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Review Article

# *in-vitro* culture of Orchids through Seeds and other plant parts- where we stand now?

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## Abstract

Orchids are blessed with spell bounding beauties, shape, size, colour and these features make orchid flowers tremendously attractive. Craze for these flowers is too high, people all over the world fantasize to have those in their flower vase and polyhouses. In nature orchid species are one of the most diverse flowering plant groups. Besides having astonishing beauty some of the orchids also possess therapeutic properties. They can be propagated through in-vitro culture of seeds and meristematic parts. Propagation through meristem parts is crucial for commercial purpose as only meristem culture can produce true to the type genotypic similarities in orchids. Tissue culture by means of seeds culture may derive progenies with heterogeneous genotypes and phenotypes as well as the plants take much longer time to flower as compared to the plants produced through meristem culture. Thus, propagation through meristem culture may be considered as the best way to produce best quality orchid planting materials as per the market demand. This review is a comprehensive synthesis of updated information pertains to orchid meristem culture aiming for quality produce which may be beneficial for all the stakeholders.

**Keywords**: Meristem culture, Commercial Orchid, PLBs, Basal media of orchid, explant.

## Introduction

Orchid is a fascinating flower all over the world. Orchid was there even during the dinosaur's period. We human beings are social so along with food we need a beautiful mind for creating a happy environment for which Orchid is counted as an astonishing flower. Many hybrid, species and other varieties of orchid are in cultivation for export and in-situ selling as a cut flower as well as potted plant. In current scenarios, world's flower industry accounts for annual sales of more than US$4 billion. The family of Orchidaceae comprises of 736 genera with more than 28,000 species which are mostly available in the wet tropics worldwide except few isolated areas. In Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura total 159 genera and 870 species whereas in in J & K, Himachal Pradesh, Uttarakhand 75 genera



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and 288 species of orchid are present. Orchid family is most diverse amongst the flowering plants. They have capacity to grow on all kind of habitats, except some aquatic systems. Based on their growth habitat they are grouped as terrestrial, epiphytic or saprophytic. Generally, most of the orchids are epiphytic in nature however, some are terrestrial. Orchids are used mainly for ornamental purpose but some are utilized for therapeutic purpose (Nand Lal, 2020). The wild orchid population basically multiplied by sexual means i.e., by seed and asexually by way of vegetative propagation. However, the traditional vegetative propagation system is very slow whereas in natural ecosystem orchid cultivation by seed germination takes greater care as only few seeds germinate in spite millions seed produced in each capsule. Traditionally orchid is propagated through *kekies* or other means of clonal propagation for large scale multiplication, however this might enhance the disease dissemination (Kunagorn *et al*., 2017). Therefore, multiplication by meristem or other in-vitro culture are attaining a greater scope for expanding its market. Orchid species preferable for cut flower are *Cymbidium eburneum*, *Pahiopedillum fairrieanum*, *Paphiopedilum hirsutissimum*, *Paphiopedilum insigni*, *Paphiopedilum venustum*, *Vanda coerulea*, *Vanda tessellate*, Cattleya, Mokara, Aranda, Oncidium etc.

In orchid regeneration plant tissue culture techniques have been apply for rapid growth and development. Only few orchid species were multiplied during last several years either by using explant or protocorm like bodies (PLBs). Seed germination give rise to heterozygous plants, whereas micropropagation through various vegetative parts of the plants overcome this problem. Usually for meristem culture stem, bulb, leaves, leaf tips, flower stalk, young flower buds, internodes segments, root, root tips, stem portion, stamens, pistil etc., are used. Basic flow chart for plant tissue culture in orchid is given below

Take any plant part then wash and clean it under running tap water for 5 minutes.

⬇

Then prepare a 10% chlorine bleach solution and add 2-3 drops of Tween twenty (wait 5 minutes)

⬇

Wash with distil water five times

⬇

Dip with 0.1% $HgCl_2$ solution (2-3 min)

⬇

then wash with distil water

⬇

Take this plant parts in Laminar Air Flow Chamber

⬇

Then dip in 0.2 % $HgCl_2$ solution (2-3 min)

⬇

Again, wash with autoclave water

⬇

Make section with sterilized blade and inoculation in suitable culture media



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Basic requirements of *in-vitro* culture of orchid have been discussed under few sub headings below.

(i) **Aseptic conditions**: It means a pathogen free environment for maintaining good health of the callus, cell or protoplast cultures. It results into recovery of health plants. The explant and glassware should be properly sterilized before their entry into the tissue culture laboratory.

(ii) **Control of temperature**: Generally, 18 to 25$^0$C temperature is essential. High temperature adversely affects the growth of the callus.

(iii) **Proper culture medium**: There are various culture media developed by various workers but it should be modified as per Orchid species requirements. Orchid culture media are composed of macronutrients, micronutrients, vitamins and growth hormones. Plant growth hormone such as cytokinin [BAP (N6 –benzylaminopurine), IBA, Kinetin, 2-iP (2-isopentyladenine), TDZ (Thidiazuron)] either alone or in combination with auxin [IAA, 2,4-D (2,4 dichlorophenoxy acetic acid), NAA (a-naphthalene acetic acid)] have been used for production of shoot and PLBs formation. PLB formation is affected by various factors among which light is one crucial factor, for example in Phalaenopsis in absence of light PLB formation induce whereas for differentiation into plantlet light is mandatory. In Orchids a perusal of literature reveals that several hundred media compositions have been used but the most commonly used media for the propagation of orchids are MS, VW and KC. Subsequent to defining the medium, other aspects are developed such as suitable pH, addition of growth regulators, and exploration of the use of alternative carbon sources. Use of activated charcoal and antioxidants for minimizing browning effect etc. (Yam, *et al*., 1989; Jayarama Reddy, 2008) is crucial.

(iv) **Sub-culturing**: It is a method of transferring of tissue or callus from old tissue culture media to fresh culture media. It is crucial to maintain proper health of callus or tissues.

The *in-vitro* propagation method of Orchid under in-vitro is discussed as below-

**A. Seed propagation**

Orchid pod or capsule may consist of thousands of dusts like seed which are devoid of food reserve (endosperm). These seeds can be grown by Orchid flasking procedure since orchid seeds usually require a symbiotic mycorrhizal fungus to germinate. There are two different techniques for flasking: symbiotic germination and asymbiotic germination. Symbiotic germination requires the isolation of a mycorrhizal fungus which is added to the agar in which the seeds are grown. Asymbiotic germination just uses the nutrients that the seed requires to grow. First innovative method for orchid seed germination was developed by David Moore (Morel, 1974). Fifty years after Moore's discovery, Noel Bernard (Bernard,1909) made another quantum



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jump when he developed a method for the symbiotic germination of orchid seeds in vitro. This is the first method derived for the in vitro propagation of any plant. Seedlings grew well on a pH 4.5 culture containing 0.8% agar.

**B. Meristem culture**

Meristem culture refers to regeneration of whole plant from tissues of an actively dividing plant part such as stem tip, root tip, auxiliary bud, flower bud, flower petal etc. Mass multiplication of orchid is possible with meristem culture technique as it maintains its genotypic value as it is. Although soma clonal and gametoclonal variation do occur in tissue culture plants but their occurrence is low.

In the initial stage of research only solid media were used in recent past researchers started using a liquid medium either in static or in moving conditions. In the latter case, a shaker was used to provide aeration for growing tissues (Steward *et al*., 1958). A distinction was also made by some authors between the starting, standard maintenance and rooting media based on their suitability. For example, in one of experiments, meristems of Cymbidium were inoculated on a liquid medium, on which PLBs were formed. The PLBs were then transferred on to a solid medium to obtain plantlets (Wimber, 1965). The choice of liquid or solid medium may depend on the type of the explant and the objective of the culture. A solid medium was found economically viable, convenient as it produces consistent results. At present Suspension culture techniques are also using by some researcher which were developed by Singh and Prakash, 1985 for the micropropagation of *Epidendrum radicans*. Example, to produce plants using apical and axillary buds of Dendrobium Caesar Red Lip, VW liquid medium were used for initial culture and KC solid medium for producing plantlets from PLBs (Jayarama Reddy, 2008).

Photo 1: Subculture under Laminar Air

Photo 2: PLB culture of Cymbidium species.



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flow chamber

Photo 3: Meristem part for induction culture under Laminar Air flow chamber

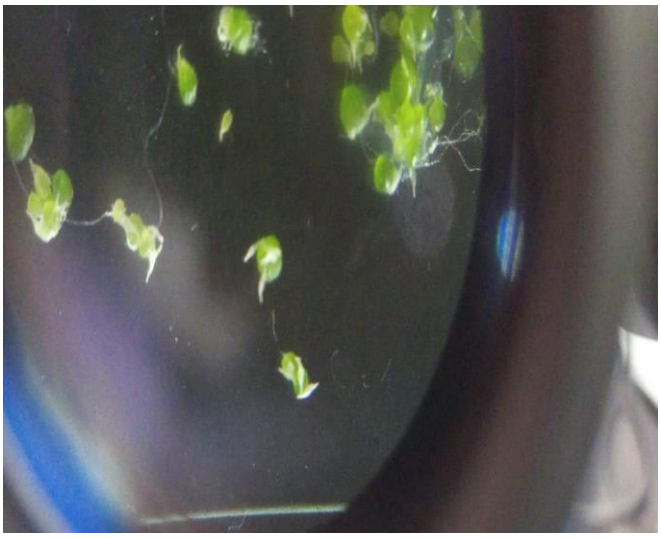

Photo 4: Protocorms Like Bodies (PLB)

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