l) + BAP (0.4 mg/l)

There are three different types of growing media used in nursery plant production. The different components of growing media are sand, peat, bark, leaf mould, sawdust, bagasse, rice hulls, perlite, pumice, vermiculite, calcined clays, expanded polystyrene, urea formaldehydes etc.

- **Seed propagation media:** These media are used for germination of seeds or establishments of germinants. The media should be sterile, and it should have a fine texture to maintain high moisture around the germinating seeds.
- **Media for rooting cuttings:** These media should be porous to prevent water logging and to allow good aeration for root formation.
- **Transplant media:** These media should be coarser and contain compost for transplantation of smaller seedlings or rooted cuttings. In some cases, 1/5th part of soil may be added to encourage the development of mycorrhizal fungi and other beneficial microorganisms.

A typical growing medium is composed of two or three organic and inorganic components with complementary physical and chemical properties. Organic components commonly used are peat moss, bark compost, rice hulls, coconut coir and sawdust. These organic materials are low in weight with high water holding capacity, high CEC and rich in nutrients. Inorganic components include gravel, sand, vermiculite, perlite, pumice and polystyrene beads. These inorganic components improve media properties by increasing aeration pore space, improving bulk density and enhancing drainage.

POTTING

Potting up plants improve health or vigour. Plants are potted due to several reasons

- Plants become pot bound and require more space to grow
- To hasten growth in short time
- To replace an old broken pot with new one
- To promote the development of new healthy feeder roots by root pruning

- To grow the plant in a better growing medium
- To transplant plants from an in-ground location into a pot

Containers

- Selection of suitable growing containers for orchids provides an opportunity for creativity and interesting aesthetics to accompany the foliage or flowering beauty of the plants. However, appearance is not the only selection criteria for growing orchids in containers. Containers may be selected based on suitability of plant's needs, suitability for the needs of the individuals and the environment, cost and availability, strength and durability, drainage, and weight.
- The style, shape, and size of the container should complement the type of orchids to be grown. Small containers are best for small slow-growing plants, while fast growing plants are better suited for large containers. Containers may be made from ceramics, plastic, fiberglass, wood, aluminium, copper, brass, and other materials.
- The pot selected for repotting tissue culture plants should be no more than 10cm larger in diameter should have at least one drainage hole and must be clean. To remove soluble salts lay pots are washed with water, soap, and a scrub brush, and wash all pots in a solution of 1 part liquid bleach to 9 parts waters. For repotting full-grown plants, pot size should be more than 15cm in diameter.

Principles of Potting

New clay pots should be soaked in water for hours to week; then dried them off before using. The condition and species of plant will determine the suitable time for potting. The best time is just after a plant has started into growth. Most of orchid plants start new growth in the spring. March, April and May are good potting or repotting months for these types of plants. After potting plants, they should be kept in dim light not in direct sun for several days. The clumpy perennial plants are best divided by using forks. The torn cut portions are cleaned with a sharp knife and treated with a proper fungicide to prevent infection. When the roots emerge from the bottom of a pot plant, it is repotted into a large container.

- A pot i.e. slightly larger is selected than the current pot
- The plant is gently removed from the smaller pot
- Any tangled and circling roots are loosened and pruned when necessary

- A layer of quality potting mix is placed at the base of new pot
- The plant is placed in the centre of new pot. The soil level must be 2.5cm below the top of the container.

Conclusions

The propagation of orchids by conventional methods is a very slow process. Vegetative propagation through division of clumps or rhizomes, cutting and separation of offshoots and keikis produced from the stem or pseudobulbs is very slow and one may not obtain more than a few plants after 2-3 years. The orchid seed although produced in a very large number i.e. 2-3 million per capsule, are non-endospermic and does not contain nutrient. They require a mycorrhizal association for their germination, which may be provided under natural undisturbed conditions. Fungal in such association are believed to provide sugar and other nutrient required for seed germination by breaking down starch (Arditti, 1977). Therefore, only 2-5% of seeds germinate in their natural environment. Tissue culture is the culture of cell, organs, tissues or plants part in aseptic condition with control environment condition and controlled nutrition to produce clone of plant with true to type of selected genome. The controlled condition includes pH of medium, favourable temperature, nutrients, proper gaseous and liquid environment. Plant parts like shoot tip, leaf segments, pseudobulbs, aerial roots, floral parts etc. have successfully been used for the *in vitro* propagation of orchids. Amongst these, meristem and leaf tip culture are however, more popular and have extensively been used for the propagation of valuable species and hybrids. Apart from propagation of large number of plants in short period of time, tissue culture technique has been effectively used for obtaining virus free clones (Gonzales *et al.* 2010). The technique of microcloning is now being used on a commercial scale for propagation of orchid's genera like *Cymbidium*, *Dendrobium*, *Oncidium*, *Odontoglossum*, *Phaius*, *Vanda*, *Epidendrum*, *Paphiopedilum* and many others.

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