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Plant Breeding Techniques in Tissue Culture to Improve the Quality of Orchids

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ABSTRACT

Conventional orchid cultivation is thought to be ineffective and time-consuming. Simple tissue culture procedures are insufficient to increase orchid quality. The need for ongoing development can be met by integrating different plant breeding strategies in orchid tissue culture, such as induction mutation, elicitor addition, and genetic transformation. The purpose of this article was to report on current improvements in the use of plant breeding techniques to orchid tissue culture to increase orchid quality. This article referenced significant scientific publications. Orchid tissue culture using mutation induction is used to develop improved variations. Giving elicitors can cause orchid plants to conserve themselves and become more resistant to diseases. The required transgene can be introduced into the genome of cultivated orchids via Agrobacterium. These advancements have the potential to revolutionize orchid cultivation.

Keywords: biotechnology, orchid, tissue culture

INTRODUCTION

Orchids (Orchidaceae) are one of the families with the most prominent members in the Angiosperm group, which has 17,000–35,000 members, including the genera *Phalaenopsis, Vanda, Dendrobium, and Bulbophyllum* (Figure 1) (Suetsugu 2020). Orchids are ornamental plants with varied colors, sizes, and flower shapes, so they have high aesthetic value. Another characteristic is that the flower has a relatively longer bloom than other ornamental plants, which impacts the high economic value of orchids (Yasmin *et al.* 2018). Orchids are propagated generatively through seeds and vegetatively through *splits* and *keiki*. Germination of orchid seeds is complicated and can only germinate when the fungus infects through the suspensor (Solichatun *et al.* 2020). Meanwhile, propagation through *split* and *keiki* must wait for mature orchid plants. Generative and vegetative propagation of orchids is still very slow and takes a long time (Lala and Singh 2020). A more effective and fast method is needed for orchid plant propagation. Using tissue culture techniques in its application can be a promising alternative for orchid plant propagation (Pujasatria *et al.*2020).

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Tissue culture is a modified technique through *in vitro* plant propagation. Plant parts (cells, tissues, and organs) can be grown in culture media under aseptic conditions. The main characteristics of tissue culture are explants selected in a healthy state, a medium with complete nutrition, and controlled environmental conditions (Vargas and Neftali 2018). Tissue culture is a well-established method for effective propagation that offers large-scale production capabilities and ensures the stability of clones. It can be cultivated in various seasons and climates (Silva *et al.* 2015).

Tissue culture applications continue to be developed by combining other strategies or protocols for orchid plant breeding. This combination can improve the quality of the orchid plant more with the expected character. Some plant breeding techniques combined with tissue culture include mutation induction, elicitors administration, and genetic transformation. Mutation induction is carried out to obtain a high diversity of variants, which will be selected based on the desired traits (Setiawan *et al.* 2015). Elicitors are given to plants to make them more resistant to the attack of specific pathogens. Plants fed with elicitors can form secondary metabolites in response to stress or pathogen attack (Ningsih 2014). Genetic transformation is a method of inserting the desired exogenous into the plant genome by involving vectors. This technique aims to obtain superior plant properties as desired (Irsyadi *et al.* 2022). Many studies have widely applied efforts to improve the quality of orchids by combining each of these techniques with tissue culture.

Figure 1 Orchid flower collection. a) *Phalaenopsis amabilis*, b) *Vanda tricolor*, c) *Dendrobium linearifolium*, and d) *Bulbophyllum lobbii.*

This article focuses on reporting the application of various plant breeding techniques through tissue culture to comprehensively improve orchid quality.

METHODS

This study conducted a review of scientific literature. The sources comprised journal articles and reference texts focused on the application of tissue culture and biotechnology in orchids. Acquisition of literature articles sourced directly from the journal website pertinent to this research topic. Articles were filtered according to their titles and abstracts in relation to the content of the discussion. The analysis of the filtered articles was conducted qualitatively, focusing on the intersection of content and context. This analysis facilitates comprehension of the acquired articles and identifies correlations among them, yielding significant information that supports the established research objectives. The results served as the foundation for the development of this article in the form of a structured report (Putri *et al*. 2023).

RESULTS AND DISCUSSION

Orchid Tissue Culture Media

Table 1 presents a variety of media for each orchid species. These media can be used to treat plant breeding techniques according to the type of orchid being cultured. MS media (Murashige and Skoog) is the most used medium in various types of orchids through tissue culture techniques (Paul *et al.* (2012). MS media with additional concentrations of appropriate growth regulators can provide a good growth response for cultured orchids. *Dendrobium aqueum* inoculated in 1/2 MS medium with the addition of cytokinin hormones (BA, 2iP, KIN, and TDZ), natural additives (BP and CW), and auxins (IBA, NAA, 2,4-D) at different concentrations was able to produce successfully acclimatized plantlets (Parthiban *et al.* 2015).

A study by Wang (2006) reported that MS media can give an excellent response to the induction of flowering. MS media with NAA combined with TDZ is the most effective combination in inducing *Dendrobium candidum* flowers. TDZ is reported to be more effective in inducing flowering than BA.

Mutation Induction in Orchid Tissue Culture

The administration of certain mutagens to PLBs is one of the steps to improve the genetic quality of orchids *in vitro*. The goal is to obtain the desired superior traits compared to their elders. The success of using mutagen in orchid plants depends on the type of mutagen, mutation technique, plant sensitivity, and resistance level. One of the mutagens induced in PLBs is ultraviolet irradiation (UV254) and ethyl methane sulfonate (EMS). UV254 light triggers mutations and the production of new genetic variations, such as variations in flower color (Li *et al.* 2021).

EMS is a chemical mutagen that triggers alkylation so that it causes mutations. UV254 irradiation induction in PLBs *Phalaenopsis* sp. with a duration of 5' on, 85' off; 10' on, 80' off produces mutants that are different from *the* wild type phenotypically (purplish leaf base, wavy leaf edges, white-spotted leaves, yellow color on some sides of the leaf, and twisted leaves). The administration of 0.05% EMS to PLBs resulted in a visually larger size than the wild type (Kurniadi *et al.* 2023). The administration of colchicine mutagen to PLBs of *Dendrobium gabriella* Suryajaya orchids has also been carried out. Colchicine is one of the chemical mutagens as an antimitotic agent. Colchicine triggers chromosomal duplication and can induce anggren explants in PLBs and calluses. Amanda *et al.* (2023) reported that 0.02% colchicine can increase plant height, root number, leaf number, and shoot number in PLBs *Dendrobium gabriella* Suryajaya.

Gamma-irradiated mutagen has been used in orchid plant breeding. This mutagen can help plants handle abiotic and biotic stress better. Gamma-irradiated

mutants leave no residue when applied to plants. Romeida *et al*. (2012) found that after gamma irradiation, the protocorm *Spathoglottis plicata* showed morphological changes at LD_{50} dosages. Gamma irradiation at 40-60 Gy results in the production of variegata PLBs. Handini *et al*. (2020) proposed that gamma irradiation at LD_{20} and LD_{50} dosages of 17.08 and 27.29 Gy produced the putative mutant *Cymbidium hartinahianum*.

Utilization of Elicitors in Orchid Tissue Culture

Elicitors are molecules that can stimulate plant protection to synthesize secondary metabolites to respond to shock conditions. There are two types of elicitors, namely abiotic and biotic. Salicylic acid is one example of an abiotic elicitor. This compound contains aromatic rings and is known to trigger increased plant resistance. This compound can increase the chlorophyll content in *Dendrobium* plantlets (Syahfitri *et al.* 2022). Chitosan, as a biotic elicitor, can also stimulate the production of secondary metabolites by signaling an enzyme to encode the synthesis of metabolites such as flavonoids, phytoalexins, and lignin. Chitosan can provide the best growth based on plant height, number of leaves, leaf size, number of roots, root length, and fresh weight *of Dendrobium sonia* (Bani *et al.* 2022).

Some microbes in nature can also be used as elixirs. *Bacillus subtilis* associated with *Vanda cristata* can synthesize auxin, ammonia, and phosphate. These biological elicitors can trigger the development of roots and shoots *of Cymbidium aloifolium* (Chan *et al.* 2023). The fungus type *Coniochaeta* sp. can also be used as an elicitor. The fungi given to plants synthesize oleic acid, D-mannitol, cyclopropane carboxylic acid, and 2H-pyran-2-one. *Coniochaeta* sp. can form root pelotones and increase the acclimatization of *Cymbidium aloifolium* plantlets (Shah *et al.* 2022). The use of elicitors for orchid growth can be seen in Table 2.

Genetic Transformation in Orchid Tissue Culture

Genetic transformation in plants is one of the alternative ways to improve quality, yield, and resistance to biotic/abiotic stress. The techniques used in the genetic transformation process are grouped into two, namely, using external energy and vectors (*Agobacterium tumefaciens*). The desired gene transformation can be done by inserting the gene in the T-DNA region of the Ti *Agobacterium tumefaciens*

Table 1 Some media compositions used in tissue cultures of various orchid species

Species	Media culture	Reference
Cymbidium goeringii	MS media with a combination of coconut water or BA and NAA	Yong *et al.* 2018
Cymbidium ensifolium	MS media with BAP, NAA, KN and IBA	Ramesh *et al.* 2019
Dendrobium houshanase	Media MS (2,4-D and TDZ)	Lun and Tsung 2014
Dendrobium candidum	MS Media with BA, NAA, ABA	Cui *et al.* 2015
Dendrobium moniliforme	Media MS (TDZ or PBZ + ABA) → Flower induction MS media (PBZ + TDZ) → Flowering	Wang *et al.* 2006
Dendrobium nobile Lindl.	MS media with NAA	Bhattacharyya *et al.* 2016
Dendrobium officinale	MS with BA NAA	Chen *et al.* 2014
Dendrobium strongylanthum	MS media with single-factor PGR and multi-factor PGR treatment	Zhao *et al.* 2012
Dendrobium palpebrae	MS media with IAA, IBA, NAA	Bhowmik and Rahman 2017
Dendrobium capra	MS media with NAA, TDZ and GA_3	Lawrie *et al.* 2021
Dendrobium primulinum	MS media with NAA and BA	Pant and Thapa 2016
Dendrobium 'Sonia 17'	MS media with vitamin B5 + 20 µM BA	Muna *et al.* 2016
Dendrobium chryseum	MS media with Kn, BAP and GA_3	Maharjan *et al.* 2020
Eulophia graminea	MS media with coconut milk, potato powder, peptone, and AC	Romeida *et al.* 2018
Gastrochilus matsuran (Makino).	MS media with coconut water, NAA, GA_3	Kang *et al.* 2020
Geodorum densiflorum (Lam.)	MS media with BAP and TDZ	Bhattacharyya and Banerje 2020
Phalaenopsis amabilis	NP media with BA and GA_3	Semiarti *et al.* 2014
Phalaenopsis Pink Leopard ("Petra")	VW Media + 22 µM BA	Khatun *et al.* 2020
Phalaenopsis Cygnus 'Silky Moon'	VW media with BA	Rojanawong *et al.* 2006
Oncidium varicosum Mericlones	VW media with coconut water and NAA	Cardoso *et al.* 2020

plasmid (Yan *et al*. 2022). Integrating foreign genes can create better results in transgenic strains than conventional breeding. According to Hxing *et al*. (2016), the most used orchid host tissues are PLB (*Protocorm Like Bodies*) and protocorm. The advantage of PLB is that it provides genetic diversity, and various somatic tissues can be used for induction. The protocol transformation method is simpler than using PLB.

Genetic transformation has been widely applied to many plants, including orchids. Some studies related to the application of genetic transformation to orchid tissue culture in the last 10 years are presented in Table 3. The *OsGA2ox6 gene* was once integrated into the Phalaenopsis genome to reduce the height of orchids to produce mini-Phalaenopsis. This technique aims to expand the appeal of the orchid to other consumer groups who prefer to keep flowers on the table (Hsieh *et al.* 2020). Dendrobium ornamental orchids are short-lived due to excess ethylene gas production. Using genetic transformation techniques, Sornchat *et al.* (2015) successfully integrated the ACO1 gene from papaya to reduce ethylene gas production in *Dendrobium* orchids.

CONCLUSION

Combining tissue culture techniques with breeding techniques can improve the quality of orchids. Granting mutagen to orchid tissue culture can increase genetic diversity so that superior traits can be selected without being constrained by germination failure. Elicitors can increase the protection of orchid plants against abiotic and biotic stress during culture. PLB and orchid protocorms can be hosts for certain gene transformations to produce better-quality GMO orchids.

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Table 2 Types of elicitor that can be used for orchid growth

Orchid species	Elicitor type	Elicitor concentration	Reference
Dendrobium	Salicylic acid	75 and 100 ppm	Syahfitri *et al.* 2022
Dendrobium sonia	Chitosan	3 ppm	Bani *et al.* 2022
Cymbidium aloifolium.	*Bacillus subtilis*	2%	Chand *et al.* 2023
Cymbidium aloifolium.	*Coniochaeta* sp.	0.4 mL	Shah *et al.* 2022

Table 3 Genetic transformation in orchid tissue culture

Species	Transformation method	Target tissue	Transgene	Transgene function	Reference
Dendrobium lasianthera	*A. tumefaciens* strain LBA4404	Protocorm	KNAT1	Development of the stem end meristem	Utami *et al.* 2018
Phalaenopsis amabilis	*A. tumefaciens* strain LBA4404	Protocorm	PaFT	Flowering induction	Semiarti *et al.* 2015
Dendrobium 5N white orchid	*A. tumefaciens* strain AGL1	PLB	AcF3H	Anthocyanin biosynthesis	Khumkarjorn *et al.* 2017.
Phalaenopsis "Sogo vivien"	*A. tumefaciens* EHA 105	PLB	RKD4	Embryo pattern formation	Mursyanti *et al.* 2015
Dendrobium phalaenopsis	*A. tumefaciens* EHA 105	PLB	RKD4	Embryo pattern formation	Zulwanis *et al.* 2019
Phalaenopsis Sogo Yukidian ‘SPM313’	*A. tumefaciens* EHA 105	PLB	OsGA2ox6	Reduce the height of the orchid to develop mini ornamental orchids	Hsieh *et al.* 2020

Bhattacharyya S, Banerjee N. 2020. Influence of cytokinins on rhizome mediated growth and morphogenesis of an endangered medicinal orchid *Geodorum densiflorum* (Lam.) Schltr. *Plant Tissue Culture and Biotechnology.* 30(1): 65–75. https://doi.org/10.3329/ptcb.v30i1.47792

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