A Review on Phytochemical Production from Orchid Culture

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Abstract

The family Orchidaceae holds quite good place in floriculture industry. Several important medicinal compounds with diverse biological activity including, antimicrobial, antiallergic, anti-inflammatory, cytotoxicity activities and phytotoxicity have been demonstrated from different species of orchids. Stilbene, denbinobin, moscatilin, dendrobin are some common phytochemicals isolated from orchids. Such phytochemicals may serve to be best sources for phytomedicine. The wild population of orchids are depleting due to extensive utilization and impact of changing environment *In vitro* culture technique provides opportunity for mass multiplication and large scale biomass production and this could be method for sustainable utilization of orchid resources and for discovery of novel compound without habitat destruction and disturbing wild population of orchids. Some recent reports on *in vitro* culture derived medicinal compounds have been sketched in this review.

Keywords: Orchid, Phytochemical, seed germination, Stilbene, Denbinobin

1. Introduction

Orchidaceae, with more than 28,000 species and about 736 genera, is considered a family having largest species of flowering plants (Zhang et al., 2018). The species of orchids are classified as terrestrial, epiphytic, lithophytic and saprophytic orchids. The long lasting, beautiful and diverse flower morphology makes several members of this family to be used extensively in floriculture trade (Kumaria and Tandon, 2001). The genus *Dendrobium*, *Cymbidium*, *Paphiopedilum* are some potted ornamental orchids and *Cattleya*, *Oncidium* are the genera of cut flower industry. The species of orchids are valued for their application in herbal medicine, food industry, flavouring agent and cultural values by tribes living in the world (Kumaria and Tandon, 2001). Vanillin obtained from *Vanila planifolia*, is a common flavouring agent of food products and is used in baking, ice-creams, custards, pudding etc. The use of orchids since long back in Chinese medicine has stated that cultivation of orchids probably initiated with its use as medicinal purpose and was first cultivated in China and Japan prior to their use as ornamental plants (Bulpitt, 2005; Singh and Duggal, 2009). The orchids including *Gastrodia alata*, *Gymnadenia*, *Bulbophyllum*, *Bletilla striata*, *D. Officinale*, *D. Nobile*, *D. fimbriatum*, *D. Chrysotoxum*, are the ingredients of Chinese medicine (Singh and Duggal, 2009). The medicinal orchids *Malaxis muscifrea*, *Malaxis acuminate*, *Habenaria intermedia* and *Habenaria edgeworthii* are constituents of Asthavarga, an ingredient of ayurvedic preparations such as Chavyanprasa (Singh and Duggal, 2009). In Sowa-Rigpa system of traditional Indian medicine *Dendrobium densiflorum*, *Cypripedium himalaicum*, *Gymnadenia conopsea* and *G. orchidis* are common ingradients (Singh and Duggal, 2009). The diverse medicinal applications are ascribed to phytochemicals accumulated in different parts of orchids. In recent past, considerable emphasis has been given to the discovery of plant based live saving therapeutic chemicals, but studies on orchids derived phytochemical are still very limited.

The wild population of orchids are depleting to low number as a result of habitat destruction and several anthropogenic pressure. A few orchids including *Pleione lagenaria*, *Aphyllorchis gollani*, *Anoectochilus rotundifolius*, *Paphiopedilum charlesworthii*, *Coelogyne treutleri*, and *Vanda wightii* are plausibly no longer existing in Indian habitat and several Indian orchids are threatened to their existence (Chug et al., 2009). Use of plant tissue culture, for large scale biomass production and use of such biomass for the production of structurally complex and high demanding useful medicinal compounds is considered preferable over the collection of orchids from their natural population. This review is an updated compilation of recent available literature of medicinal orchids with emphasis on their *in vitro* regeneration and phytochemical production, which in future can help in exploring plants derived therapeutic drugs.

2. Difficulties in orchid multiplication

Orchids are considered highly threatened among the angiospermic plants. Orchids can multiply both asexually and sexually. However, multiplication of orchid is quite slow in

contrast to their demand for commercial need. Vegetative propagation methods such as cutting, division, back bulb are some common methods of asexual propagation (Chug et al., 2009). The seeds lack cotyledons and endosperm and shows availability of insufficient nutrient reserves which is necessary for their germination. They have varied suspensor morphology. For germination seeds of orchid, their association with suitable fungus is necessary to fulfill the essential physical and chemical stimulus required for germination of seeds and development of seedlings (Chug et al., 2009). Germination of a few seed in spite of millions produced sexually in each capsule restricts fast multiplication of most orchid species. Like other plants, orchid species are also affected by unsustainable harvest for commercial applications, pressures such as habitat destruction, climate change. However, their complex interactions with mycorrhizal fungi, pollinators, and plants on which they inhabit, makes them at very high risk as they are dependent on other organisms that are also being affected by climatic change, anthropogenic activity or habitat destruction. Thus, orchids show very high challenges than many other plant groups.

2. Plant tissue culture and phytochemical production

The utilization of plant resources in phytochemical production has constrains such as slow growth of several medicinal plants, many plants are susceptible to environmental conditions. The extensive use of natural plant resources is resulted in extinction of some medicinal plants and several plants are endangered and cannot be harvested for isolation of phytochemical. Moreover, the amounts of extracted phytochemicals are inconsistent and usually show large fluctuations. Plant tissue culture technique is the aseptic *in vitro* culture of plant cell tissue or organ which provides large scale propagation of plants under controlled experimental conditions without any seasonal variations and environmental hazards. This technique has been utilized for production of phytochemicals since past several years. The plant tissue culture could overcome the danger of overexploitation of medicinally useful plants growing in nature, by using the alternative method for production of plants biomass at large scale in laboratory under controlled conditions. In cultures, phytochemical production can be carried out throughout the year. Cell cultures provide the possibility of bioconversion of low value compounds into high value compounds. Production of some novel compounds not produced in natural plants is a promising application of biotransformation (Singh et al., 2013).

4. Phytochemicals from orchid cultures

The increasing demand for natural chemical compound from orchids has focused on the utilization of *in vitro* cultures for accumulation and production of bioactive secondary metabolites. Production of bioactive phytochemicals from plant tissue culture is an efficient and alternative method to conventional practices for orchid multiplication and bioactive metabolite production. The increasing demand on natural phytochemical has focused attention on *in vitro* plant materials as potential factories for secondary phytochemical products. The potential of plant cell, tissue, and organ cultures to accumulate and produce a wide range of the similar valuable chemical compounds as in nature by the parent plant has been reported almost since the establishment of *in vitro* tissue culture technology (Singh et al., 2013). Although, a significant number of orchids of medicinal application are multiplied by plant tissue culture technique but practice of *in vitro* cultures for valuable chemical compound production from orchids is infrequent. Accumulation of chemical compounds with biological activity in cultures of orchids is demonstrated in some studies and a few are listed in table 1.

Table 1: Some phytochemicals produced from *in vitro* cultures of orchids

Orchid Name	Culture type	Medium	Phytochemical/Activity	Reference
Ansellia africana	PLBs	MS + 5 µM TDZ + 15 µM *m*T and 5 µM NAA	Phenolic acids, antioxidant activity	Bhattacharyya et al., 2019
Dendrobium aphyllum	Complete plantlets	Shoot: MS + 15 µM meta-topolin + 10 µM TDZ + 10 µM $AgNO_3$	antioxidants	Bhattacharyya et al., 2018

		Root: MS + 15 µM IBA		
D. candidum	Protocorm suspension culture	MS + 0.5 mg/lNAA+5.25mM $NH_4NO_3$2.5% sucrose1% banana homogenate	Polysaccharides, polyphenolics, flavonoids, coumarins	Cui et al., 2015
Dendrobium fimbriatum	Shoot, root and leaves from plantlets	MS + 8.88 µM NAA	β-sitosterol	Paul et al., 2017
D. nobile	Plantlet	MS + 3.5 mg/l TDZ	Phenolic, flavonoid,tannin	Bhattacharyya et al., 2014
Dendrobium thyrsiflorum	Plantlet	MS + 1 mg/l BA + 0.5 mg/l phloroglucinol.	Antioxidants	Bhattacharyya et al., 2018

Numerous factors including age of culture, concentration and type of carbon source, phosphorus and nitrogen concentration in the medium, concentration of growth regulator etc. effect the biosynthesis and accumulation of secondary metabolites in cultured cells and tissues. The *in vitro* culture has advantage of consistent production of valuable compound with culture of selected high yielding cell line under controlled condition, moreover production of compound can be carried out throughout the year independent of any microbial contamination and free of seasonal variation. The approaches such as immobilization of cultured cells, elicitation, hairy root culture and biotransformation reactions in which a low value compound can be converted into a high value product provides efficient opportunities for optimization of compound under culture conditions (Bhojwani and Dantu, 2013). In some species, accumulation of high quantity of metabolite in culture condition in comparison to intact plant is a boon of tissue culture technique. A significantly higher amount of β-sitosterol accumulation is reported from *in vitro* regenerated plantlets of *D. fimbriatum* (Paul et al., 2017).

Cell suspension culture and bioreactors has been successfully employed for secondary metabolite production from several plant species (Bhojwani and Dantu, 2013). Optimum production of secondary metabolite in cell suspension culture generally depends on composition of culture media, carbon source and its concentration, organic additives, inoculums and cell density in culture, agitation speed, and addition of precursors or elicitors. Agitation of cell suspension causes proper aeration and facilitates proper mixing of nutrient medium and homogenous availability of nutrient to the cells, resulting in more biomass generation and accumulation of secondary metabolite (Bhojwani and Dantu, 2013). They found that the addition of caffeic acid resulted in high accumulation of phenolic and flavonoid content while with p-coumaric acid highest amount of tannin was recorded. Cell suspension culture is established and characterised from friable callus of *V. planifolia* (Mosqueda and Andreu, 2017).

The use of bioreactors for large-scale biomass cultivation has become feasible for the phytochemical production. Different types of bioreactors, such as airlift balloon, bubble column and temporary immersion bioreactors with a few modifications in their structure and working are being used for enhanced biomass and phytochemical production. Protocorms derived from nodal segments of *D. candidum* resulted in maximum biomass and optimum production of coumarins polyphenolics, polysaccharides, and flavonoids when inoculated in balloon-type bubble bioreactor (Cui et al., 2015). In continuous immersion type of bioreactors, bioactive compounds like kinsenoside and polysaccharides were produced on a large-scale by using rhizomes of *Anoectochilus roxburghii* (Jin et al., 2017). There are limited reports available on the use of bioreactors for large-scale biomass production and an increase in secondary metabolites.

5. Conclusion and future prospects

Orchids are the source of several therapeutically useful chemical compounds. Medicinally and ornamentally useful orchids are needed at very high scale. Natural and conventional methods of multiplication of orchids are quite slow and to fulfil the demand there is necessity to multiply them on large scale through alternative method. Number *in vitro* multiplication protocols of orchids have been developed. Studies on production of medicinally useful chemical compounds from cultured cells and tissues are limited. Extensive research is needed to culture medicinally demanded species of orchids and explores the possibility of accumulation of useful metabolites from the established cultures. Cost effective protocols can be developed by manipulating the constituent of culture medium. Only a few reports are available on metabolic pathway engineering. Studies should also be focussed to explore the metabolic engineering techniques including over-expression of gene(s) associated with the metabolic pathway for enhanced production.

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