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Tissue culture of *Phalaenopsis*: present status and future prospects

Khadiza Khatun[1*], Ujjal Kumar Nath[2], Md. Shafikur Rahman[1]

[1]Department of Biotechnology, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh
[2]Bangladesh Argicultural University, Mymensingh, Bangladesh

[*]Corresponding author: Dr. Khadiza Khatun, Department of Biotechnology, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh, Phone: +8801763405303, Email: kkr@pstu.ac.bd.

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ABSTRACT: *Phalaenopsis* one of the popular cut-flower among the orchid species. The improvement/multiplication of this orchid is very difficult through conventional breeding due to delay flowering and uneven flower characteristics. Therefore, tissue culture techniques have been extensively used for improvement of *Phalaenopsis* by inducing and selecting somaclonal variants. However, it is difficult to get stable regenerations techniques of *Phalaenopsis* due to production of phenolic compounds, arising somaclonal variation in the culture and less recovery in the field of the regenerated plantlets. Improved and modified tissue culture techniques providing regeneration from various vegetative parts of plant are needed for industrialization and *ex situ* conservation of this valuable orchid. In this paper we have reviewed various *in vitro* propagation methods of *Phalaenopsis* culture which will be helpful for commercialization of this valuable orchid.

KEYWORDS: *Phalaenopsis*, organ culture, somatic embryogenesis

INTRODUCTION

Orchids considered as the most popular ornamental crop species in the world due to their unique use as cut flower and pot plants. Their ubiquitous beauty fascinated people since ancient times. Orchids are widely grown as ornamental cut flowers because of their exotic beauty and long shelf life [1]. Orchid cultivation is one of the most economically global trade nursery industries constituting a multi-billion dollar exchange among different countries [2, 3]. In contrast to world run-up, Bangladesh is in initial stage of orchid cultivation and starting orchid production commercially just few years ago along with the development of floriculture. In Bangladesh, BRAC, Proshika and Wonderland Toys etc. NGO's are commercially planting orchids in a large scale. Orchids are bisexual plants and produced fruits after pollination and fertilization. They are normally produced large numbers of capsule, that are highly fragile and possess virtually no stored food material or endosperm [4].

Phalaenopsis (moth orchids) is one of the most popular among orchid species because of their specially beautiful and long-lasting flowers, and can cultivate quite easily in the artificial conditions [5-7]. The nomenclature of *Phalaenopsis* derived from Greek words *phalaina*, meaning moth, and *opsis*, meaning look-alike and the name describes the flowers apparently look like to flying moth [8]. In international flower market, these orchids have high economic value as cut flower. Today, *Phalaenopsis* are the most widely grown orchids. Statistical data from Netherlands show that *Phalaenopsis* market prospects increased from 5% to 66% in the year 1983 to 1994%, respectively [9]. As a monopodial plant *Phalaenopsis* are traditionally propagated by the cutting or division of off-shoots, however, these methods results low multiplication rate and hamper the growth of the mother plant, making them ineffective for large scale production. Therefore, their vegetative propagation is difficult and seedling characteristics are not uniform. It needs at least 3 years for flowering under greenhouse condition which is one of the vital problems in commercial production of *Phalaenopsis*. Therefore, tissue culture may be an efficient and alternative tool for propagation of this orchid species [10]. Thereafter, scientists in different corner in the world are trying their level best for commercial *Phalaenopsis* production through tissue

culture technique (Figure 1). Inflorescence nodes of *Phalaenopsis* were induced to form plantlet in aseptic seed germination media by which laid the landmark of *Phalaenopsis* tissue culture [11]. Based on the findings of Rotor (1950), *in vitro Phalaenopsis* propagation and multiplication protocols have been developed by many researchers [12]. In this paper, we have tried to review the explant-based *Phalaenopsis* tissue culture starting from the pioneering works of Rotor (1950) to date and also the future perspectives of the tissue culture techniques for improvement of *Phalaenopsis* as well as *in vitro* conservation of the varietal purity of this species.

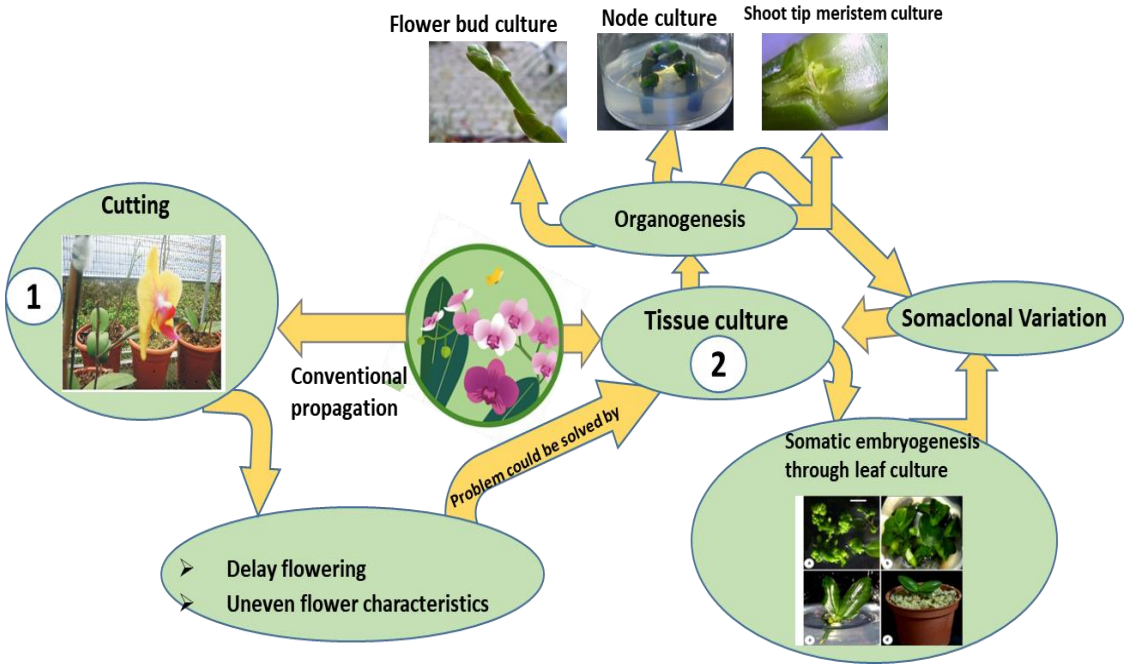

Figure 1. Schematic diagram of *Phalaenopsis* propagation method: 1) *Phalaenopsis* propagation can be done by cutting which may result delay flowering and uneven flower characteristics. 2) Propagation can also be done by tissue culture. Tissue culture of *Phalaenopsis* is done by using different method e.g. organogenesis and somatic embryogenesis.

THE TRADE IN PHALAENOPSIS AND ITS CONTRIBUTION IN ECONOMY

Phalaenopsis are the second most important orchid marketed as both cut and potted flower. It is one of the most popular and economically important orchid genera at commercial scale production [13]. "Orchid growing has not fully achieved the transition from a hobby to an industry" stated by James Shoemaker in 1957. However, today, orchid growing is an international business and more than just an industry. *Phalaenopsis* are about 75% of all orchids sold [14]. Many countries like Germany, Netherlands, United States, China, Japan and Taiwan commercially grown the *Phalaenopsis* in large scale. Currently *Phalaenopsis* young plants production may have extended more than 300 million per year over the world [14]. Germany, Japan, United States, Netherlands and Taiwan commercially grown *Phalaenopsis* and Taiwan ranks tops in the world production [15]. In Taiwan *Phalaenopsis* export value increased from $8 million to $13 million in 2005 to 2006 [16], where worldwide turnover of Taiwanese *Phalaenopsis* raised from $27.5 million to $35.4 million from 2005 to 2006 [17]. Bangladesh is in very beginning stage of *Phalaenopsis* production. The Government of Bangladesh is now giving concern to meet up the local demand and can be participated in export market of *Phalaenopsis*.

PHALAENOPSIS REGENERATION THROUGH ORGANOGENESIS

Orchid could be propagated rapidly via protocorm like body (PLB) formation from explants rather than direct regeneration. However, recently PLB formation is considered as limited condition due to identify some crucial deleterious factors of orchid tissue culture. Therefore, scientists are seeking alternatives of the PLB formation in commercial orchid production. However, plant regeneration through the formation of PLB has been still practiced for mass propagation of monopodial orchid *Phalaenopsis*. Rotor, 1950 pioneered of vegetative propagation of *Phaleonopsis* gave the first documented report on micropropagation of

Phaleonopsis using flower stalk cutting as explant [11]. Several reports have provided the indications regarding the flower-stalk cuttings would be very promising approach for clonal propagation of *Phalaenopsis* [11, 18-27]. Micropropagation of *Phalaenopsis* using flower stalk cuttings is the most widely used technique for mass propagation since the explants can be collected without damaging the mother plant [1]. In contrast , Tanaka *et al.* (1988) claimed that the flower stalk cuttings could not be used for large scale clonal propagation since the propagation rate is very low [28]. They suggested that the large scale propagation and multiplication of *Phalaenopsis* would be possible through the formation of PLB [22, 23, 29-34]. Murdad *et al.* 2006 reported protocorm is the unique structure for *Phalaenopsis* production and observed multiplication capability of trimmed and untrimmed protocorms using coconut water and activated charcoal on XER medium contain 20 gl^{-1} fructose [35]. Though Murdad *et al.* 2006 did not suggest that this protocol could be used for mass clonal propagation; they did stated that trimmed protocorm obtained from germinated seed is much better than untrimed one and trimmed protocorm cultured on coconut water and activated charcoal could be used for high frequency multiplication of *Phalaenopsis gigantae.* Chen *et al.* 2000 developed a reliable *Phalaenopsis* regeneration protocol using seed derived protocoms [36]. They used seed of *Phalaenopsis nebula* for the formation of protocorm in ½ MS basal medium and found that seed derived protocorm performed better for callus induction and subsequent plant regeneration from the induced callus. Whereas Tanaka and Sakanishi, 1985 recommended efficient seed germination of *Phalaenopsis* in MS medium through PLB formation using different leaf segment (distal, middle and proximal) from *in vivo* grown mature plant [37]. Park et al. 2002 reported an efficient *in vitro Phalaenopsis* regeneration protocol through PLB formation using flower stalk nodes derived leaf segments and recommended that modified hyponex medium is suitable for optimal number of PLBs [10]. They optimized the growth regulators combination, to obtain the highest regeneration of PLBs on½ MS medium with BA (88.8 µM) and NAA (5.4 µM) and for the first time they used the raft culture along with solid and liquid culture for proliferation of PLBs. Gonokbari, 2007gave an account for *Phalaenopsis* regeneration via protocorm formation through thin cell layer culture [38].They used ½ MS media with 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA along with coconut water and activated charcoal for PLB formation and used L-glutamine instead of plant hormone for shoot development from PLB. By using this method, they obtained large number of plantlet within a short period. Whereas, Tanaka, 1977 and Tanaka and Sakanishi, 1980 used both solid and liquid VW media with 20% coconut water for the proliferation of PLBs [39, 40]. MS medium used by Hass-Von, 1983 for proliferation and differentiation of PLBs. PLBs derived from ½ MS medium were cultured on solidified Hyponex medium (1 gl^{-1} 6.5 N- 4.5 P- 19 N + 1 gl^{-1}20 N - 20 P - 20 K + 2 gl^{-1} peptone + 0.05% activated charcoal + 30 gl^{-1}sucrose) for plantlets development [41]. They found that use of simple Hyponex medium during the proliferation and conversion of PLBs into plantlets was always advantageous. Among different liquid media VW liquid medium was effective for PLB multiplication [42]. Though Park *et al.* 2002 did not suggest any selective method that could be used on commercial scale vegetative propagation of orchid. Tokuhara and Mii, 1993 used New Dogashima Medium (NDM) instead of ½ MS medium for PLB formation containing 0.1 mgl^{-1} naphthaleneacetic acid (NAA) and 1mgl^{-1} 6-benzylaminopurine (BAP) suggesting that their method could be used for vegetative propagation of *Phalaenopsis* and *doritaenopsis* on a commercial scale [43]. TDZ and auxins combination in culture medium found to be best for the induction of callus and PLBs from leaf of *Phalaenopsis* [44]. Maximum seed germination was observed in VW medium containing coconut water, with 1mgl^{-1} BAP and 2 mgl^{-1} kinetin. Callus and PLB were induced from the leaf of germinated plantlets on NDM medium containing TDZ, BAP and combination of TDZ and NAA. They found that TDZ in combination with NAA produce good quality and higher quantity PLBs than TDZ alone. Their result is contradictory with the findings of Soe et al. 2014. They found that PGR free MS medium was efficient for PLB formation [45]. In the propagation of *Phalaenopsis* Dora and *doritaenopsis* from inflorescence axis section, TDZ alone found to be more effective [46]. Arditti and Ernst, 1993 used modified MS medium with NAA and BA, Young et al. 2000 used MS medium with NAA and BA for PLB induction from leaf explants but these medium did not give any good result in case of *Phalaenopsis gigantae* so they used NDM medium for PLB induction [12]. They harvested plantlet after culturing the PLB and callus in hormone free NDM medium. Homma and Asahira, 1985 used inter-nodal section of flower stalk as explants to regenerate shoot of *Phalaenopsis* through PLB formation and PLB were produced from basal end of explants which touch the media [34]. Intermodal section of flower stalk was better than using flower stalk node and leaf culture in terms of duration of PLB formation and rate of contamination [34]. Among the different parts (tip, middle and basal) of PLB, the basal parts showed highest PLB formation in the PGR free medium [45]. Kobayashi *et al.* 1993 cultured protoplast derived from callus of lateral bud on flower stalks for regeneration of shoot and established a plant regeneration system from protoplasts culture in

Phalaenopsis [47]. They pre-cultured the bud on P basal medium without coconut water and sucrose for 30 days for callus formation. The protoplasts were isolated from callus enzymetically and then cultured in the medium supplemented with 0.05-1.0 mgl^{-1} 2,4-D and 10% cw. They found that 2,4-D was more important than CW for colony formation. Then the protoplast derived PLBs were placed on P basal regeneration medium (10% CW, 3% gelrite) for shoot regeneration. Tokuhara and Mii, 1993 developed an efficient PLB formation and subsequently plantlet regeneration method from PLB using shoot tip of flower stalk bud through cell suspension culture by selecting suitable carbohydrate source and concentration. They found that glucose produce the highest PLB than other carbohydrate sources used and lactose was not suitable for cell proliferation or PLB formation. Among the carbon sources, sorbitol was most suitable for plantlet initiation and development from PLB on *Phalaenopsis* regeneration [48]. They cultured lateral bud from young flower stalk in new *Phalaenopsis* medium (NP) with $10gl^{-1}$ sorbitol for callus induction. The PLBs were than cultured on NP medium supplanted with 20 gl^{-1} sucrose, 20 gl^{-1} maltose and $10gl^{-1}$ sorbitol and found that sucrose containing medium showed some necrosis while maltose and sorbitol medium have no necrosis and plantlet production was higher in sorbitol medium than sucrose and maltose medium.

Many researchers try to get regeneration without formation of PLB. Myint et al. 2001 have developed a rapid *Phalaenopsis* propagation technique through PLB (protocorm like body) formation using leaf as explant [49]. Plantlet was successfully regenerated via the adventitious bud without PLB formation from vegetative bud of flower stalk for avoiding somaclonal variation [50].They used Vacin and Went medium with 15% coconut water along with different concentration of TDZ and BAP and found that TDZ was more effective than BAP in stimulating the axillary buds for induction of shoots. Rotor, 1950 initiated *in vitro Phalaenopsis* cultures using flower stalks without disturbing the whole plant [11]. This technique found to be used extensively for mass propagation of *Phalaenopsis*. Dormant buds of the inflorescence were most advantageous among the other explants for *in vitro* propagation of *Phalaenopsis*; where Indole Acetyl amino Acids (IAA) used for the propagation of plantlets [33]. Flower stalk buds were cultured and achieved reproductive shoots from upper node and vegetative shoots from lower node [19, 22, 51]. Effect of bud position, temperature and BAP on the growth mode of bud studied in *P. amabilis* [51]. Lin, 1986 reported the influence of developmental stage and age of flower stalk on plantlet regeneration in *Phalaenopsis* and *doritaenopsis* and marked that the flower stalk with first flower and intermodal section near the tip of stalk have the highest regeneration capacity [52]. Kosir *et al.* 2004 used nodes with dormant bud of flower stalks for rapid shoot regeneration of *Phalaenopsis* [53]. They used six media with little difference in composition and found that none of the media was appropriate for mass generation and their result was contradictory with Arditii and Ernst (1993). They suggested that media with higher BAP and lower nitrogen content would be suitable in tissue culture and later for *in vivo* flower induction. BA was mandatory for floral bud induction where high nitrogen concentration inhibits the development of floral buds and shortening nine months growth period of *Phalaenopsis* for flowering [54]. Flower stalk culture is most frequently used as explants but it takes a long time to come out plantlets, leaf culture also need more time to produce protocorm and frequent release of phenolic compound is also a major problem. Plantlet regeneration through PLB formation is not easily reproducible [55]. Alternatively elongated stem node was used as explants for regeneration of plantlets by Duan et al. 1996 [55]. The elongated stems were cut into 4 section as top, second, third and basal node and placed on Hyponex medium supplemented with various concentration of BA. Shoots and multiple adventitious buds were produced after 70 days of culture and the highest number of shoots was obtained from third nodes and second nodes.

Phalaenopsis propagation through PLB formation was efficient but in many cases occurrence of somaclonal variation is a major problem for large scale production of plantlets. Chen et al. 1998 found considerable variation in flower colour and shape of *Phalaenopsis* "True Lady- B79-19" regenerated through tissue culture [56]. Tokuhara and Mii, 1998 also found somaclonal variation in flower and inflorescence axis of the micropropagated plants derived from flower stalk bud via protocorm like body formation of *Phalaenopsis* [57]. The variation ranges from 0 to 100% but maximum cultivars showed less than 10% somaclonal variation. Release of high phenolic compounds is another major problems of tissue culture of *Phalaenopsis* which is toxic for *in vitro* growing plantlets [58]. Use of bioreactor systems: continuous immersion system (air-lift type) and temporally immersion system could solve this problem. Temporary immersion bioreactor with activated charcoal filter attached was most suitable for multiplication of PLBs which was effective to remove phenolics. 83% PLBs regenerated into plantlets in 8 weeks and fresh weight of the plantlets and rooting percentage was also very high of the regenerated plantlets [58].

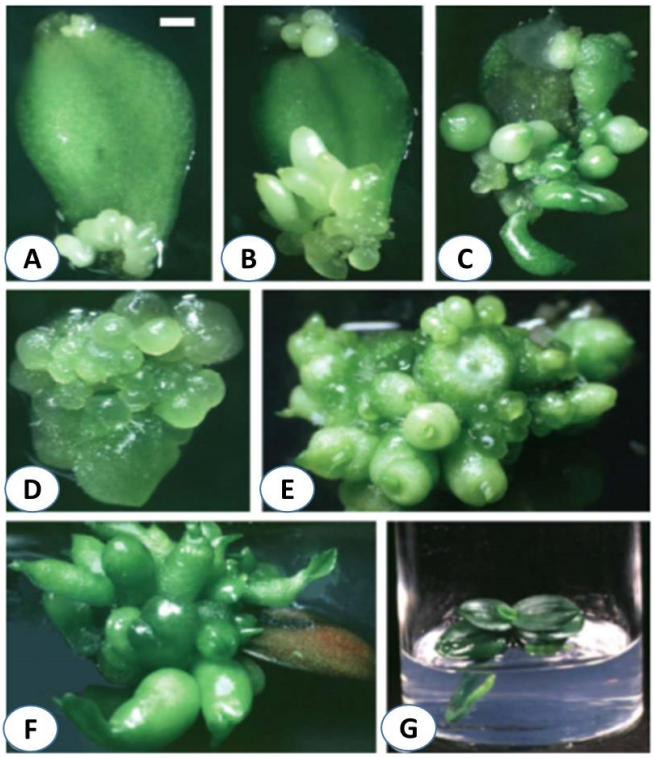

Figure 2. Direct somatic embryogenesis and Plant regeneration of *Phalaenopsis amabilis* using leaf explants : (A) somatic embryos after 20 days of culture (bar = 700 mm); (B) enlarged and elongated embryos after 30 days of culture (bar = 750 mm); (C) green embryos under light and young somatic protocorm after 45 days of culture (bar = 850 mm); (D) somatic embryos developed from leaf-derived nodular masses (bar = 950 mm); (E) shoots and some formed secondary embryos from the developed embryos (bar = 1.2 mm); (F) leaf-derived embryos formed shoots (bar = 2 mm);(G) a plantlet from the leaf derived embryos (bar = 7.2 mm) [62].

PHALAENOPSIS REGENERATION THROUGH SOMATIC EMBRYOGENESIS

Somatic embryogenesis has often been considered efficient techniques for plant regeneration and for obtaining transgenic plant. Currently somatic embryogenesis protocols have been successful studied in *Phalaenopsis* [59-62]. Successful regeneration of *Phalaenopsis* through somatic embryogenesis depends on many factors like source of explant, nutrient composition, the growth hormones and part of the explant taken, explant orientation etc. Kuo *et al.* 2005 reported plant regeneration using leaf explants through direct somatic embryogenesis after 20-30 days of culture on half-strength MS medium supplemented with BA and TDZ [61]. The frequency of embryogenesis is affected by explant orientation usually adaxial surfaces near wounded regions gave the highest embryogenic competency compared to other regions of explants though the authors have not clarify the reason for this. However Gow *et al.* 2008 found that the cut ends of leaf had the highest embryogenic competence than adaxial and abaxial sides in *Phalaenopsis amabilis* and *Phalaenopsis nebula* [63]. Cytokinin is effective for the somatic embryo induction. BA and TDZ has been reported to promoted embryogenesis mostly from the epidermal cell layers [61]. TDZ has also been reported to promote direct embryo formation from the epidermal cells and secondary embryogenesis from the leaf explants of *Phalaenopsis amabilis* [62] (Figure 2). Whereas NAA, 2,4-D highly retarded the somatic embryo formation from leaf explants of *Phalaenopsis* [61, 62].Concentrations of different plant growth regulators had effect on somatic embryogenesis from leaf explant of *Phalaenopsis [64]*. N6-benzyl adenine (6-BA) had better performance than adenine sulphate (Ad) in embryoid induction [64]. They reported that upper epidermis and single cell of mesophyll were the starting source of somatic embryos origination. Chen and Chang, 2004 reported TDZ promoted the formation of embryo from protocorm like bodies derived from seed; whereas NAA retarded the embryo formation of *Phalaenopsis amabilis* var. *Formosa* [65]. When protocorms derived from seed were cultured on ½MS medium without plant growth regulators except TDZ, 100% of the protocorms were produced embryos from the posterior regions. Regeneration of plantlets thorough somatic embryogenesis has also been achieved by Samson et al. 1998 [66]. They had used internodal flower stalk segment with an axillary bud to develop protocorms. They cultured the nodal cutting on Vacin-Went medium to develop vegetative shoots which were cultured on solid New Dogashima Medium (NDM1) supplemented with NAA and 4,4,4 tri-fluro-isopentenyl-adenine for the initiation of protocorm regeneration and histological study permit these protocorms as somatic embryo. They have recommended their methods for commercial propagation of *Phalaenopsis.* In the similar way, Tokuhara and Mii, 2001 developed a method for embryogenic calli; subsequently, plantlets from the calli using flower stalk bud by changing the sucrose concentration in NDM medium following liquid cell suspension culture [60]. Although Sajise and Sagawa, 1991 were first reported on embryogenic calli formation but they did not give any clear-cut protocol for callus induction [67]. However, Tokuhara and Mii, 2001 found that high sucrose concentration in media inhibit initial callus induction, but high sucrose plays vital role of callus proliferation, whenever callus being established in media [60]. Their proposed method could be efficiently utilized for the microprogation of *Phalaenopsis* despite about 10% somaclonal variations. Embryogenic cell suspension culture for the regeneration of plantlets from protoplast of *Phalaenopsis wataboushi* were followed using ½ NDM medium containing 0.06M sucrose, 0.44M sorbitol and 0.1g/l glutamine [68]. They

established a plant regeneration protocol from protoplast without any plant growth regulators and coconut water as supplement. They used shoot tip for the induction of embryogenic calli following the protocol of Tokuhara and Mii, 2001, whereas one year old cells of suspension culture was used to isolate protoplast. As a carbohydrate source sorbitol (10gl^{-1} sorbitol in hormone free NDM medium with 0.3% gellun gum) considered most suitable for the regeneration of plantlets from PLBs and sucrose was most suitable for shoot development [68]. Ishii *et al.* 1998 has been reported that sucrose was effective for callus induction but somatic embryos were formed upon subculture in medium without sucrose indicated that the growth of monopodial orchid *Phalaenopsis* influenced by the sugar in medium [59]. Table 1 represents the brief scenario of tissue culture of Phalaenopsis.

Table 1. Brief scenario of the success in micropropagation of *Phalaenopsis* orchid species using different explants and media by different researchers.

Variety/ cultivar	Material/Explant	Media composition	Results	References
Phalaenopsis (Clyde×Malibu) ×josephHampton)×Anne cavaco *Phal.* Bright light×*Dritaenopsis* Odorika *Phal.* Mount Kaala×Hamaoka	Intermodal section of flower stalk from in vivo grown plant	Thomale GD (macroelement)+ Ringe and Nitsch 1968 (microelement, organic element) + 8 gl^{-1} agar +20 mgl^{-1} BA+ 5 mgl^{-1} NAA+10% coconut milk	Protocorm	[34]
Phalaenopsis	Leaf from mature plant	Modified MS+10 ppm adenine+1 pmm NAA+10 ppm BA	PLB	[37]
Phalenopsis hybrid	Flower stalk	Modified VW medium+ 1mg/1(73.4%) or 5mg/1 BA (46.8%) + 20g/1 sucrose	PLB	[52]
	PLB	Modified VW medium+ 1mg/1BA+ 20g/1 sucrose	Plantlet	
*(Phalaenopsis (*Grand City x Texas Thunder) x *Phalaenopsis* (Mikawa White x Wataboushi)).	callus-derived protoplasts	P basal medium+ 0. 05 mg/l 2, 4-D + 10% (v/v) CW	PLB	[47]
	PLB	P basal medium+10% (v/v) CW + 0. 3% (w/v) Gelrite	Plantlet	
	Plantlet	Hyponex (N: P: K, 6. 5: 6: 19) + 2. 0% (w/v) sucrose+ 0. 05% (w/v) activated charcoal+0.3% (w/v) Gelrite)	Shoot and root formation	
Phalaenopsis and *Doritaenopsis*	shoot tips of flower stalk buds (*in vivo*)	NDM+0.1 mg/ l NAA + 1 mg/l BAP+10 gl^{-1} sucrose	PLB	[43]
	PLB	NDM medium+10 gl^{-1} sucrose	plantlet	
Phalaenopsis Happy Buddha selfed	Seed (Explant source)	XER medium	Plantlet	[46]
	Flower stem section	XER+0.23-11.35 µM TDZ	Multiple shoot	
	Flower stem section	XER+0.45 µM TDZ XER+1.14 µM TDZ	Multiple shoot and PLB Clumbs of protocorm & callus	
Phalaenopsis Pink Leopard Petra	Flower stalk node from in vivo grown plant	Hyponex(6.5N-6.0P-19.0K)+22µM BA+25 gl^{-1}sucrose+2 gl^{-1}peptone	Adventitious shoot	[54]
	Adventitious shoot	VW+22µM BA+25gl^{-1} sucrose+10 gl^{-1} agar	Floral bud	

Phalaenopsis hybrid	-Flower stalk node with bud from in vivo grown plant	VW+ 15% coconut water+ 5 to 40 µM TDZ+7 gl^{-1}sucrose	Adventurous shoot	[50]
	-Shoot tip	Liquid VWC medium	PLB	
Phalaenopsis hybrid Morning M-28XGladys Read St. Louis	-Plant without root (explants donar)	Hyponex +5 mg/l BA 5	Stem	[55]
	-Stem with node	Hyponex+ $2gl^{-1}$	Shoot	
	-Shoot	peptone+different BA conc. Hyponex medium	Root	
Doritaenopsis×Phalaenopsis cultivar	-Flower stalk with one node	MS agar medium	Vegetative shoot	[42]
	-Leaf	MS agar medium	PLB	
	-PLB	MS+5 mg/l kinetin+5 mg/l NAA+30 g/l sucrose	PLB multiplication	
		Hyponex+ 1 mg/l NAA+10 mg/l BA		
		VW+10% CM+15 g/l sucrose		
		VW+20% CM+ 20g/l sucrose		
Phalaenopsis (Phal.) orchid (True Lady 'B79-19')	-flower stalks derived dormant buds	1/2 MS +2.6 µM BAP+ 0.5 µM NAA+ 0.8% (w/v) agar + 10% (v/v) coconut milk.	adventitious shoots	[56]
	-Shoot			
	-Leaf	0.2% active charcoal medium	Plantlet	
		Liquid VW +0.4 m*M* Ca3(PO4)2+ 1.9 µMadenine+0.9 µ*M* BAP+0.3 µM NAA+1.5% (w/v) sucrose+2% (v/v) coconut milk.	PLB	
	-PLB	VW mineral salts+0.4 m*M*Ca3(PO4)2 +8.5 µM adenine+4.5µMBAP+ 1.8 µMNAA+ 0.5% (w/v) sucrose+ 10% (v/v) coconut milk + 0.8%(w/v) agar.	Plantlet	
		KCpc (cited Tsai et al. 1992) medium		
		L8 medium (cited Chen et al. 1994)		
Phalaenopsis Richard Shaffer 'Santa Cruz'	-Leaf(explants donar)	Media cited by Tanaka & Sakanishi 1980)	PLB	[59]
	Leaf derived PLB			
		VW+0.1 mgl^{-1} 2,4-D +0.01 Mgl^{-1} BA +2 gl^{-1} gellun gum+40 gl^{-1}Sucrose+200 ml l^{-1}CW	Callus	
	Flower stalk shoot derived leaf	VW+1.0 mgl^{-1} 2,4-D +0. 1Mgl^{-1} BA +2 gl^{-1} gellun gum+40 gl^{-1}Sucrose	Callus	
	-callus	VW+0.1 mgl^{-1} 2,4-D +0.01 Mgl^{-1} BA +2 gl^{-1} gellun gum+200 ml l^{-1}CW	PLB(somatic embryo0	
Phalaenopsis weeding Promenade P4 and Phal.Hanaboushi×Pha. euuestris llocos(P5)	-Embryogenic callus derived from lateral bud of flower stalk	NP+10gl^{-1} sorbitol+3gl^{-1} gelrite	PLB	[48]
	-PLB			
		NP+20gl^{-1} Sucrose(Induce CLB)/ 20gl^{-1} maltose/10gl^{-1} sorbitol(BEST)	Plantlet	
Phalaenopsis	-Internodal flower stalk segment with axillary bud	VW medium+NAA+BAP	Flower stalk/ vegetative shoot	[66]
	-Flower stalk/ vegetative shoot	VW medium+NAA+4,4,4	PLB	
	-PLB	VW medium+NAA+BAP	Plantlet	
Phalaenopsis Nebula	-Seed	1/4 MS salts +1000 mg l^{-1} peptone + 2000 mg l^{-1} activated charcoal + 50	Protocorm	[36]

		000 mg l^{-1} banana pulp + 100 mg l^{-1} myo-inositol + 20 000 mg l^{-1} sucrose + 3000 mg l^{-1} gelrite		
	-Protocorm	1/2 MS+0-1.0 mg l^{-1}TDZ+0-1.0 mg l^{-1}2,4-D(TDZ 4.52µM gave heist PLB)	Callus	
	-Callus	1/2 MS+0.1-1.0 mg l^{-1}TDZ	PLB/plantlet	
Phalaenopsis hybrid (pink stripped flower)	-Leaf	MS+ 45gl^{-1} sucrose+15mgl^{-1} BA+ I mgl^{-1} NAA	PLB	[58]
	-PLB	Hyponex(6.5N-4.5P-19K 1gl^{-1}+20N-20P-20K 1gl^{-1}+1%ne potato homogenate	PLB proliferation Plantlet	
	-PLB	MS+45gl^{-1}+7 gl^{-1} VW+45gl^{-1}+7 gl^{-1} KC+45gl^{-1}+7 gl^{-1} Hyponex(6.5N-4.5P-19K 1gl^{-1}+20N-20P-20K 1gl^{-1})+45gl^{-1}+7 gl^{-1}(best medium)		
Phalaenopsis red strain	-Leaf from In vivo grown plant	Modified VW medium+10 mg l^{-1}NAA+10 mg l^{-1}BA+200 ml l^{-1}CW+20gl^{-1} sucrose with cotton supporter	PLB	[49]
	-PLB	Modified VW medium+0.1 mg l^{-1}NAA+1mg l^{-1}BA+0.8% agar	PLB proliferation Plantlet	
	-PLB	Hyponex+1.0 mgl^{-1}NAA+1.0mgl^{-1}BA+3% potato homogenate+2% sucrose VW+1.0 mgl^{-1}BA+1.0 mgl^{-1}NAA +3% potato homogenate+2%sucrose ND+1.0mgl^{-1}BA+1.0mgl^{-1}BA +3% potato homogenate+2%sucrose (best)		
Phalaenopsis [(Baby Hat_Ann Jesica)_equestris] (PM292)	-Shoot tip of flower stalk buds	NDM+ 0.5 mM NAA+ 4.4 mM BA+ 29.2 mM sucrose	Embryogenic callus	[60]
	-Embryogenic callus	NDM+29.2 mM sucrose+ 2gl^{-1} gelun gum	PLB	
	-PLB	NDM + 10 g l^{-1}Potato Granule+10 g l^{-1} apple juice+ 58.4 mM sucrose +2 g l^{-1} gellan gum	Plantlet	
Phalaenopsis hybrid	-Flower stalk bud	NDM+0.1mg/l NAA+1mg/l BAP+10g/l sucrose	PLB	[69]
Phalaenopsis 'Taisuco Hatarot', *P*. Tinny Sunshine Annie, *P*. Taipei Gold 'Golden Star	-Flower stalk node(explants donar)	MS + 20.2mM + BA+ 45 g l^{-1} sucrose +7.0 g l^{-1} Phyto agar	Plantlet	[10]
	-Leaf	½ MS + 88.8 µM BAP + 5.4 µM NAA	PLB	
	-PLB	Hyponex+ 30 g l^{-1} sucrose+2 g l^{-1} peptone +3% (w/v) potato homogenate + 0.05% activated charcoal+ 7 g l^{-1} Phyto agar	Plantlet	
Phalaenopsis snow parade, wedding promenade	-Shoot tip	NDM+0.5µM NAA+4.4µM BA+2gl^{-1}gellun gum+29.2 mM sucrose	Callus	[70]
	-Callus	Liquid + NDM+0.5µM NAA+4.4µM BA+2gl^{-1}gellun gum+58.4 mM sucrose	PLB	
Phalaenopsis amabilis var. formosa Shimadzu	-Seed	Modified ½ MS	Protocorm	[65]
	-Prottocorm	½ MS+13.62µM TDZ	Somatic embryo	
	-Somatic embryo	½ MS	Plantlet	

Phalaenopsis hybrid	-Flower stalk nodes	A. Sigma(P 6793) medium+ 2 mg/l BAP+ 0.5 mg/l NAA+2.6 mg/l gellun gum B. MS medium+ 4.41 mg/l BAP+ 1 mg/l NAA+2.6 mg/l gellun gum C. B5 +2 mg/l BAP+2.6 mg/l gellun gum D.B5 medium without banana homogenate+2.6 mg/l gellun gum E and F. Media cited by Arditti and Ernst (1993)	Shoot	[53]
Phalaenopsis Little Steve	-Flower stalk bud (explants donar)	1/2 MS+100 mg l^{-1} myo-inositol+0.5 mg l^{-1} niacin+ 0.5 mg l^{-1} pyridoxine HCl +0.1 mg l^{-1} thiamine HCl+ 2.0 mg l^{-1} glycine+1000 mg l^{-1} peptone + 170 mg l^{-1} NaH_2PO_4+ 20 000 mg l^{-1}Sucrose + 2200 mg l^{-1} Gelrite	Plantlet	[61]
	-Leaf	1/2 MS + 4.54 µM TDZ+20 000 mg l^{-1}Sucrose + 2200 mg l^{-1} Gelrite	Somatic embryo	
	-Somatic embryo	1/2 MS medium+20 000 mg l^{-1}Sucrose + 2200 mg l^{-1} Gelrite	Plantlet	
Phalaenopsis amabilis var. formosa	-Seed(explants source)	½MS+100 mg l^{-1} myo-inositol+0.5 mg l^{-1} niacin+ 0.5 mg l^{-1} pyridoxine HCl +0.1 mg l^{-1} thiamine HCl+ 2.0 mg l^{-1} glycine+1000 mg l^{-1} peptone + 170 mg l^{-1} NaH_2PO_4+ 20 000 mg l^{-1}Sucrose + 2200 mg l^{-1} Gelrite	Plantlet	[62]
	-Leaf	½MS+ 0.1(6.6%), 1(7.5%), 3(19.4%) mg dm^{-1} TDZ	Somatic embryos	
	-Somatic embryos	½MS+3mg dm^{-1} TDZ	Secondary embryos	
Phalaenopsis gigantea	-Seed(explants donar)	XER+10% CW+0.2% AC	Protocorm	[35]
	-Protocorm	XER+0,10,15(highest) ,20% CW+0,1,2,2.5(highest) g l^{-1}AC+20gl^{-1}fructose	Protocorm multiplication/proliferation	
	-Protocorm	XER+10 gl^{-1}agar+2 ACl^{-1}+20gl^{-1}fructose	Plantlet	
Phalaenopsis var. Hawaiian Clouds x Phalaenopsis Carmela's Dream,	-Flower stalk derived PLB	½ MS+ 30g/l sucrose+9 g/l agar	Callus	[71]
	-Callus	NDM+1 mg/l TDZ+10 g/l matose	PLB	
	-PLB	½ MS+20g/l sucrose+1g/l AC+2.8 g/l gelrite	Plantlet	
Phalaenopsis amabilis (L.) Bl. cv. Cool Breeze	-Inflorescence axis	MS + 2 mg/l BAP + 1 mg/l NAA + 10% (w/v) CW + 2 g/l peptone + 1 g/l activated charcoal	PLB	[38]
Phalaenopsis Wataboushi	-Protoplast	½ NDM salt+0.06 M sucrose+0.44 M sorbitol+ 0.1 g/l L-glutamine	Colony formation	[68]
	-Callus	NDM+10 g/l maltose+0.3% gellun gum	PLB	
	-PLB	NDM+10 g/l sorbitol+0.3% gellan gum	Plantlet	
P. amabilis and P. Nebula	-Leaf from in vivo grown plant	1/2 MS+ 13.32 µM BA +4.92 µM 2iP + 20µM ACC	Somatic embryo	[63]
	-Somatic embryo	½ MS	Plantlet	
P. amabilis and P. Nebula	-Leaf from in vivo grown plant	1/2 MS+ 3 mg dm^{-3} TDZ	Somatic embryo	[72]
	-Somatic embryo	1/2 MS	Plantlet	

Phalaenopsis 'Silky Moon	-Seed	Hyponex (6.5N-6P-19K; + 2 g l^{-1} peptone+100 g l^{-1} potato juice + 20 g l^{-1} sucrose.	Plantlet shoot	[73]
	-Shoot	Hyponex+ 22.2 µM BA+100 g l^{-1} potato juice+2.0 g l^{-1} peptone+30 g l^{-1} sucrose+1.0 g l^{-1} activated charcoal + 2.0 g l^{-1} Phytagel.	protocorm	
4 hydrid Dark purpled flower (DP), Pink purpled flower (PP), white flower with red leaf (WR), white cultivar queen emma	-Flower stalk node	Ms+20.2µM BA+ 45 g/l sucrose+2.3 g/l gelrite	Plantlet	[74]
	-Leaf	½ MS+ 9.1µM TDZ	Shoot	
Phalaenopsis	-Seed derived protocorm	NDM+0.1-0.3 mg/l TDZ	Protocorm multiplication	[35]
Phalaenopsis amabilis (L.) BL. cv. 'Golden Horizon	-Young leaf segments	1/2 MS+ 2.0 mg l^{-1}BA+ 0.5 mg l^{-1}NAA+ 2 g l^{-1} peptone+ 1 g l^{-1} activated charcoal+2%sucrose+10% CW	PLB	[75]
	-PLB	1/2 MS+2 g l^{-1} peptone+ 1 g l^{-1} activated charcoal+2%sucrose+10% CW+150 mgl^{-1}L-glutamone	New PLB with leafy shoots	
	-Shoot	1/2 MS+2 g l^{-1} peptone+2% sucrose+10% CW+1 g l^{-1} activated charcoal	Rotted plantlet	
Phalaenopsis gigantea	-Seed(explants source)	VW+20gl/l sucrose=200ml/l CW+1mg/lBAP+1mg/l kinetin	plantlet	[44]
	-Leaf			
	-PLB	NDM+ 1 mg/L NAA +0.1 mg/L TDZ NDM medium	Callus/PLB Plantlet	
Phalaenopsis cornu-crevi blume and Rchb.	-Seed	MS+15% CW	Protocorm	[76]
	-Wounded Protocorm	½ MS+0.1 mg/l NAA+0.1 mg/l TDZ	PLB	
	-PLB	MS+15% CW+0.2% AC	Plantlet	
Phalaenopsis amabilis (L.) Bl. cv. Cool Breeze	-Flower stalk node	MS+ 4.40 mgl^{-1}BA + 1 mgl^{-1}NAA	Plantlet	[77]
	-Plantlet	1/2MS+1mg/l NAA	Root	
P. aphrodite subsp. formosana	-Seed	1/4 MS +1 g/L +5 g/L coconut powder + 20 g/L sucrose+ 8.5 g/L Bacto agar	Seedling	[78]
	-Seedling	1/2 MS + 1 g/L peptone+20 g/L sucrose+ 4 g/L Gelrite+1 mgl^{-1} TDZ	Somatic embryo	
Phalaenopsis	-PLB	MS + NAA(0,0.1)+ BA(0,5.0,10.0) But MS without PGR showed highest result	PLB	[45]

CONCLUSION AND FUTURE PROSPECTS OF PHALAENOPSISIS

*Phalaenopsis*is one of the most popular orchid and has immense economic value as ornamental cut flower. To date, the seed derived propagation of *Phalaenopsis* is very rapid and easy approach. Therefore, their uniform flower characteristics are one of the important criteria for commercialization. This could only possible by following tissue culture techniques despite very little somaclonal variation up to 10% within acceptable limit. Meanwhile, tissue culture method need less time to develop and maintain varietal purity compare to conventional breeding. In this paper we have tried to collect together most of the commercially important *in vitro* propagation of *Phalaenopsis* using different explants and different growth condition which will be helpful for rapid clonal propagation, industrial exploitation and also improvising the currently available method for *in vitro* mass propagation of this valuable orchid. The commercial demand of *Phalaenopsis* has been increasing day by day and *Phalaenopsis* production is now international in scope. Based upon the advanced tissue culture techniques new types might be developed

with a compact growth habit, variegated foliage and uniform flower characteristics. Therefore, the *Phalaenopsis* trade might be increased both in volume and value throughout the world and possible to earn lots of foreign exchange by exporting the orchids.

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AUTHOR CONTRIBUTIONS

Dr. Khadiza Khatun drafted the manuscript and revised the final draft. Dr. Ujjal Kumar nath revised the initial manuscript. Md. Shafikur Rahman helped to write the manuscript.

CONFLICTS OF INTEREST

Authors declared that they have no conflict of interest.

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