CELL BIOLOGY & DEVELOPMENT
Volume 5, Number 2, December 2021 E-ISSN: 2580-4499
Pages: 51-62 DOI: 10.13057/cellbioldev/t050201

Review: Factors affecting mass propagation of *Vanda* orchid in vitro

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Manuscript received: 18 June 2021. Revision accepted: 9 July 2021.

Abstract. *Setiaji A, Annisa RRR, Santoso AD, Kinasih A, Riyadi ADR. 2021. Review: Factors affecting mass propagation of Vanda orchid in vitro. Cell Biol Dev 5: 51-62.* For the past decade, *Vanda* has been the primadonna of ornamental orchids in the south and southeast Asia, along with *Phalaenopsis* and *Dendrobium*. Along with the increase in demand for *Vanda*, this genus has faced several threats, from illegal collection to habitat loss. Therefore, mass propagation through in vitro culture is a promising strategy to ensure sustainable business in horticulture and conservation. This review provides an overview and synthesizes various *Vanda* in vitro culture literature. We showed the researchers' preferences on several aspects for growing *Vanda*, including species, basal medium, plant growth regulators, explant, and culture conditions. The most commonly used as explants are seeds or protocorms, growing on Murashige & Skoog or Vacin & Went medium. This medium can be added banana homogenate to increase its nutritional value. *Vanda* seedlings can be incubated at 25 ± 1-3°C, with a lighting intensity of 50 µmol m^{-2} s^{-1} at 12/16 h PP. Choosing a medium that is cheaper but still rich in nutrients and its additives, especially during the subculture phase; selecting explants that are responsive and minimizing the possibility of contamination; as well as seeing the target market in particular, can make *Vanda*'s propagation efforts more effective, efficient, and profitable.

Keywords: Efficient protocols, in vitro culture, Orchidaceae, *Vanda*

Abbreviations: KC: Knudson's C medium (Knudson 1922); Mitra: Mitra et al. (1976) medium; MS: Murashige and Skoog (1962) medium; ND: New Dogashima medium (Tokuhara and Mii 1993); Nitsch: Nitsch and Nitsch (1969) medium; NP: New Phalaenopsis medium (Ichihashi 1992); P723: P723 Orchid Seed Sowing Medium (PhytoTechnology Laboratories, Inc.); RT: Raghavan and Torrey (1964) medium; SH: Schenk and Hildebrandt (1972) medium; VW: Vacin and Went (1949) medium; 2,4-D: 2,4-dichlorophenoxyacetic acid; 2-iP: N6-isopentenyladenine; BAP: 6-benzylaminopurine; IAA: indole-3-acetic acid; IBA: indole-3-butyric acid; Kn: kinetin; NAA: a-naphthaleneacetic acid; TDZ: thidiazuron; BP: banana pulp; CH: casein hydroxylate; CM: coconut milk; CW: coconut water; PP: potato pulp; TJ: tomato juice; YE: yeast extract; PVP: polyvinyl pyrrolidone; PLB: protocorm like bodies; RH: Relative Humidity; PP: photoperiod

INTRODUCTION

Orchidaceae is the second largest family of angiosperms after Asteraceae. There are many approximate versions of orchid species numbers because almost every expedition in biodiversity hotspots is reported to discover new species. A provisional checklist suggests 28,000 species of orchids, including 736 recognized genera, represent 8% of species of the angiosperms (Chase et al. 2015; Christenhusz and Byng 2016; Willis 2017). It exceeded the estimated number (25,000) (Atwood 1986).

The fascinating charisma of orchids defines them as ornamental plants in terms of color and the uniqueness of the flower shape. Orchid flowers could be kept indoors in fresh conditions for a long time as a symbol of beauty (Rahman et al. 2008). Cut flowers of the hybrids of *Mokara*, *Dendrobium*, and *Vanda* remain fresh for 7-30 days, while *Phalaenopsis* and *Cattleya* remain fresh for 1-4 weeks and 18-28 days for *Aranda* (De et al. 2014). In addition, orchid flowers have persistent perianth characters, unlike other cut flowers that easily fall off (Rahman et al. 2009). Today, orchid cultivation is an international business with great potential to participate in countries' economic growth. In the world floriculture trade, around 8% of sales are covered by orchids (Martin and Madassery 2006). In the ornamental plant industry, they are the second favorite cut flowers and potted plants (Hossain 2008).

One of the widely cultivated orchids in Southeast Asia and the Indian subcontinent is the genus *Vanda*, which was established by Sir W. Jones in 1795. His type species of this genus is *Vanda roxburghi*. *Vanda* is a monopodial orchid and mostly epiphytic (Islam et al. 2014). About 184 plant species are native to China, the Himalayas, Bangladesh, Indonesia, and northern Australia, of which 62 are accepted names, 122 are synonyms, and 5 remain unresolved (The Plant List 2019).

The name of *Vanda* came from an Indian language called Sanskrit (Garay 1974), which means that people like these plants by their fragrance, color, and flower shape. Many *Vanda* hybrids have characteristics preferred to mass

consumption, such as variable color pallets, fragrant flowers, free-blooming, long-lasting flowers, multiple inflorescences, compact growth habits, and cold tolerance. These superior traits make *Vanda* become great potential to dominate the American and European markets. In the 1950s, Hawaii, United States, became the center of *Vanda* orchid development, where they produced primary and secondary hybrids of *Vanda* with round and large-sized flowers. Later in the 1960s, Hawaii was replaced by Thailand. *Vanda* orchid breeders in Thailand produce more complex *Vanda* hybrids due to the segregation of progeny genes with new flower colors and shapes (Motes 2004).

One of the obstacles of *Vanda* cultivation is it requires three or more years of maintenance to reach flowering size since deflasking (compared to *Phalaenopsis*, which requires only 18–24 months). In addition, small-scale production and duration of *Vanda* culture cause the relatively high per-unit cost of production, which causes high selling prices at the farm level (Johnson and Kane 2007). Like some other orchid genera, Vanda was also threatened by habitat destruction, climate change, and unsustainable harvest (often illegal) for horticulture, food, or medicine (Fay 2018).

Mass production of orchids is important to meet the demand of orchid consumers and innovation for the world floriculture industry. There are many ways to propagate orchids. The conventional propagation was the separation of pseudobulb clumps and keiki. Still, these methods are unsuitable for mass production because they are inefficient in time and space and have high risks of the parent plant's death. Tissue culture is now an established effective propagation method, offers large-scale productions, and ensures clonal stability, irrespective of season and weather (Singh and Duggal 2009; Teixeira da Silva et al. 2015). Knudson (1922) developed a protocol for asymbiotic in vitro orchid seed germination on a medium containing mineral nutrients and sugar. The first experiments on *Vanda* in vitro culture were carried out at the University of Singapore with callus derived from seedlings in undefined media containing tomato juice and 2,4-Dichlorophenoxy acetic acid (2,4-D) (Rao 1963; Rao 1967). This technique continues to be developed, including applied along with genetic engineering, and becomes an important method for mass-scale propagation and conservation of orchid species. The main objective of this review is to provide a thorough understanding of *Vanda's* germplasm response to in vitro conditions by compiling what is known from various published literature and research. Data representation in the chart is based on the proportion of the number of times a study is to the total number of outcomes. Detailed information is shown in Table S1.

IN VITRO PROPAGATION FOR CONSERVATION AND SUSTAINABLE UTILIZATION

Vanda is important in the ecosystem, especially related to the host plant and its symbiotic microorganisms. Its aerial roots can absorb and retain moisture, attracting various bacteria and fungi for symbiosis, beginning with seed germination. These microorganisms provide nutrients and increase resistance against pathogens through induced systemic response (ISR) mechanisms and metabolites excreted by these microbes (Pieterse et al. 2014). These microorganisms, which are generally bacteria, known as plant growth-promoting rhizospheric bacteria (PGPR), can be isolated and applied to other plants as biocontrol agents and biostimulants (Glick 2015). *Vanda*'s ethnobotany is about aesthetics and promises the exploration of various compounds for health benefits.

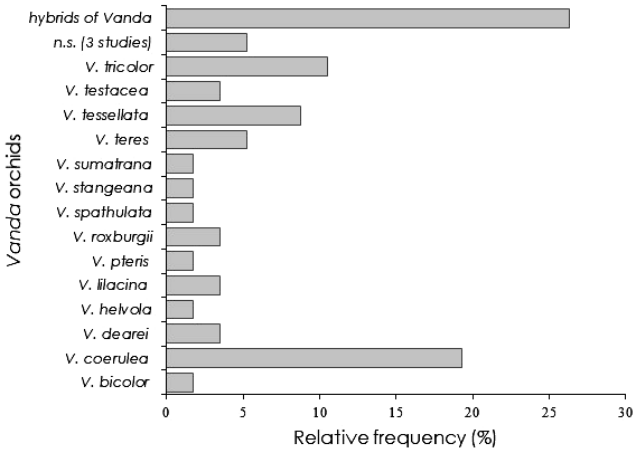

Figure 1. Micropropagation studies' relative frequency (%) shows various *Vanda* species and hybrids. Some of the studies use more than one type of orchid. n.s. not specified.

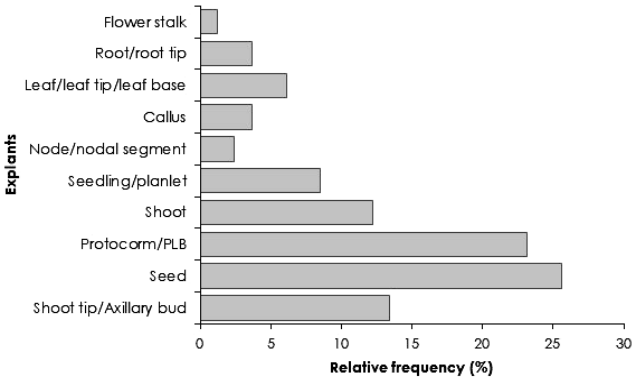

Figure 2. The relative frequency (%) of micropropagation substudies shows different explants used in *Vanda* in vitro culture. Some studies used more than one explants.

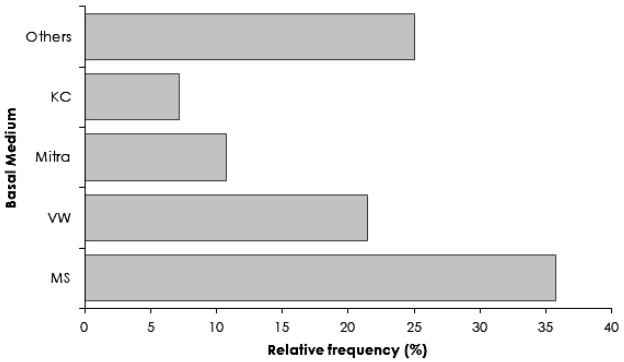

Figure 3. Relative frequency (%) of micropropagation substudies showing basal medium used in *Vanda* in vitro culture. Some of the studies used more than one basal medium

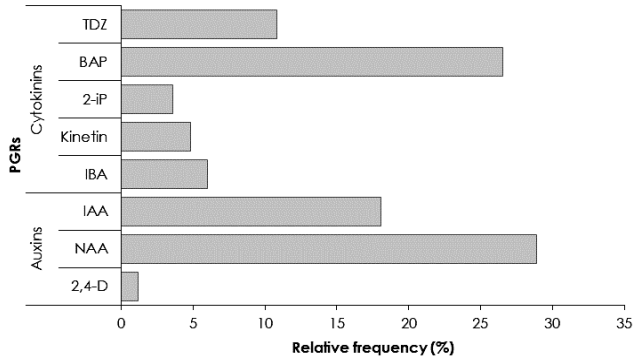

Figure 6. The relative frequency (%) of micropropagation substudies shows different plant growth regulators (PGRs) used in *Vanda* in vitro culture. Some of the studies may use more than one substudies

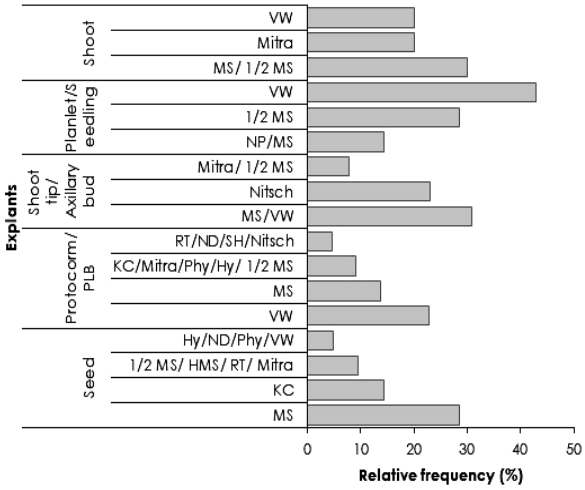

Figure 4. Relative frequency (%) of micropropagation studies showing basal medium used based on explant types in *Vanda* in vitro culture. A study may have one or more types of explant

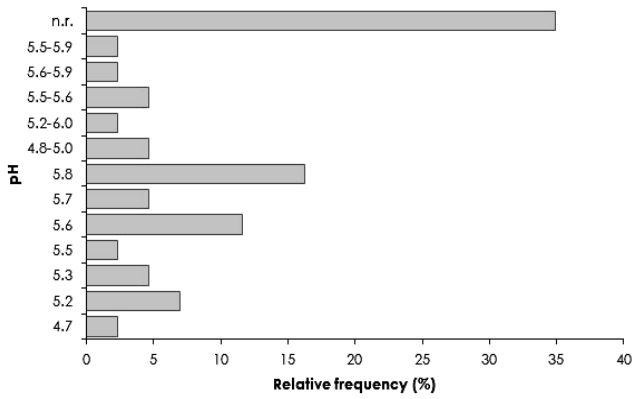

Figure 5. Relative frequency (%) of micropropagation studies showing different pHs used of the basal medium in *Vanda* in vitro culture. n.r. not reported

In addition to medicinal uses, especially in India, Nepal, China, and Bangladesh, Khan et al. (2019) have summarized the use of *Vanda* in traditional medicine and its bioactive compounds. The class of compounds detected included eucomic acid and its derivatives from *V. teres* (Simmler et al. 2011); Phenanthrene derivatives from *V. tessellata*, *V. parviflora*, and *V. coerulea* (Anuradha et al. 2008; Anuradha and Rao, 1998; Simmler et al. 2010); Bibenzyl derivatives from *V. coerulea* and *V. roxhburghii* (Simmler et al. 2010; Uddin et al. 2015); Phenolic compounds from *V. roxburghii*, *V. parishii*, and *V. tessellata* (Chawla et al. 1992; Dahmén and Leander 1976; Prakash and Bais 2016); Anthocyanins from *V.* hybrid (*V. teres* x *V. hookeriana*) (Junka et al. 2012); Alkaloids from *V. hindsii* (Brandange and Granelli 1973); Steroids and triterpenoids from *V. roxburghii* (Mohammed-Usman et al. 2012). Based on the examination of *Vanda* extracts of various species, these orchids are known to have pharmacological activities like anti-inflammatory, antioxidant neuroprotective, membrane stabilizing, anti-aging, hepatoprotective, antimicrobial, and wound healing activities (Khan et al. 2019). Traditional uses usually treat rheumatism, dyspepsia, indigestion, piles, wounds, bronchitis, and hepatitis (Khan et al. 2019). However, more research is needed to select unique compounds with strong bioactivity potential. A further prospect is the extraction of materials with whole plants that need to be replaced with plant materials from in vitro cultures, such as callus cultures and cell suspensions. In vitro culture can provide optimal conditions for synthesizing these compounds with additional precursors, and the amount of the yield can be standardized (Setiaji et al. 2020).

Due to the limited population size and providing expected properties in medicine, and the rise of vulnerability in the future, conservation efforts are important. The reintroduction of *Vanda* is mostly undertaken by returning plants taken from nature and donations from certain nurseries. Mass breeding through tissue culture promises to speed up the reintroduction and translocation of orchids. However, these two processes are important, and conservation programs' primary goals

should include maintaining, managing, and restoring habitats that support orchid populations. In vitro propagation studies provide a starting point for conservation efforts. Unfortunately, research publications about *Vanda* have not been as much as *Dendrobium*, *Phalaenopsis*, *Oncidium*, *Cattleya*, and another popular genus of orchids that may have reached hundreds. Still, this information could be used as initial information to develop in vitro *Vanda* cultivation.

Vanda breeding, both with genetic transformation and interspecific hybrids, is the consumer's favorite since they have various choices in flower colors and other superior properties. On the other hand, wild *Vanda* tends to have some disadvantages, such as difficulty adjusting the growth to the local climate, usually does not meet the dosage of commercial fertilizers and hormones (maintainers need to determine the optimal dose for the orchid by themselves), and more expensive. So far, the relative frequency of research conducting on *Vanda* hybrid micropropagation is 26.3%.

Based on the number of papers that have been published, species of *V. coerulea* (19.3%), *V. tricolor* (10.6%), and *V. tesselata* (8.8%) are the most widely studied (Figure 1). *V. coerulea* is one of the most popular native orchids found in the northeastern region of India, with a range of distribution extending to China (southern Yunnan). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) listed this species in appendix 2 (removed from appendix 1 due to the discovery of new populations, especially in the Himalayas region (Christophe 2012) and the global conservation status is vulnerable (Walter and Gillet 1998). Habitat loss and degradation, mainly from human activities and illegal hunting, were the major cause of the decline in the population of these orchids. In other regions, *V. tricolor* (appendix 2) also faces the same threats. It is widespread and highly cultivated in South East Asia, while the wild populations are small and highly fragmented, especially in Java and Bali (Gardiner 2007). Gardiner (2007) reported that this species has been rare in nature due to over-collecting and natural disasters such as Mount Merapi eruption, one of the most active volcanoes in Indonesia with a 4-year eruption cycle. Anticipating a similar threat, the researchers developed an in vitro propagation technique for *V. tesselata* earlier to maintain a population whose trend tended to decline even though it still had the least concern status (Khela and Chadburn 2014). The distribution of V. tesselata is broad enough to cover Bangladesh, India, Myanmar, Nepal, Sri Lanka, and Thailand.

A successful campaign of *Vanda* conservation efforts has been carried out by Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), India. The successful restoration of the *Smithsonia maculata* orchid, carried out by JNTBGRI in 1993, was expanded to other species, including *Vanda coerulea*, *Vanda wightii*, and *Vanda thwaitesii*. The success of in vitro propagation which can provide mass and quality of plants, has an important role in the success of this conservation program. Its reintroduction success reaches 80-95% after establishment for 1-2 years (Rajasekharan and Wani 2020). An integrated conservation program involving various professionals in ecology, pollination biology, tissue culture, microbiology, and genetic populations has proven effective in planning and implementing real conservation efforts.

EXPLANT, CULTURE MEDIUM AND ITS CONSTITUENTS

Explants used in *in vitro* propagation

Explant selections are an important factor to consider before initiating a culture method. Explants taken from potted plants in the greenhouse (ex vitro) may carry fungal and bacterial infections due to exposure to open environments. In monopodial orchids, such as *Vanda*, choosing the shoot tip as an explant could be caused death to the mother plant since monopodial orchid relies their growth on their apical dominance. Furthermore, a flower stalk could only be obtained during the flowering season (the flowering could be induced but cannot be continuously carried out). Indeed, the flower stalk of *Vanda* has limited plantable parts; for comparison, they have a shorter length than the stalk of *Phalaenopsis*, which is commonly used as an explant for *Phalaenopsis* micropropagation. The flower stalks of *Vanda* also mature rapidly, whereas young flower stalks are known better to use as explants. In general, choosing juveniles and other young tissues over mature parts needs to be considered. Other than that, the flowering plants of *Vanda* are 2-3 times more expensive than their vegetative plants.

Seeds are the most commonly used explant for *Vanda* propagation (25.6%) (Figure 2). The next preference explants used are protocorm (23.2%), shoot/ axillary tip (13.4%), shoot (12.2%), and others below 10% (seedling, nodal segment, callus, leaf, root, and flower stalk). Seeds could provide large quantities of explants where adult orchids plant are limited. Seeds could germinate even using a basal medium without adding hormones or complex organic matter, only. Seeds will grow into protocorms and become seedlings later (Yildiz 2012). Protocorms have the flexibility to induce shoots and roots and/or reproduce secondary protocorms/ PLBs (Sujjaritthurakarn and Kanchanapoom 2011; Setiaji et al. 2018). The protocorm phase usually begins when the bipolar structure cannot be distinguished between basal and apical (Setiari et al. 2016). By definition, protocorms are produced by seeds, whereas protocorm-like bodies (PLB) are produced by explants (Lee et al. 2013).

Seed culture is probably the most effective technique so far to get lots of new seedlings, despite the long maturity time of *Vanda* fruit capsules, which could reach 6-9 months or even up to 20 months (PhytoTech Labs 2019). This problem could be overcome by applying 6-Benzylaminopurine (BAP) and gibberellic acid (GA) hormones to stimulate flowering in plants, continued by spraying 6-30-30 sodium-phosphate-potassium fertilizer after pollination for fruit ripening. However, unripe seeds can still be planted and show better results in some cases. When the seeds are ripe, the inner coat surrounding the embryo may be thickened, making it difficult for water and

nutrients to reach the seeds. In addition, some seeds may carry some poor traits that lead to nonuniformity clones. That could be avoided by choosing superior breeds and maintaining their genetic content stability. The optimum preference for explants, medium, and incubation conditions will be explained later.

Culture media

In general, the most commonly used basal medium for *Vanda* cultures is MS (35.7%) and VW (Vacin and Went, 1949) (21.4%) (Figure 3). On the other hand, 28.5% of the sub-studies using seeds were planted on Murashige and Skoog medium (1962) (Figure 4). MS is widely used in a variety of plants, including orchids. This medium contains high concentrations of ammonia, potassium, and nitrates; and is relatively cheaper than other mediums, such as the White medium (Stewart Jr 2016). On the other hand, VW media was specifically intended for orchid species at the beginning of the formulation, especially for *Cymbidium*. In this medium, $Ca_3(PO_4)_2$ is added in abundant quantities, providing phosphate to increase the formation of PLB (protocorm-like bodies) (Teixeira da Silva 2012). The seeds of 18 different orchid genera, planted on the VW medium, produce a chance of more than 70% of protocorms formation (Kartikaningrum et al. 2017).

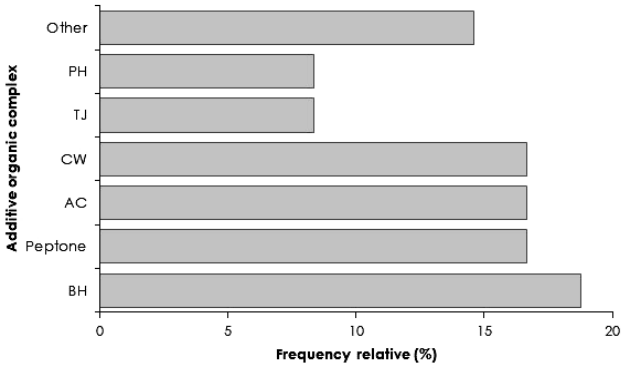

Figure 7. The relative frequency (%) of micropropagation substudies shows various additives used in *Vanda* in vitro culture. A study may have one or more substudies. PP potato pulp, TJ tomato juice, CW coconut water, AC activated charcoal, BP banana pulp

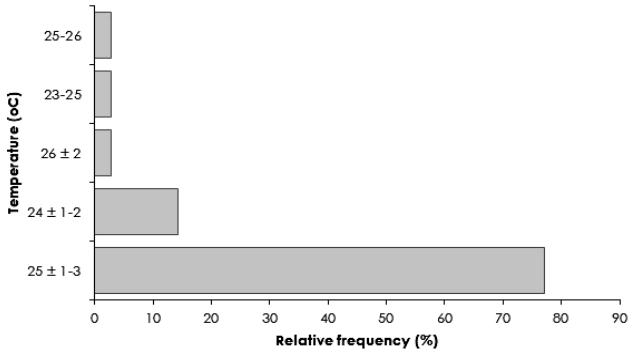

Figure 8. Relative frequency (%) of micropropagation studies showing different temperature conditions in *Vanda* in vitro culture

Table 1. Number of micropropagation studies showing different lighting conditions during the *Vanda* in vitro phase

		Photoperiod (h)				
		0	**12**	**16**	**24**	**nr**
Light intensity (µmol m^{-2} s^{-1})	25	-	-	1	-	
	30	-	-	2	-	1
	35	-	1	1	-	-
	40	-	1	1	-	-
	45	-	-	2	-	-
	50	-	4	3	-	-
	NR	1	-	2	1	-
	28-35	-	-	1	-	-
	30-50	-	1	-	-	-
	NR	4	-	-	-	-
	20-50	-	1	-	3	-
	37	-	-	1	-	-
	10	-	1	-	1	1
	9	-	-	-	2	-
	20-30	-	-	1	-	-
	56	-	-	1	-	-
	15	-	-	1	-	-
	100	-	-	-	-	1

The pH set for *Vanda* culture varies from 4.7-5.9 (Figure 5). The greatest preference was at pH 5.8 (16.3%) and after that, 5.6 and 5.2. The pH of the MS medium is usually set between 5.6-6.3. Adjusting the pH of the culture medium is important to ensure the plant's physiological processes are not disturbed. Acidic medium prevents the uptake of phosphoric acid, Ca^{2+}, and Mg^{2+}; alkalic medium prevents the uptake of iron, Cu^{2+}, Zn^{2+}, Mn^{2+}, and boron (Bell et al. 2020; Jakobsone and Osvalde 2019; Ichinose et al. 2018). In addition, pH affects the solubility and absorption of nutrients by activating certain enzymes and solidifying gelling agents; and preventing the absorption of toxic substances (Sahu et al. 2017; Lager et al. 2010). A slightly acid medium seems to be preferred by most orchids and is important for auxin action (Sarkar et al. 2009). Sachin (2015) reported that the highest protocorm formation on *V. tesselata* was observed at a temperature of 20°C and pH 5.5. It is difficult to determine whether the pH of the medium could affect the orchid seedlings because it is related to other culture media components.

Plant growth regulators

Plant growth regulators (PGRs) can be used simultaneously to match *Vanda* growth stages. The most commonly used PGRs in *Vanda* cultures, either as combined or in a single dose, are the cytokinins (6-Benzylaminopurine (BAP), kinetin (Kin), N6-isopentenyladenine (2-iP), and thidiazuron (TDZ); and auxin (indole-3-acetic acid (IAA)), indole- 3-butyric acid (IBA), 2,4-Dichlorophenoxyacetic acid (2,4-D), and a-naphthaleneacetic acid (NAA) (Fig. 6). In combination, 15.47% used higher concentrations of cytokinins such as BAP (4.44-66.6 µM), while 10.71% used a higher concentration of auxin such as NAA (0.27-8.06 µM). Single auxin (15.47%) is generally used to induce roots or germination with optimum concentrations ranging from

0.54-22.80 µM for NAA, while single cytokinin (10.71%) is generally used to induce shoots with optimum concentrations range of 0.91-11.35 µM for TDZ. The rest 41.66%, do not use any PGRs, and generally prefer to add complex organic materials for germination or seedling maintenance.

Organic complex sources

Organic complex materials contain different sucrose, fructose, agar, peptone, nicotinic acid, biotin, folic acid, auxin, glutamic acid, glycine, adenine, niacin, and nitrogen levels (Park and Yeung 2018; Acemi and Ozen 2019). Any of these components are responsible for promoting the growth and development of the cultures (Islam et al. 2015). The most commonly used complex organic materials in *Vanda* cultures are banana homogenate (18.8%), peptone (16.7%), activated charcoal (16.7%), coconut water (16.7%), tomato juice (8.3%), and potato homogenate (8.3%) (Figure 7). In *Vanda*, few papers explain the function of adding these additives because they may have complex effects and focus more on the effects of PGRs. However, the beneficial effects of complex organic materials (BH, CW, peptone) on the growth and differentiation of protocorms and seedlings have been carried out by Arditti (1979).

Banana is rich in carbohydrates, certain vitamins, minerals, carotenoids, and polyphenols. Usually, Studies that employed BH used a concentration of 3.5-15% (v/v) combined with auxin and/or auxin-cytokinin and other additives. BH might help stabilize the pH of the medium, which may change due to activated charcoal. The pH of the medium could drop due to the acid residues of HCl in AC since AC needs to be washed by HCl solutions in its production (George et al. 2008). Peptone generally consists of high tryptophan, a low molecular weight protein, vitamins, and plant growth factors. These factors may induce changes in *Vanda*, which can give plant cells an easily absorbed nitrogen source (George et al. 2008). CW can induce cell division, thus promoting early protocorm differentiation and a wide spectrum of growth factors, and has been successfully used in some orchid production (Intuwong and Sagawa 1973; Pyati et al. 2002). In epiphytic orchids, the addition of 15% CW to the basal medium can increase growth performance in various parameters: shoot length, number of roots, leaf width, leaf area, fresh and dry weight of shoots and roots, and stimulating new shoots (Baque et al. 2011; Yong et al. 2009; Paris et al. 2019). The main hormone contained in CW is IAA, while cytokinin, gibberellin, and abscisic acid are also detected (Yong et al. 2009; Tan et al. 2014).

The addition of activated charcoal improves the growth of *Vanda*. Some of the positive effects of AC are improved aeration, established polarity of microelements, stabilized substrate temperature, and adsorbs toxic substances (phenolic compounds), all because of the nature of AC which has small pores and a large surface area (Thomas 2008; Zeng et al. 2015). In addition, AC is suitable for root induction because it creates dark conditions of the medium in accordance with the underground root's original environment. AC and BAP can increase flowering frequency from 65% to 100%, increase in vitro germination and plantlet development, increase rhizome production and fresh weight gain during micropropagation, and increase the formation of orchid buds and promote bud induction of orchid seeds effectively (Thomas 2008). However, in some cases, adding 1% activated charcoal to culture media caused acidification, largely due to an increase in the hydrolysis of sucrose during sterilization (Saad and Elshahed 2012). Another disadvantage of AC is the adsorption is not selective; some beneficial substances may also be adsorbed.

INCUBATION CONDITION

Lighting, temperature, and humidity are important aspects for maintaining the incubation chamber to support plant growth and adapt to in vivo environments. However, this review does not explain the humidity conditions because too few papers have mentioned it since measuring humidity inside culture bottles may be difficult.

The studies on *Vanda* in vitro culture used the temperature ranging from 23-26ºC, but 25 ± 1-3ºC (77.1%) was most commonly used. The light intensity varies from 25 to 100 µmol m^{-2} s^{-1}, but the most widely used is 50 µmol m^{-2} s^{-1} at 12/16 h PP (7 studies). Only 5 studies report using dark culture; the light intensity is not reported (Tab. 1). All studies employ light-emitting diodes.

In conclusion, the growth of *Vanda* orchids during the in vitro phase requires optimal controlled conditions. It ensures seedlings' viability during acclimatization and uniformity during flowering induction in the greenhouse. This review attempts to infer the basic needs for in vitro culture in *Vanda* based on the preferences of previous studies. The most commonly used source of explants is seeds or protocorms planted on MS or VW medium with a pH of 5.8. Banana homogenate 3.5-15% is the most used additive. *Vanda* seedlings were mostly incubated at 25 ± 1-3ºC, with a lighting intensity of 50 µmol m^{-2} s^{-1} at 12/16 h PP. *Vanda's* in vitro culture technique still needs to be developed and expanded with the application of molecular biotechnology. The potential and uniqueness of ornamental, horticultural, and medicinal values are also slightly mentioned. This review can temporarily serve as a basis for *Vanda* producers to avoid confusion in choosing culture procedures from the various studies conducted.

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Table S1: Remarks on micropropagation of *Vanda* species and/or hybrids

Authors	Media	pH	Explant	PGR (µM)	Outcome	Additives	Incubation conditions	Spesies and/or hybrids
Aini et al. (2015)	MS	n.r.	Shoot tip	6.66 BA	PLB			*Vanda sumatrana*
Puspasari et al. (2018	NP	5.2-6.0	Seed		development of protocorm to seedling	0.1% P		*Vanda tricolor*
David et al. (2015)	KC	n.r.	Seed		development of protocorm to seedling	0.1% P	24-h PP, 25 ± 2 °C	*Vanda helvola*
	KC		Seed		Germination	10 or 15% TJ		
Begum et al. (2002)	MS	5.8	Axilary bud		Protocorm		12-h PP, 50 µmol m^{-2} s^{-1}, 26 ± 2°C, 78% RH	*Vanda pteris*
	MS		Protocorm	8.88 BA + 5.37 NAA	Shoot			
	1/2 MS		Shoot	9.84 IBA	Shoot produce root	0.1-0.2% AC + 15% BP along + 15% PP + 15% CM		
Bembecha et al. (2016)	1/2 MS	5.6-5.9	Seed		Germination		16-h PP, 28–35 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda stangeana*
	1/2 MS		Protocorm	2.7 NAA + 2.3 Kin	Protocorm multiplication			
	1/2 MS		Seedling		Planlet	3% BP		
	1/2 MS		Micropropagated shoots	5.7 NAA	Shoot produce root			
Bhattacharjee and Islam (2014)	MS	5.6-5.8	Shoot segment	5.37 NAA + 4.44 BA	Multiple shoot		16-h PP, 25 ± 2 ºC	*Vanda tessellata*
	MS		Seed		Germination			
	1/2 MS		Shoot	5.71 IAA	Shoot produce root			
Malabadi et al. (2004)	VW	5.8	Shoot tip	11.35 TDZ	PLB	0.2% CH + 0.05% L-glutamine + 0.025% P	100 µmol m^{-2} s^{-1}, 25 ± 2 ºC, 55-60% RH	*Vanda* coerulea
	VW		Shoot tip	11.42 IAA / 14.76 IBA / 16.11 NAA	Shoot produce root	0.2% CH + 0.05% L-glutamine + 0.025% P		
Deb et al. (2018)	MS	n.r.	Seed	3 NAA + 3 BA	PLB, planlet		12-h PP, 40 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda* bicolor
Decruse et al. (2003)	Mitra	5.6	Node	44.4 BA + 17.1 or 28.5 IAA and 66.6 BA + 28.5 or 40.0 IAA	Shoot/node		12-h PP, 30–50 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda spathulata*
	Mitra		Shoot	5.7 IAA	Shoot produce root	7.5% BP		
Dwiyani et al. (2015)	NP	n.r.	Seed		development of protocorm to seedling	10-20% TJ		*Vanda tricolor* var. *suavis*
	NP		Seedling		Planlet	10-20% TJ		
Gnasekaran et al. (2012)	VW	4.8-5.0	PLB		Secondary PLB	20% TJ + 10% CW	16-h PP, 30 µmol m^{-2} s^{-1}, 25 ± 1 ºC	*Vanda* Kasem's Delight
Hardjo and Savitri (2016)	1/2 MS	n.r.	Callus	0.27 NAA + 0.04 BA	Embryogenic callus		30 µmol m^{-2} s^{-1}, 24 ± 1 ºC	*Vanda tricolor* var. *pallida*
Hrahsel and Thangjam (2015)	MS	5.8	Seed		PLB		16-h PP, 35 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda coerulea*
	MS		PLB	22.80 IAA	shoot			
	MS		Shoot		Shoot produce root	0.075% banana extract		
Islam et al. (2011)	Hyponex	5.6	seed		Germination	20% PP	16-h PP, 45 µmol m^{-2} s^{-1}, 25 ± 1 ºC	*Vanda* roxburgii
	Hyponex		Protocorm		Planlet	10% PP		
Islam et al. (2014)	MS	n.r.	Seed	0.54 NAA	Germination	15% CW	16-h PP, 25 ± 1 ºC	*Vanda roxburghii*

Jawan et al. (2014)	1/4 Mitra, 1% sucrose	n.r.	Calli induced from leaf segment	4.54 TDZ + 0.54 NAA.	Callus maintain		24-h in the dark, 25±2°C	*Vanda dearei*
Jawan et al. (2010)	1/2 MS	5.3	Seeds germination		Protocorm	0.2% YE	24-h PP, 20-50 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda dearei*
Jitsopakul et al. (2013)	VW, 1% sucrose	5.2	Shoot tip	4.44 BA	Shoot and root		16-h PP, 37 µmol m^{-2} s^{-1}, 25 ± 3 ºC	*Vanda coerulea*
	VW, 1% sucrose		Seedling	2.69 NAA + 9.08 TDZ	Root multiplication			
Jhonson and Kane (2007)	½ MS	5.7	Seed		Germination		12-h PP, 50 µmol m^{-2} s^{-1}, 23 ± 2 ºC	*Vanda* Paki · (*Vanda* tessellata · *Vanda* cristata)
	½ MS		Seed		Germination		16-h PP, 50 µmol m^{-2} s^{-1}, 23 ± 2 ºC	(*Vanda* Joan Warne · *Vanda* Paki) · *Vanda* Loke
	P723		Seed		Germination		8 or 16-h PP, 50 µmol m^{-2} s^{-1}, 23 ± 2 ºC	*Vanda* Motes Primrose · Ascocenda Tavivat
David et al. (2008)	KC	n.r.	Protocorm	8.88 BA + 2.69 NAA	PLB		24-h PP, 20-50 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda helvola*
Jualang et al. (2014)	KC, 1% sucrose	5.3	Seed		Germination	0.5% YE	24-h PP, 20-50 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda dearei*
	KC		Protocorm		Seedling	20% CW		
Karyanti (2017)	MS	n.r.	Planlet	2.32 Kin	Shoot induction		10 µmol m^{-2} s^{-1}, 25-26 ºC	*Vanda douglas*
	MS		Planlet	2.27 TDZ	Shoot and leaf			
Kaur and Bhutani (2009)	Mitra	5.7	Foliar	4.44 BA alone/with 5.37 NAA	PLB proliferations and plantlet development	2% AC	12-h PP, 35 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda testacea*
Lang and Hang (2006)	MS	5.2	Root	13.62 TDZ + 13.63 2,4-D	Callus			*Vanda coerulea*
	MS		Callus derived stem	0.54 NAA + 13.62 TDZ	Somatic embryogenesis			
Manners et al. (2010)	MS	5.8 ± 0.02	Root	30 BA + 15 IAA	PLB		12-h PP, 50 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda coerulea*
Manners et al. (2011)	MS	5.8 ±	Seed	5 BA or 5 IAA	Germination		12-h PP, 50 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda coerulea*
	MS	0.02	Protocorm	5 BA + 15 IAA	Seedling			
Mathews and Rao, 1980)	RT or Mitra	5.5	Protocorm		Planlet		12-h PP, 10 µmol m^{-2} s^{-1}	*Vanda* TMA (*Vanda* Josephine Van Brero X *Vanda sanderiana*),
	RT or Mitra		Seed		Germination			*Vanda* TMA × *Vanda roxburghii*
	RT or Mitra		Seed		Germination			*Vanda* TMA × *Vanda* Miss Joaquim
Mathews and Rao, 1985)	Nitsch	5.5-5.6	Protocorm		Protocorm differentiation	10-15% CM	24-h PP, 9 µmol m^{-2} s^{-1}, 25 ± 2 ºC, 55-60% RH	*Vanda* TMA × *Vanda* Miss Joaquim
Mathews and Roy, 1985)	Nitsch	5.5-5.6	Leaf base	5.71 IAA + 9.84 2iP	PLB		24-h PP, 9 µmol m^{-2} s^{-1}, 25 ± 2 ºC, 55-60% RH	
			Shoot tip	4.92 2iP + 5.71 IAA	protocorm			V TMA x V Miss Joaquim
			Shoot tip	4.92 2iP	protocorm			V TMA x V Miss Joaquim

			Shoot tip	5.71 IAA	green nodular protuberances			V TMA x V Miss Joaquim
Jain and Ochatt (2010)	VW	5.6	Nodal segment	9.29 Kin + 2.69 NAA	PLB	0.2% peptone + 0.1% AC	16-h PP, 50 µmol m^{-2} s^{-1}, 24 ± 2 ºC	*Vanda* teres
	VW		PLB		Shoot	15% CW + 0.2% peptone + 1.03 mM l-glutamine + 0.1% AC		
	VW		Shoot	4.92 IBA	Planlet	15% CW + 0.2% peptone + 0.1% AC, 5% BP		
Obsuwan and Thepsithar (2014)	VW	4.8-5.0	Planlet		Planlet maintain	10% 'Gros Michel' BP	16-h PP, 30 µmol m^{-2} s^{-1}, 25 ± 1 ºC	*Vanda* Tokyo Blue
	VW		Planlet		Planlet maintain	15% CW		
Pimda and Bunnag (2010)	ND	n.r.	Seed		Protocorm to planlet	1% PP	16-h PP, 40 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda lilacina*
	ND		Protocorm	13.32 BA	Protocorm proliferation			
Prakash et al. (2012)	MS	5.5-5.9	Seed		Germination and protocorm formation		16-h PP, 25 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda tessellata*
Rahman et al. (2009)	MS	5.8	Shoot tip	8.06 NAA + 4.44 BA	Shoot formation		16-h PP (20-30 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda tessellata*
	MS		Shoot	2.69 NAA + 4.92 IBA	Root formation			
Rineksane and Sukarjan (2015)	ND	n.r.	*Ex vitro* leaf	4.44 BA + 0.54 NAA or 8.88 BA + 0.54 NAA	Callus		24-h PP, 10 µmol m^{-2} s^{-1}, 23-25 ºC	*Vanda tricolor*
	ND		*In vitro* leaf	2.27 TDZ	Callus			
Roy et al. (2011)	Phytamax	5.6	Seed		Protocorm development		16-h PP, 56 µmol m^{-2} s^{-1}, 24 ± 2 ºC	*Vanda coerulea*
	Phytamax		Protocorm	5.36 NAA + 3.80 BA	PLB			
	Phytamax		PLB		Planlet	0.3% AC		
Sebastinraj et al. (2014)	1/2 MS	5.6	Seed		Protocorm		16-h PP, 15 µmol m^{-2} s^{-1}, 25 ± 2 ºC	*Vanda testacea*
				0.91 TDZ	Shoot			
				0.98 IBA	Root			
Seeni and Latha (2000)	Mitra	5.2	Proliferating buds / PLB	1.08 NAA	new bud or PLB, rapid growth of buds into shoot and emergence of shoot	3.5% BP + 30% CW	12-h PP (20-50 µmol m^{-2} s^{-1}, 24 ± 2 ºC	*Vanda coerulea*
	Mitra		Shoot	1.08 NAA	root	3.5% BP		
Sinha and Roy (2004)	VW	5.8	Seed	4.44 BA + 2.69 NAA	Germination	0.2% P	16-h PP, 50 µmol m^{-2} s^{-1}, 24 ± 1 ºC	*Vanda teres*
	VW		Protocorm	4.44 BA + 2.69 NAA	Shoot	0.2% P + 10% CW		
	VW		Shoot		Shoot multiplication	0.2% banana powder + 100 mg L^{-1} CH		
	1/2 MS		Planlet		Planlet maintain			
Widayanti et al. (2014)	MS	n.r.	Shoot tip		Shoot multiplication			*Vanda tricolor*
Tanaka et al. 1975)	VW/Hy	4.7	PLB	5.37 NAA + 44.40 BA	Planlet	800 mg L^{-1} PVP + 0.2% AC		*Vanda*
Khaw et al. 1978)	VW/SH		PLB	1.34-2.69 NAA + 0.44 BA	Apical/axillary bud			*Vanda*
Chaturvedi and Sharma, 1986)	VW		Root/leaf tip	0.57 IAA	PLB/planlet			*Vanda*
Valmayor et al. 1986)	Kn->VW		Flower stalk/bud	4.44 BA + 4.65 Kin	PLB/planlet			*Vanda coerulea*