OPEN ACCESS Freely available online

Journal of Plant Biochemistry & Physiology

Research Article

Standardization of Tissue Culture Techniques in *Phalaenopsis* Orchids

S Parvathy*

Department of Agriculrural Sciences, Savitribai Phule Pune University, Pune, India

ABSTRACT

Every year, hundreds of new species with diverse floral traits, lifestyles, habitat distributions, and trophic patterns are discovered and evolved in the Orchidaceae, the biggest and most fascinating family of flowering plants with over 800 genera and roughly 30,000–35,000 specie. Due to their distinctive use as cut flowers and pot plants, orchids are arguably the most popular decorative crop species in the world. It is a vast and diverse family of flowering plants with important ornamental, medicinal, conservation, and evolutionary research applications. Since ancient times, their ubiquitous beauty has captivated humans. Because of their exotic beauty and long shelf life, orchids are commonly grown as ornamental cut flowers.

Orchid cultivation has really been prominent in tropical and subtropical regions, with many large, mechanized, and well-managed greenhouses. *Phalaenopsis* is the world's most significant potted orchid, with enormous breeding and *in vitro* propagation technology achievements in countries such as Belgium, the Netherlands, Taiwan, and Thailand. Another reason for its popularity is the simplicity with which floral induction may be regulated for year-round output. Cut orchids such as *Cymbidium, Dendrobium, Oncidium*, and *Vanda* are widely used as cut flowers all around the world. Orchids have become increasingly popular around the world market as a result of their long shelf life, diverse colour palette, and other desirable traits that purchasers want based on their location or culture.

Keywords: Monopodial orchids; *Phalaenopsis*; Sympodial orchids

INTRODUCTION

Orchids are classified into two main categories depending on their development habits: monopodial and sympodial. Monopodial orchids, such as Phalaenopsis and *Vanda*, have the main stem that grows year after year, but sympodial orchids, such as *Cattleya* and *Cymbidium*, have a main stem that stops growing at the end of each season. Due to the vast spectrum of floral character uniqueness and the access to a large number of varieties and hybrids, monopodials have gained in popularity around the world. The monopodial orchids *Phalaenopsis*, *Aranda*, *Mokara*, and *Vanda* are the most popular [1-20].

Phalaenopsis, often known as moth orchid, is among the most recognized orchid species because of its beautiful and long-lasting flowers and simplicity of growth in artificial environments. The name *Phalaenopsis* comes from the Greek terms 'phalaina', which means 'moth', and 'opsis', which means 'look-alike', and refers to the flowers' resemblance to a fluttering moth. The deep, damp, and warm woods of India, Indonesia, and the Philippines are habitats to about 60 species of this genus. They can be found between 200 and 400 metres above sea level. These orchids have a great commercial value as cut flowers on the worldwide flower market. *Phalaenopsis* orchids have long fibrous roots, short leafy shoots, and long-lasting flat blooms grouped in a floral stalk that frequently bends at the terminal. *Phalaenopsis* is usually propagated by cutting or dividing off-shoots as it is a monopodia plant. These methods, however, have a low multiplication rate and impede the growth of the mother plant, making them ineffective for large-scale production. Vegetative propagation is difficult, and seedling quality varies [21-28].

Based on their floral characteristics, *Phalaenopsis* orchids can be divided into two categories: grandiflora (cut flower) and multiflora (pot plant). Multiflora varieties have short, multiple inflorescences with many tiny blooms, and grandiflora types

Correspondence to: S Parvathy, Department of Agriculrural Sciences, Savitribai Phule Pune University, Pune, India, Tel: 6238930675; E-mail: parvathyvld05@gmail.com

Received: 11-Mar-2022, Manuscript No. JPBP-22-16244; **Editor assigned:** 14-Mar-2022, PreQC No. JPBP-22-16244 (QC); **Reviewed:** 28-Mar-2022, QC No. JPBP-22-16244; **Revised:** 11-May-2022, Manuscript No. JPBP-22-16244 (R); **Published:** 18-May-2022, DOI: 10.35248/2329-9029.22.10.289.

Citation: Parvathy S (2022) Standardization of Tissue Culture Techniques in *Phalaenopsis* Orchids. J Plant Biochem Physiol. 10:289

have long, bowing inflorescence with huge flowers. Long coarse roots, short leafy stems, and long-lasting flat blooms organized in a flowering stem that frequently branches at the end characterizes *Phalaenopsis* orchids [29-36]. Since *Phalaenopsis* is a monopodial plant, it is traditionally propagated by cutting or dividing off-shoots. However, these methods result in a low multiplication rate and slow the mother plant's growth, making them ineffective for large-scale production. As a result, vegetative propagation is difficult, and seedling qualities vary. Flowering takes at least three years in a greenhouse, which is one of the major challenges in commercial *Phalaenopsis* production. As a result, tissue culture may be a viable alternative for propagating this orchid species.

Cross pollination involving selected orchid plants has increased the quality of commercial orchids. The hybrid will then be rebuilt through seed germination [37-45]. The hybrid will exhibit a wide range of phenotypes; therefore this method has various disadvantages. Although a considerable amount of time is required to further select hybrids with favourable characteristics. In vitro culture, also known as micropropagation, can be used to regenerate specific orchids in order to produce uniform plants in a short period of time. In most cases, the propagule exhibits similar features of its parent [46-54].

A number of researchers have attempted to rejuvenate Phalaenopsis orchids. The same orchid genus has been successfully propagated using PLBs. Various plant hormones and explant sources have been used to induce PLBs [55-67]. Utilizing leaf cells from propagules, PLB generation on MS media with BAP and NAA or TDZ has been described. 5.4 M NAA and 4.5 M TDZ were used to produce PLBs and plantlets from *Doritaenopsis* orchid flower stalks.

In light of the foregoing, the goal of this work was to establish a procedure for in vitro mass multiplication of superior *Phalaenopsis* genotype.

MATERIALS AND METHODS

The study entitled "Morphological studies and standardization of tissue culture technique in *Phalaenopsis* orchids" was conducted in the Department of Plant Biotechnology and Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram, during 2020-2021. The objectives of this research were to standardize tissue culture technique in *Phalaenopsis* genotype. The following are the most important studies that were undertaken.

- Identification of the best explant from *Phalaenopsis* genotype for propagation.
- Standardization of tissue culture media for *Phalaenopsis* genotype.

Experiment: *Phalaenopsis* genotype with high flower yield attributing quantitative vegetative and floral characters and commercially valuable qualitative floral traits were chosen for *in vitro* mass multiplication studies. This study aimed to identify the best explant from the selected genotype for the standardization of tissue culture protocol for the same. The materials and methods used in this experimental are described in detail below [68-87].

Materials

Source of explants: *Phalaenopsis* genotype with good cut flower quality selected based on statistical analysis of morphological traits from eight commercially available moth orchids maintained in the green house of Department of Plant Biotechnology and Department of Plant Breeding and Genetics, College of Agriculture, Vellayani was used as explants source. These are monopodia orchids that produce fleshy and conspicuous leaves. From the selected genotype, inflorescence stalk with axillary bud, inflorescence stalk and intermodal segment of inflorescence stalk were taken as explants for *in vitro* propagation of orchid.

Design: CRD

Treatments: 3

Replications: 5

Treatments (explants), Inflorescence stalk with axillary bud, Inflorescence stalk, and Inter nodal segment of inflorescence stalk.

Composition of media

In this investigation, plant tissue culture was performed using ½ MS medium supplemented with several growth regulators. The MS medium's composition is listed.

Experimental method

Fully developed inflorescence stalk, intermodal segment of inflorescence stalk and axillary bud with inflorescence stalk before flowering were taken as explants. The procedure used to prepare explants for inoculation differed depending on the type of explant [88-96].

Inflorescence stalk

Individual inflorescence stalks were gathered from mother plants by cutting them at the base using a sharp knife, leaving the basal node on the stalk for future inflorescence formation. The stalks were cleansed with cotton soaked in 70% alcohol before being cut into nodal sections and cleaned in distilled water. The sections were surface sterilized after the bracts protecting the dormant buds were removed.

Intermodal segment of inflorescence stalk

Individual inflorescence stalks were harvested from mother plants by cutting them at the base with a sharp knife, leaving the basal node for later inflorescence production. The stalks were cleaned with cotton soaked in 70% alcohol before being trimmed into intermodal sections and cleaned in distilled water. The sections were then surface sterilized.

Axillary bud with inflorescence stalk

Shoot tips (Axillary bud) with inflorescence stalk was collected from the parent plant under aseptic condition using sterile blades and cleaned with cotton soaked in 70% alcohol followed by surface sterilization before removing the leaf sheath.

Standardization of surface sterilization

Explants were surface sterilized to ensure that they were free of microbial contamination. Prior to surface sterilization, explants were exposed to the various pretreatments. Explants were carefully cleaned under running tap water. They were then soaked in a 0.1 percent baiting solution with two drops of pill along with 0.1 per cent streptomycin for 40 minutes followed by repeated washing with distilled water before surface sterilization [97-117].

Surface sterilization of explants was performed inside a laminar air flow chamber. The explants were washed thoroughly in sterile double distilled water before applying treatments. The inflorescence stalk explants were then cut into smaller pieces of 4-5 cm with a sharp blade for easy handling during sterilization.

The cut explants were treated with surface sterilization agents with continuous shaking for different durations as per (Table 1). Each treatment was replicated five times. After each surface sterilization, the explants were rinsed thrice with sterile double distilled water and 1-2 cm length explants were inoculated on half strength MS medium to study the explant survival. Axillary bud with inflorescence stalk was rinsed thoroughly with double distilled water after separating them from leaf sheath.

0.1 percent Mercuric chloride ($HgCl_2$) followed by alcohol (70 %) were employed with all the three explants for varied time durations (5 min, 8 min, 10 min, and 12 min) in order to design an effective surface sterilization approach [118-120].

Table 1: Treatments to evaluate the effectiveness of surface sterilization.

Treatment No.	Treatments	Duration (minutes)	Explant used
ST_1	0.1% $HgCl_2$	5	IS, IIS, ABIS
	70% alcohol	5	
ST_2	0.1% $HgCl_2$	8	IS, IIS, ABIS
	70% alcohol	8	
ST_3	0.1% $HgCl_2$	10	IS, IIS, ABIS
	70% alcohol	10	
ST_4	0.1% $HgCl_2$	12	IS, IIS, ABIS
	70% alcohol	12	

ST-Surface sterilization Treatment; IS-Inflorescence;IIS-Intermodal segment of inflorescence stalk; ABIS-Axillary bud with inflorescence stalk

Following mercuric chloride treatment, explants were washed three to four times with sterile double distilled water inside the laminar air flow cabinet to remove any traces of $HgCl_2$. The end portions of the inflorescence (nodal and intermodal) segments on both sides were removed and cut into a 2 cm long piece.

Axillary bud with flower stalk will be separated from leaf sheath under aseptic condition using sterile blade and trimmed to 2 cm long piece.

Observations recorded

- Percentage survival of explants at culture initiation stage
- Percentage propagation of explants
- Days required for response

Preparation

Plant tissue culture media used for the study was half strength Murashige and Skoog medium preparation. Major and minor nutrient stock solutions were made by dissolving the needed amount of chemicals in a precise volume of double glass-distilled water. Plant growth regulators were first dissolved in 1N NaOH or 95 percent ethanol, then double glass-distilled water was added to make up the volume, and the stock solutions were kept refrigerated at 4 degrees Celsius [121-134].

The stock solutions of plant growth chemicals were only kept for one week, but the other stock solutions were kept for up to one month. Six stock solutions for macro nutrients, micro nutrients and organic supplements (A, B, C, D, E, and F) were prepared and kept chilled in pre-cleaned glass bottles. The stock solutions of various growth regulators were kept in the refrigerator in order to make the media preparation procedure easier. Plant growth substance stock solutions could only be kept for a week,

although other stock solutions could be kept for up to a month. All of the glassware used in the culture media preparation were washed with tap water containing a few drops of lanoline and then rinsed with double glass-distilled water.

All stock solutions were pipetted into a 1000 ml beaker in proportionate volume. Sucrose was added and dissolved immediately. Other chemicals were added directly to the media while it was being prepared for certain treatments. Then, using double glass-distilled water, the volume was increased to 950 ml. After adding all of the medium components except the agar, the pH was adjusted using an electronic pH metre between 5.5 and 5.7 using 0.1 N NaOH and 0.1 N HCl. The medium was then added with 8.0 g L-1 agar, and the final volume was increased to 1000 ml using double glass-distilled water. The beaker with media was then placed in a microwave oven to boil, with intermittent stirring with a glass rod, until the agar melted and a clear solution was obtained. Then 15-20 ml of hot medium was transferred into pre-sterilized glass culture tubes measuring 25 mm x 150 mm with proper labels, which were then closed with non-absorbent cotton plugs and autoclaved [135-142].

Inoculation of explants

Inoculation of explants was carried out in Laminar Air Flow Chamber as it ensures aseptic condition for safe transfer of plant material to sterile medium. Swabs with 70% alcohol were used to sanitize the work table of the laminar air flow cabinet. Prior to operating in the laminar air flow cabinet, the UV lamp was turned on for 20 minutes to create an aseptic atmosphere within the cabinet. Wash the glassware (Petri dishes and beakers) and tools (blades, scalpels, and tweezers) required for inoculation thoroughly, rinse with double glass distilled water, wrap with plastic film and store in a polypropylene cover that can be autoclaved, and autoclaved at 121°C and 1.06 Kg cm^{-2} pressure for 40 minutes. After surface sterilization treatments, the trimmed explants were carefully transferred to culture medium with the help of sterile forceps. The culture vessels should be opened and closed in front of the spirit lamp flame during explant inoculation for ensuring sterile condition and thereby minimizing the chance of contamination. Explants were inoculated in such a way that their cut end had as much contact with the medium for maximum utilization of media components. Inoculated culture vessels were then incubated in culture room.

Culture conditions

In an air-conditioned culture room with a 16 hours photoperiod, the cultures were kept. To meet the explants' light requirements (2000 lux), white fluorescent tubes were used. The humidity in the culture chamber ranged from 60 to 80 per cent. Sub culturing was done at two week intervals. The culture vessels and racks were wiped with 70 per cent ethanol at an interval of three days to avoid the entry of contaminants [143-148].

Identification of best explant

The explants were selected based on certain criteria such as maximum percentage survival at culture initiation stage observed one week after inoculation, fastest response in basal medium (½ MS) with different hormonal combinations (2-3 mg/L BAP and 0.5-1 mg/L NAA) with maximum percentage propagation (Table 2). Explants selected from this experiment were used for further *in vitro* micro propagation studies of selected superior *Phalaenopsis* genotype.

Table 2: Different explants of Phalaenopsis and basal media with different hormonal combinations.

Sl. No.	Explant used	Media composition
1	Inflorescence node Inflorescence internode Axillary bud with inflorescence stalk	½ MS (control)
2	Inflorescence node Inflorescence internode Axillary bud with inflorescence stalk	½ MS+2 mg/L BAP+0.5mg/L NAA
3	Inflorescence node Inflorescence internode Axillary bud with inflorescence stalk	½ MS+2.5 mg/L BAP+0.5mg/L NAA
4	Inflorescence node Inflorescence internode Axillary bud with inflorescence stalk	½ MS+2.5 mg/L BAP+1mg/L NAA
5	Inflorescence node Inflorescence internode Axillary bud with inflorescence stalk	½ MS+3 mg/L BAP+0.5 mg/L NAA

Observations recorded

- percentage propagation of each explant after three week
- presence of phenolic
- explant response
- days required for explant response in culture media

Experiment -1

- Explants showing maximum percentage survival at culture initiation stage with minimum contamination as well as fastest response in culture media selected from experiment ii was used for further studies.

Design: CRD

Treatments: 5

Replication: 5

Experimental method: The explant selected from experiment II which showed the fastest response in half strength Murashige and Skoog media with different combination of BAP and NAA was used for the standardization of tissue culture technique in *Phalaenopsis* genotype. Those explants showed positive responses was sub cultured in the same media and maintained for the formation of Protocorm Like Bodies (PLBs). The cultures were incubated under a 16-hour photoperiod. Subculture was performed every two week. Developed PLBs were then transferred into half-strength MS medium without any plant growth regulator. The pH of the media was adjusted to 5.6-5.8. These cultures were then incubated under a 16-hour photoperiod. Mean number of PLBs were calculated after tenth week of inoculation.

Observations recorded: Comparison of percentage propagation of explant in selected basal media with different combinations of BAP and NAA.

- Days for emergence of PLBs in different media combination
- Days for emergence of plantlets from protector like bodies
- Number of plantlets generated per explant

RESULTS

The outcomes of the present study are reported in the following sections. The most important studies that were undertaken are listed below.

- Identification of best explant from selected genotype for propagation
- Standardization of tissue culture media for selected Phalaenopsis orchid.

Experiment-2

Inflorescence stalk, intermodal segment of inflorescence stalk and inflorescence stalk with axillary bud were used for *in vitro* culture. Flowering mother plants of *Phalaenopsis* Chian Xen diamond x Tinny Honey selected based on inflorescence characters kept at the College of Agriculture, Vellayani, were used as explant sources.

Standardization of surface sterilization technique

Table 3 shows observations on the percentage of explant survival one week after inoculation. Explants were subjected to pretreatments prior to surface sterilization. Under running tap water, the explants were carefully cleaned. They were then immersed in a 0.5 percent bavistin solution containing two drops of pill and 0.5% bactericide for 40 minutes before being thoroughly rinsed with distilled water. Surface sterilization treatments with mercuric chloride (0.1%) and alcohol (70%) were applied to the inflorescence nodal, intermodal segments of inflorescence node, and axillary bud with inflorescence stalk for varying time durations (5 min, 8 min, 10 min, and 12 min).

On half strength MS media with different combination of BAP and NAA, four treatments were evaluated to examine the effect of surface sterilization treatments on microbial contamination and percentage explant survival (Table 3). Surface sterilization treatments had different impacts on different explants of P8. The treatment TS3 (0.1 percent mercuric chloride for 10 minutes+70% alcohol for 10 minutes) was determined to be the most effective for surface sterilization, with an explant survival rate of 80 percent (inflorescence stalk and intermodal segment of inflorescence stalk) and 60 per cent (axillary bud with inflorescence) (Figures 3-10).

Table 3: Standardization of surface sterilization treatments for inflorescence stalk.

Treatment number	Treatments	Duration (min.)	Percentage survival at culture initiation stage		
			Inflore scence stalk	Internodal segment of inflorescen ce stalk	Axillary bud with inflorescenc e stalk
ST_1	0.1% $HgCl_2$	5	20	20	0
	70% alcohol	5			
ST_2	0.1% $HgCl_2$	8	40	40	20
	70% alcohol	8			
ST_3	0.1% $HgCl_2$	10	80	80	60
	70% alcohol	10			
ST_4	0.1% $HgCl_2$	12	60	60	40
	70% alcohol	12			

Effect of different hormonal combinations on various explants

For the study, explants were inoculated in ½ MS with various combinations of growth regulators BA (2-3 mgl^{-1}) and NAA (0.5-1 mgl^{-1}) (Figure 1). Response of each explant along with percentage establishment in culture and days required for response were recorded in the (Table 4).

Figure 1: *Phalaenopsis* genotype selected for further studies-*Phalaenopsis* Chian Xen Mammon x Tinny Honey.

Figure 2: Stage of explants used for inoculation.

Table 4: Response of various explants to combinations of growth regulators.

Sl. No.	Media composition	Explant used	Explant response	Percentage of culture establishment	Days required for sprouting
1	½ MS (control)	Inflorescence stalk with axillary bud	No Change	Nil	Nil
		Inflorescence stalk	No Change	Nil	Nil
		Inter nodal segment of Inflorescence stalk	No Change	Nil	Nil
2	½ MS+2 mg/L BAP +0.5mg/L NAA	Inflorescence stalk with axillary bud	No Change	Nil	Nil
		Inflorescence stalk	Sprouting	66	34
		Inter nodal segment of Inflorescence stalk	No Change	Nil	Nil

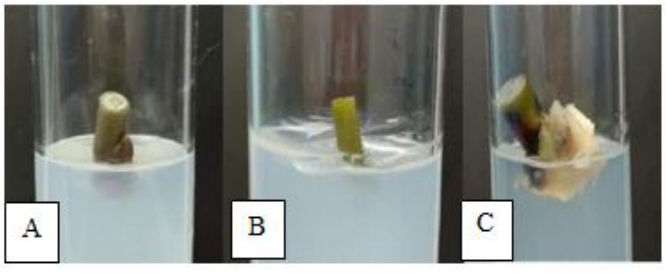

Figure 3: Inoculation of explants on culture medium; (A) Infloescence stalk; (B) Internodal segment of inflorescence stalk; (C) Axillary bud with inflorescence stalk.

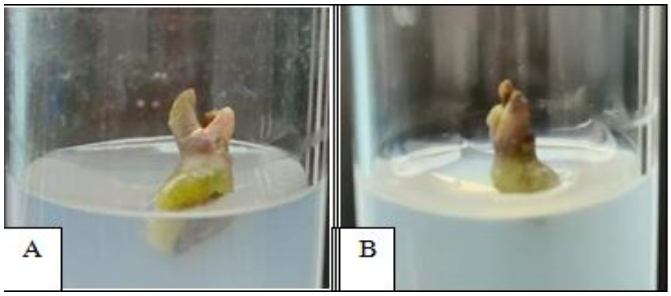

Figure 4: Response of inflorescence stalk to combination of growth regulators. (A) Bulging of inflorescence stalk 22 days after inoculation in ½ MS+ 2.5 mg/L BAP+1 mg/L NAA; (B) Bulging of inflorescence stalk 16 days after inoculation in ½ MS+3 mg/L BAP+0.5 mg/L NAA.

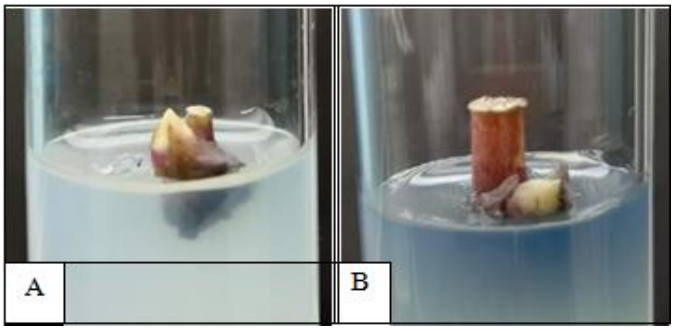

Figure 5: Response of inflorescence stalk to combination of growth regulators. (A) Sprouting of inflorescence stalk 34 days after inoculation in ½ MS+2 mg/L BAP+0.5mg/L NAA; (B) Sprouting of inflorescence stalk 25 days after inoculation in ½ MS+2.5 mg/L BAP+0.5 mg/L NAA.

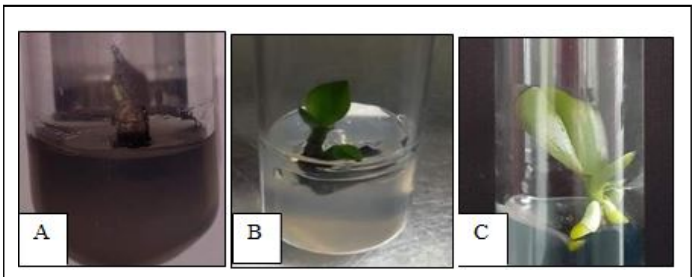

Figure 6: Regeneration of shoots and roots from inflorescence stalk in ½ MS+2 mg/L BAP+0.5 mg/L NAA. (A) Shoot initiation seven weeks after inoculation; (B) Shoot elongation 12 weeks after inoculation; (C) *In vitro* rooting 18 weeks after inoculation.

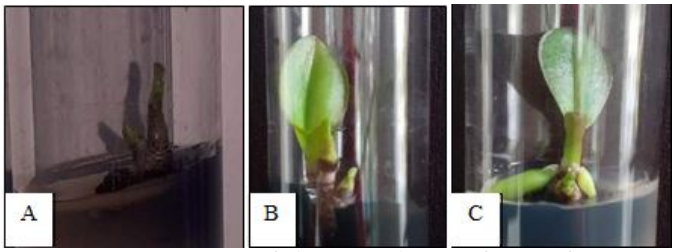

Figure 7: Regeneration of shoots and roots from inflorescence stalk in ½ MS+2.5 mg/L BAP+0.5 mg/L NAA. (A) Shoot initiation three weeks after inoculation; (B) Shoot elongation seven weeks after inoculation; (C) *In vitro* rooting 13 weeks after inoculation.

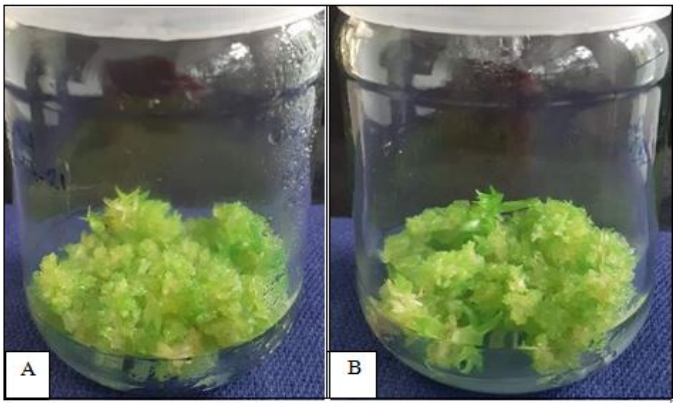

Figure 8: PLBs formation from inflorescence stalk. (A) 14 weeks after inoculation in ½ MS +3mg/L BAP+0.5 mg/L NAA; (B) 16 weeks after inoculation in ½ MS+2.5 mg/L BAP+1 mg/L NAA.

Figure 9: PLBs subcultured on basal medium without.

Figure 10: Plantlets regenerated from PLBs after 12 week of.

Among the explants studied, only the inflorescence stalk responded by bulging and sprouting. In culture media, the inflorescence stalk with axillary bud and the intermodal section of the inflorescence stalk remained intact. Within 16 days of inoculation, bulging of the inflorescence stalk was observed with 50 per cent propagation in ½ MS with 3 mg/l BAP and 0.5 mg/l NAA. Within 25 days of inoculation, sprouting from the node of a mature inflorescence stalk was observed in with 2.5 mg/l BA and 0.5 mg/l NAA with 83 per cent propagation. As a result, the inflorescence stalk was used as an explant in further research (Figures 3 and 4).

Experiment 2

Inflorescence stalk was inoculated in half strength Murashige and Skoog (½ MS) medium with different concentrations of BAP and NAA for Protocorm Like Bodies (PLB) formation.

Design: CRD

Treatments: 5

Replication: 5

Treatments

T1-½ MS (control)

T2-½ MS+2 mg/L BAP+0.5mg/L NAA T3-½ MS+2.5 mg/L BAP+0.5mg/L NAA T4-½ MS+2.5 mg/L BAP+1 mg/L NAA T5-½ MS+3 mg/L BAP+0.5 mg/L NAA

Morph genic responses of inflorescence nodal explant cultured in media containing different combinations of growth regulators were recorded (Table 5). Within 14 weeks of inoculation, Protector Like Bodies (PLBs) were distinguished from the inflorescence stem in ½ MS with 3 mg/L BAP and 0.5 mg/L NAA (Figure 8). Average number of PLBs that arose from inflorescence stalk was found to be maximum in ½ MS+3 mg/L BAP+0.5 mg/L NAA (Table 6). Within three weeks of inoculation, ½ MS with 2.5 mg/L BAP and 0.5 mg/L NAA displayed inflorescence stalk shoot initiation response (Figure 5). From the same medium, shoot elongation needed only seven weeks while root regeneration taken thirteen weeks. ½ MS with 2 mg/L BAP and 0.5 mg/L NAA displayed inflorescence stalk shoot initiation response within seven weeks after inoculation (Figure 6).

Table 5: Morph genic responses of inflorescence nodal explant cultured in media containing different combinations of growth regulators.

Media	Explant response	Percentage propagation of explant	Days for emergence of shoots/ PLBs
T_1	Nil	Nil	Nil
T_2	Shoots initiation	66	49
T_3	Shoots initiation	83	21
T_4	PLB formation	22	112
T_5	PLB formation	50	98

Table 6: Average number of PLBs that arose from inflorescence stalk.

Media	Number of PLBs per explant
½ MS+2.5 mg/L BAP+1 mg/L NAA (T_4)	6
½ MS+3mg/L BAP+0.5 mg/L NAA (T_5)	9

Regeneration of plantlets

PLBs were transferred to basal medium without plant growth hormones (Figure 9). After 12 weeks of being placed into media without plant growth regulators, all PLBs germinated into plantlets (Figure 10). When a Protector-like Body (PLB) is grown in basal medium without growth regulators, it retains its ability to develop new leaves, roots, or even shoots since it has leaf and root primordial. Around 9 plantlets were regenerated per inflorescence stalk (Table 7).

Table 7: Regeneration of plantlets from flower stalk node.

Media	Days for emergence of plantlets from protector like bodies	Number of plantlets regenerated per inflorescence stalk
½ MS w/o PGR	84	9

DISCUSSION

Floriculture has emerged as a massive entrepreneurial opportunity for small and marginal farmers, as well as a means of earning foreign currency. Cut flowers, pot plants, cut foliages, seeds, bulbs; tubers, rooted cuttings, and dried flowers or leaves are all products of the floriculture industry. Floriculture has grown in importance as the demand for flowers has increased, making it one of the most important commercial trades in Indian agriculture. Rose, carnation, chrysanthemum, gerbera, gladiolus, orchids, anthodium, tulip, and lily are the most important floricultural crops in the international cut flower trade. Orchids account for a significant portion of the global floriculture trade, both as cut flowers and as potted plants, and are estimated to account for approximately 10% of the international fresh cut flower trade.

Tropical and subtropical countries, many of which have automated and effectively managed big greenhouses, have produced the majority of the world's orchids. *Phalaenopsis* is the most widely traded potted orchid, with outstanding breeding and micro propagation technological achievements in countries such as Belgium, the Netherlands, Taiwan, and Thailand. The ease of controlled floral introduction for scheduled and year round production is another reason for its popularity. Cut orchids, such as *Indicium, Vanda, Cymbidium*, and *Dendrobium* are widely used as cut flowers around the world.

Dendrobium and *Phalaenopsis* plants were the major orchid genera for exports. Thailand is the world's leading producer and exporter of tropical orchids. Orchid exports generate approximately 2.5 billion baht in revenue for Thailand each year. Taiwan's orchid kingdom expanded its global leadership by adding Australia and Brazil as export markets. Orchids from the Taiwan are exported to 36 countries in North America, Northern Europe, and South Africa. Thailand is the largest exporter of orchid cut flowers to India, accounting for 80.67 percent of total imports, followed by the Netherlands (15.54 percent), New Zealand (2.29 percent), and China (1.5%). Singapore's imports of fresh orchids from India were only US$ 1379.3, accounting for 0.02 percent of the country's total imported products in 2007. As a result, considering the country's proximity and India's East Policy, there are numerous opportunities to increase India's exports to Singapore.

Due to the severe coronavirus pandemic and global economic disruption since the beginning of 2020, orchid marketing has shifted to a greater share of digitalization or E-commerce, coexisting with traditional flower stores and supermarkets. Non-traditional technologies, such as biotech-oriented efficient breeding, aid in the development of more orchid products or novel new orchid hybrids, which may open up new avenues for orchid production in the future.

Standardization of surface sterilization treatment for explants

The biggest obstacle encountered during the establishment of tissue culture explants was contamination induced by bacteria and fungi. Because the mother plants had been exposed to field conditions for a long period, microbes harbored the plant tissues, resulting in systemic infection during the culture of explants taken from them. During the early phases of culture, a high rate of bacterial and fungal contamination was detected in the current study. Various surface sterilization methods were used in order to get contamination free culture.

When surface sterilization was performed using 0.1 percent mercuric chloride in combination with 70% alcohol for 10 minutes, the percentage survival of inflorescence stalks and intermodal segment of inflorescence stalk on half strength MS medium was determined to be the highest (80%). The sterilization treatment with 0.1 per cent mercuric chloride and 70 per cent alcohol (ST3) was shown to be very successful regardless of the explants. In comparison to other surface sterilization techniques, sterilized explants had a higher proportion of survival in the culture medium. Most of the strategies discovered for *in vitro* propagation of Phalaenopsis, according to and, involve propagation via seedling or culturing the dormant buds found at the basal section of the inflorescence. Axillary bud with inflorescence stalk showed 60 per cent survival in culture media after surface sterilization with TS3 treatment.

Response of different explants on culture medium: Only the inflorescence stem responded by bulging and sprouting when explants were inoculated in ½ MS with various combinations of growth regulators BA (2-3 mgl^{-1}) and NAA (0.5^{-1} mg l^{-1}). The axillary bud and the intermodal region of the inflorescence stalk remained intact in culture conditions. In ½ MS with 3 mg/l BAP and 0.5 mg/l NAA, bulging of the inflorescence stem was seen 16 days after inoculation, with 50% propagation. In ½ MS with 2.5 mg/l BA and 0.5 mg/l NAA, sprouting from the node of a mature inflorescence stalk was observed with 83 percent propagation within 25 days after inoculation. As a result, the stalk of the inflorescence was employed as an explant in the further studies. Explants were utilized in the culture of orchid species in a variety of ways. Four mass propagation protocols have been established. PLB regeneration from inflorescence node, shoot tips, root tips, and stem segments through direct embryogenesis was determined to be one of the finest techniques in *Phalaenopsis*, according to Chen et al. (2000) and Zhang and Fang (2005).

Standardization of tissue culture protocol for selected *phalaenopsis* genotype

Protocorm-Like Bodies (PLBs) were differentiated from the inflorescence stalk in ½ MS with 3 mg/L BAP and 0.5 mg/L NAA within 14 weeks of inoculation. According to Stacey D.

Novak et al., hormone-depleted medium or decreased levels of auxin in combination with cytokinins generally create PLBs from callus. Arditti and Ernst, reported that auxins, particularly NAA, alter the process of regeneration in monopodial epiphytic species, acting synergistically on the production of PLBs. ½ MS with 2.5 mg/L BAP and 0.5 mg/L NAA showed shoot initiation response from inflorescence stalk within three weeks of inoculation. Shoot elongation was observed within seven weeks and root regeneration within 13 weeks from same medium. Shoot development from nodes of a stem or stem like tissue, such as rhizomes or inflorescence stalks, is a common *in vitro* response to exogenously given cytokine and auxin in many orchid species. In addition, some orchid tissue culture studies found that axing alone or in combination with cytokines increased overall shoot growth. The influence of auxin on new root production has been widely investigated in model plant systems, according to report that several techniques of propagation for *Phalaenopsis* have been developed through *in vitro* culture of various parts including shoot tips, flower stalk nodes, buds, root tips, and rhizome segments with varying responses. Although other portions of the plant can be utilized as initial explants for growing PLBs, foliar explants are more convenient to obtain and do not require the mother plant to be sacrificed. According to, unlike the utilization of inflorescence components, foliar explants are not limited in availability. Suggested that, in order to produce more PLBs, half strength MS was utilized, which produced an average of 9 PLBs per explant.

CONCLUSION

PLBs per explant for VW: The formation of Protector Like Bodies (PLBs), according to, is a significant strategy for micro propagating orchids. All PLBs germinated into plantlets 12 weeks after being transferred into basal media without plant growth regulators. Exogenous Auxins (NAA and IAA) and Cytokinin (BAP) can induce cell proliferation and differentiation to create somatic embryos or PLBs, in studies conducted by. PLBs can germinate into plantlets when grown in media without plant growth regulators, according to studies by. Around 9 plantlets were regenerated per inflorescence stalk nodal segment. A continuous sub culturing of PLBs can generate more number of plantlets.

REFERENCES

1. Abbaszadeh SM, Miri SM, Naderi R. Optimization of asymbiotic germination of Phalaenopsis orchid in *in vitro* conditions. International Conference on Agricultural Science, Medicinal Plants and Traditional Medicine, Mashhad, Iran. 2018.
2. Abbaszadeh SM, Miri SM, Naderi R. Optimization of *in vitro* seed germination of Phalaenopsis orchid. M. Sc. Thesis of Horticulture, Islamic Azad University-Karaj Branch. 2018.
3. Aktar S, Nasiruddin KM, Huq, H. *In vitro* root formation in *Dendrobium* orchid plantlets with *IBA*. J Agric Rural Dev. 2008;5(2):48-51.
4. Al-Khayri JM. Optimization of biotin and thiamine requirements for somatic embryogenesis of date palm (*Phoenix dactylifera L.*). *In vitro* Cell Dev Biol Plant. 2001;37: 453-456.
5. Allard RW. Principles of Plant Breeding. John Wiley and Sons. New York. 1960;485.
6. Aloni R, Aloni E, Langhans M, Ullrich CI. Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann Bot. 2006;97(5):883-893.
7. Arditti J, Ernst R. Micropropagation of Orchid. John Wiley & Sons, Inc., New York. 1993;682.
8. Arditti J, Harrison CR. Vitamin requirements and metabolism in orchid. Orchid Biology: Reviews and Perspectives (USA). Cornell University Press, New York. 1997;159-175.
9. Batchelor SR. Orchid culture. 5. Air pollution, air movement, and humidity.A. O. S. B. 1981.
10. Bazand A, Otroshy M, Fazilati M, Piri H, Mokhtari A. Effect of plant growth regulators on seed germination and development of Protocorm and seedling of *Phalaenopsis amabilis* (L.) Blume (Orchidaceae). Annu Res Rev Biol. 2014;4(24):3962-3969.
11. Blanchard MG, Runkle ES. Temperature during the day, but not during the night, controls flowering of Phalaenopsis orchids. J Exp Bot. 2006;57(15):4043-4049.
12. Bhandari HR, Bhanu AN, Srivastava K, Singh MN, Shreya HA. Assessment of genetic diversity in crop plants-an overview. Adv Plants Agric Res. 2007;7(3):00255.
13. Bhattarcharjee S. Effect of growth regulating substances on in vitro seed germination of Phalaenopsis hybrid. Indian Agric. 1999;43:79-83.
14. Brown HT. On the culture of excised embryos of barley on nutrient solutions containing nitrogen in different forms. Trans. Guinness Research Lab. 1906;1:288-299.
15. Budavari S, O'neil MJ, Smith A, Heckelman PE, JR, Gallipeau JAR, D'Arecea MA. The Merck index: An encyclopedia of chemicals, drugs, and biologicals. Whitehouse Station, NJ: Merck & Co. Inc. 1996;450:1674.
16. Burton GW, Devane DE. Estimating heritability in tall fescue *(Festuca arundinacea)* from replicated clonal material. J Agron. 1953;45(10):478-481.
17. Cabral PDS, Soares TCB, Gonçalves LSA, Amaral Júnior ATD, Lima ABP, Rodrigues R, et al. Quantification of the diversity among common bean accessions using Ward-MLM strategy. Pesqui Agropecu Bras. 2010;45(10):1124-1132.
18. Cha-um S, Ulziibat B, Kirdmanee C. Effects of temperature and relative humidity during *in vitro* acclimatization, on physiological changes and growth characters of Phalaenopsis adapted to *in vivo*. Aust J Crop Sci. 2010;4(9):750–756.
19. Chen YC, Chang C, Chang WC. A reliable protocol for plant regeneration from callus culture of Phalaenopsis. *In Vitro* Cell Dev Biol Plant. 2000;36:420–423.
20. Chen TY, Chen JT, Chang WC. Multiple shoot formation and plant regeneration from stem nodal explants of *Paphiopedilum orchids*. *In Vitro* Cell Dev Biol Plant. 2002;38(6):595-597.
21. Chen JT, Chang WC. Direct somatic embryogenesis and plant regeneration from leaf explants of *Phalaenopsis amabilis*. Biol Plant. 2006;50(2): 169-173.
22. Chen JT, Chang WC. Effects of tissue culture conditions and explant characteristics on direct somatic embryogenesis in *Oncidium 'Gower Ramsey'*. Plant Cell Tissue Organ Cult. 2002;69:41–44.
23. Chen C, Chien MY. The leaf growth model and influencing factors in Phalaenopsis orchid. Afr J Agric Res. 2012;7(28):4045–4055.
24. Chen JT, Chang C, Chang WC. Direct somatic embryogenesis on leaf explants of *Oncidium 'Gower Ramsey' and subsequent plant regeneration*. Plant Cell Rep. 1999;19:143–149.
25. Chen JT, Chang WC. Induction of repetitive embryogenesis from seed derived protocorms of Phalaenopsis amabilis var. Formosa Shimadzu. *In Vitro* Cell Dev Biol. Plant. 2004;40: 290–293.

26. Chen YQ, Piluek C. Effects of thidiazuron and N-6-benzyl aminopurine on shoot regeneration of Phalaenopsis. Plant. Growth Regul. 1995;16:99-101.
27. Choong CW, Choong SW, Thong WH. A defined culture medium suitable for suitable for sensitive Phalaenopsis species seedlings. Biotechnol. 2013;12(3):163-167.
28. Chou CC, Chen WS, Huang KL, Yu HC, Liao LJ. Changes in cytokinin levels of Phalaenopsis leaves at high temperature. Plant Physiol Bioch. 2000;38(4): 309–314.
29. Chugh S, Guha SI, Rao U. Micropropagation of orchids: Are view on the potential of different explants. Scientia Horti. 2009;122:507–520.
30. Cybularz-urban T, Hanus-E, Swiderski A. Effect of light wave length on *in vitro* organogenesis of a Cattleya hybrid. Acta Biol Crac Ser Bot. 2007;49(1): 113–118.
31. De LC, Singh DR, Barman D. Evaluation of some Phalaenopsis hybrids at Sikkim Himalaya. Intl J Agrl Sci Res. 2019;6(5): 189-196.
32. Dueck T, Trouwborst G, Hogewoning SW, Meinen E. Can a high red: Far red ratio replace temperature-induced inflorescence development in Phalaenopsis?. Environ Exp Bot. 2016;121:139-144.
33. Endo M, Sugi Y. Growth and nutrient absorption of Hydroponic Phalaenopsis during a year. J Japan Soc Hort Sci. 1992;61(2): 532-533.
34. Ernst R. The use of activated charcoal in asymbiotic seedling culture of Phaphiopedilum. Am Orchid Soc Bull. 1974;43:35–38.
35. Ernst R. Studies in asymbiotic culture of orchids. Am Orchid Soc Bull. 1975;44:12–18.
36. Eymar E, Alegre J, Toribio M, Lopez-Vela D. Effect of activated charcoal and 6 benzyl adenine on *in vitro* nitrogen uptake by *Lagerstroemia indica*. Plant Cell Tissue Organ Cult. 2000;63:57–65.
37. Falconer DS. Introduction to quantitative genetics 3rd ed. Harlow Longman Sci Tech. 1989.
38. George RM. Genetic variability studies in Phalaenopsis orchids. J pharmacogn Phytochem. 2020;9(5):377-379.
39. George FE, Debergh PC. Micropropagation: Uses and Methods. U George, FE, Hall, MA, Klerk, GD Plant Propagation by Tissue Culture. 2008;29-65.
40. Gheisari M, Miri SM. *In vitro* callus induction and bulblet regeneration of hyacinth (*Hyacinthus orientalis* L.). Plant Cell Biotechnol Mol Biol. 2017;18(3):145-155.
41. Goh CJ, Wong PF. Micropropagation of the monopodial orchid hybrid Aranda 'Deborah' using inflorescence explants. Sci Hortic. 1990;44:315–321.
42. Gopi R, Thomas B, Sathishkumar S. Genetic variability among diverse genotypes of *Anthurium andreanum* Linden. Adv Life Sci. 2016;5(12):4886-4888.
43. Gow WP, Chen JT, Chang WC. Enhancement of direct somatic embryogenesis and plantlet growth from leaf explants of *Phalaenopsis by adjusting culture period and explant length*. Acta Physiol Plant. 2010;32:621–627.
44. Griesbach RJ. The use of indole acetyl amino acids in the *in vitro* propagation of Phalaenopsis orchids. Sci Hortic. 1983;19:363-366.
45. Griesbach RJ. Development of Phalaenopsis orchids for the mass market. In: Jainick, J., Whipkey, A. (Eds.), Trends in New Crops and New Uses. ASHS Press, Alexandria, VA. 1983.
46. Guo WJ, Lee N. Effect of leaf and plant age, and day/night temperature on net CO2 uptake in *Phalaenopsis amabilis var. formosa*. J Am Soc Hortic Sci. 2006;131(3):320-326.
47. Guo WJ, Lin YZ, Lee N. Photosynthetic light requirements and effects of low irradiance and daylength on *Phalaenopsis amabilis*. J Am Soc Hortic Sci. 2012;137(6):465-472.
48. Hall RD. An introduction to plant-cell culture. Plant cell culture protocols. 1999;1-18.
49. Hew CS, Yong JW. Physiology of Tropical Orchids In Relation To The Industry, The. World Scientific Publishing Company. 2004.
50. Hsiao YY, Pan ZJ, Hsu CC, Yang YP, Hsu YC, Chuang YC, et al. Research on orchid biology and biotechnology. Plant Cell Physiol. 2011;52(9):1467-1486.
51. Hsuan HW, Chiang TM, Fure CC. Effects of supplemental chemicals and light quality onadventitious root induction of *in vitro* grown Phalaenopsis and Doritaenopsis shoots. J Taiwan Soc Hortic Sci. 2009;55(2): 127-135.
52. Huang LVT, Takamura T, Tanaka M. Callus formation and plant regeneration from callus through somatic embryo structures in *Cymbidium orchid*. Plant Sci. 2004;166:1443–1449.
53. Hurst CC. Curiosities of Orchid Breeding. Nature. 1898;59:12-21.
54. Ichihashi S. Micropropagation of Phalaenopsis through the culture of lateral buds from young flowerstalks. Lindleyana. 1898;7:208-215.
55. Ichihashi S. Orchid production and research in Japan. In Orchid Biology, Springer. 1997; 171-212.
56. Intuwong O, Sagawa Y. Clonal propagation of sarcanthine orchids by aseptic culture of inflorescence. Am Orchid Soc Bull. 1973;42:209–215.
57. Ishii Y, Takamura T, Goi M, Tanaka M. Callus induction and somatic embryogenesis of Phalaenopsis. Plant Cell Reports. 1998;17(6):446-450.
58. Intuwong O, Sagawa Y. Clonal propagation of Phalaenopsis by shoot tip culture. Am orchid Soc Bull. 1974;43:893-895.
59. Jo MH, Ham IK, Park SK, Seo GS, Han GH, Woo IS. Effect of Plant Growth Regulators on Clonal Production through Basal Stem Explant Cultures of a Phalaenopsis Hybrid. Plant Resour. 2003;6(2): 89-93.
60. Kanchanapoom K, Anuphan T, Pansiri S. Effects of total nitrogen and BA on *in vitro culture of Phalaenopsis*. International Symposium on Orchids and Ornamental Plants. 2012;243-245.
61. Khatun K, Nath UK, Rahman MS. Tissue culture of Phalaenopsis: Present status and future prospects. J Adv Biotechnol Exp Therap. 2020;3:273-285.
62. Khoddamzadeh AA, Sinniah UR, Kadir MA, Kadzimin SB, Mahmood M, Sreeramanan S. *In vitro* induction and proliferation of protocorm-like bodies (PLBs) from leaf segments of *Phalaenopsis bellina (Rchb. f.)* Christenson. Plant Growth Regul. 2011;65(2):381-387.
63. Kim KW, Kako S. Morphological and histological studies on the formation of Protocorm like bodies and explants development in Cymbidium shoot apex culture *in vitro*. J Kor Soc Hort Sci. 1984;25:156-163.
64. Kluge M, Ting IP. Crassulacean Acid Metabolism. Analysis of an Ecological Adaptation. Springer-Verlag, Berlin. 1978.
65. Knudson L. A new nutrient solution for the germination of orchid seed. Amer Orchid Soc Bull. 1946;15:214-217.
66. Knudson L. The use of amino compounds by orchid embryos. NY Agr Exp Sta Rep. 1932;111.
67. Kamemoto H. Status report on breeding for superior anthurium and dendrobium cultivars. Research extension series-College of Tropical Agriculture and Human Resources, University of Hawaii (USA). 1983.
68. Kosir P, Skof S, Luthar Z. Direct shoot regeneration from nodes of Phalaenopsis orchids. Acta agriculturae slovenica. 2004;83(2): 233-242.
69. Kubota S, Kokubu T, Mori N, Yoneda K. Effects of light intensities before temperature treatments on temperature sensitivity for flower stalk emergence in cultivated Phalaenopsis. J Jpn Soc Hort Sci. 1992;62(1):462-463.

70. Kubota S, Yoneda K. Effect of light intensity preceding day/night temperatures on the sensitivity of Phalaenopsis to flower. J Jpn Soc Hort Sci. 1993;62(3):595-600.

71. Kumar N, Reddy MP. *In vitro* plant propagation: a review. J Environ sci. 2011;27(2):61-72.

72. Kunisaki JT, Kim KK, Sagawa Y. Shoot tip Culture of Vanda. Am Orchid Soc Bull. 1972;41:435-439.

73. Kuo HL, Chen JT, Chang WC. Efficient plant regeneration through direct somatic embryogenesis from leaf explants of Phalaenopsis 'Little Steve'. Dev Biol Plant. 2005;41(4):453-456.

74. Lin CC. *In vitro* culture of flower stalk internodes of Phalaenopsis and Doritaenopsis. Lindleyana. 1986;1:158-163.

75. Liu YC, Tseng KM, Chen CC, Tsai YT, Liu CH, Chen WH, et al. Warm-night temperature delays spike emergence and alters carbon pool metabolism in the stem and leaves of Phalaenopsis aphroide. Sci Hortic. 2013;161:198-203.

76. Lopez R, Runkle E, Wang YT, Blanchard M, Hsu T. Growing the best phalaenopsis. Part 3: Temperature and light requirements, height, insect and desease control. Orchids, March 2007;76(3): 182-187.

77. Luo JP, Wawrosch C, Kopp B. Enhanced micropropagation of Dendrobium huoshanense CZ Tang et SJ Cheng through protocorm-like bodies: the effects of cytokinins, carbohydrate sources and cold pretreatment. Scientia Hortic. 2009;123(2):258-62.

78. Mahendran G, Bai VN. Direct somatic embryogenesis and plant regeneration from seed derived protocorms of Cymbidium bicolor Lindl. Scientia Hortic. 2012;135:40-44.

79. Maziah M, Chern CY. Agrobacterium-mediated Genetic Transformation of *Phalaenopsis bellina* Using GFP and GUS Reporter Genes. Pertanika J Sci Technol. 2008;16(2):129–139.

80. McConnell J, Kamemoto H. Characteristics of four sets of reciprocal crosses in Dendrobium. J Am Soc Hort Sci. 1983;108(6):1003-6.

81. McDonald GJ. Disa Hybridization – Part II: Breeding characteristics. Am Orchid Soc Bull. 1991;60:748-753.

82. Mitchell FW. Heating, Cooling and Humidity Part I and II. Amer Orchid Soc Bull. 1963;32:531-537.

83. Mitsukuri K, Mori G, Johkan M, Shimada Y, Mishiba KI, Morikawa T, et al. Effects of explant position and dark treatment on bud formation in floret culture of *Ponerorchis graminifolia* Rchb. F Scientia Hortic. 2009;121(2):243-247.

84. Mohammadi SA, Prasanna BM. Analysis of genetic diversity in crop plants—salient statistical tools and considerations. Crop science. 2003;43(4):1235-1248.

85. Morel GM. Producing virus-free Cymbidium. Am Orchid Soc Bull. 1960;29:495-497.

86. Morel GM. Tissue culture-a new method of clonal propagation of orchids. Am Orchid Soc Bull. 1964;33:473-478.

87. Mohammadi SA, Prasanna BM. Analysis of genetic diversity in crop plants salient statistical tools and considerations. Crop Sci. 2003;43(4):1235-1248.

88. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Pl. 1962;15(3):473-497.

89. Nayak NR, Rath SP, Patnaik S. *In vitro* propagation of three epiphytic orchids, *Cymbidium aloifolium (L.) Sw., Dendrobium aphyllum (Roxb.) Fisch. and Dendrobium moschatum (Buch-Ham)* Sw. through thidiazuron-induced high frequency shoot proliferation. Scientia Hortic. 1997;71(4):243-250.

90. Niedz RP, Bausher MG. Control of *in vitro* contamination of explants from greenhouse-and field-grown trees. *In Vitro Cellular & Developmental Biology-Plant*. *In Vitro* Cell Dev Biol Plant. 2002; 38(5):468-471.

91. Niknejad A, Kadir MA, Kadzimin SB. *In vitro* plant regeneration from protocorms-like bodies (PLBs) and callus of *Phalaenopsis gigantea*. Afr J Biotechol. 2011;10(56):11808-11816.

92. Ninitha NC. Compatibility studies in monopodial orchids. M.Sc. thesis, Kerala Agricultural University, Thrissur, India. 2003;115.

93. Novak SD, Luna LJ, Gamage RN. Role of auxin in orchid development. Plant Signal Behav. 2014;9(10):e972277.

94. Nuraini I, Shaib MJ. Micropropagation of orchids using scape nodes as the explant material. Acta Hortic. 1990;292:169-72.

95. Onwubiko NC, Nkogho CS, Anyanwu CP, Onyeishi GC. Effect of different concentration of sterilant and exposure time on sweet potato (Ipomoea batatas Lam) explants. Int J Curr Microbiol Appl Sci. 2013;2(8):14-20.

96. Oota K, Yamamoto Y, Morioka K. Effects of leaf age, moisture condition, temperature and light intensity on *Phalaenopsis photosynthesis*. Abstr Jpn Soc Hort Sci Autumn. 1988;534-535.

97. Oota K, Morioka K. CAM photosynthesis in *Phalaenopsis*. Proc Nagoya Int Orchid. 1990;177-179.

98. Oota, K., Kawai, A. and Ichihashi, S. Studies on useful fertilizer application method and CAM photosynthesis in *Phalaenopsis*. J Jpn Soc Hort Sci. 1992;61:534-535.

99. Paek KY, Murthy HN. Temperate Oriental Cymbidium species. In: Kull, T., Arditti, J. (Eds.), Orchid Biology: Reviews and Perspectives, VIII. Kluwer Academic Publishers. 1977;287.

100. Pant B, Thapa D. In vitro mass propagation of an epiphytic orchid, *Dendrobium primulinum Lindl.* through shoot tip culture. Afr J Biotechol. 2012;11(42):9970-9974.

101. Paradiso R, Maggio A, De Pascale S. Moderate variations of day/night temperatures affect flower induction and inflorescence development in *Phalaenopsis*. Scientia Hortic. 2012;139:102-117.

102. Paradiso R, De Pascale S. Effects of plant size, temperature, and light intensity on flowering of *Phalaenopsis* hybrids in Mediterranean greenhouses. Sci World J. 2014.

103. Park SY, Murthy HN, Paek KY. Rapid propagation of *Phalaenopsis from floral stalk-derived leaves*. *In Vitro* Cell Dev Biol Plant. 2002;38(2):168-172.

104. Parvin MS, Haque ME, Akhter F, Moniruzzaman M, Khaldun AB. Effect of different levels of NAA on *in vitro* growth and development of shoots of *Dendrobium* orchid. J Agril Res. 2009;34(3):411-416.

105. Pollet B, Kromwijk A, Vanhaecke L, Dambre P, Van Labeke MC, Marcelis LF, et al. A new method to determine the energy saving night temperature for vegetative growth of *Phalaenopsis*. Ann Appl Biol. 2011;158(3):331-345.

106. Pridgeon A. The Illustrated Encyclopaedia of Orchids, Timber Press, Portland, Ore, USA. 2000.

107. Pridgeon AM, Cribb PJ, Rasmussen F, Chase MW. Genera Orchidacearum: Volume 2. Orchidaceae. Oxford University Press on Demand. 1999;2.

108. Puchooa D. Comparison of different culture media for the in vitro culture of Dendrobium. Int J Agric Biol. 2004;6:884-888.

109. Rahi D. Induction of genetic variability in *Phalaenopsis* orchids through hybridization and embryo culture. M. sc. Thesis. Kerala Agricultural University, Thrissur, Kerala. 2017;93.

110. Rotor G. A method of vegetative propagation of *Phalaenopsis species and hybrid*. Am Orchid soc Bull. 1949;18: 738-739.

111. Runkle ES. December. Innovative production systems for ornamental potted plants: a case study for *Phalaenopsis orchids*. In International Conference on Quality Management in Supply Chains of Ornamentals. 2007;55-60.

112. Sagawa Y, Sehal OP. Aseptic stem propagation of Vanda jaaquim. Bull Pacific Orchid Soc. 1967;25: 17-18.

113. Saieed NT, Douglas GC, Fry DJ. Induction and stability of somaclonal variation in growth, leaf phenotype and gas exchange characteristics of Poplar regenerated from callus culture. Tree Physiol. 1994;14:1–16.

114. Sakanishi Y, Imanishi H, Ishida G. Effect of temperature on growth and flowering of *Phalaenopsis amabilis*. Bull Univ Osaka Pref Ser B. 1980;32:1-9.

115. Schween G, Schwenkel HG. Effect of genotype on callus induction, shoot regeneration, and phenotypic stability of regenerated plants in the greenhouse of Primula ssp. Plant Cell Tissue Organ Cult. 2003;72(1):53-61.

116. Seeni S, Latha PG. *In vitro* multiplication and ecorehabilitation of the endangered Blue *Vanda*. Plant Cell, Tissue and Organ Cult. 2000;61(1):1-8.

117. Shen X, Kane ME, Chen J. Effects of genotype, explant source, and plant growth regulators on indirect shoot organogenesis in Dieffenbachia cultivars. *In Vitro* Cell Dev Biol Plant. 2008;44(4): 282-288.

118. Singh F. Exquisite orichids from Western Ghats (India)-Aerides crispum. Am Orchid Soc Bull. 1982;51:937-939.

119. Singh F. Orchids. In: Ornamental Horticulture in India. Chadha, K.L. and Chaudhary, B. (eds.). Indian Council of Agricultural Research, New Delhi. 1986;127-153.

120. Singh F, Sagawa Y. Vegetative propagation of Dendrobium by flower stalk cuttings. Hawaii orchid.. 1972;19.

121. Sinha P, Alam MF, Hakim ML. Micropropagation of *Phalaenopsis* blume. InProtocols for *In Vitro* Propagation of Ornamental Plants 2010;77-85.

122. Soleimani V, Mohammadi A, Miri SM. Study of *in vitro* propagation of *Phalaenopsis* orchid. M.Sc Thesis of Horticulture, Islamic Azad University-Karaj Branch. 2014.

123. Stancato GC, Tucci ML. Monitoring the end of the *in vitro phase of Anthurium andreanum Lindl.* plantlets. Brazilian J Plant Physiol. 2010;22:61-68.

124. Stewart J, Button J. Tissue culture studies in Paphiopedilum. In: proceeding of the 8th world orchid conference. 1976;372-378.

125. Suto K, Tutui K. C02 absorption of *Phalaenopsis* and *Dendrobium*. Abstr Jpn Soc Hort Sci. 1978;310-311.

126. Suto K, Ito H, Sinoda K. Estimation of nutrient levels in orchids growing media. J Jpn Soc Hort Sci. 1991;60:490-491.

127. Suto K, Sinoda K, Ito H, Usui T. Effects of frequency and concentration of liquid fertilizer on the growth of *Phalaenopsis* hybrid. J Jpn Soc Hort Sci. 1991;60:524-525.

128. Talukder SK, Nasiruddin KM, Yasmin S, Hassan L, Begum R. Shoot proliferation of Dendrobium orchid with BAP and NAA. J Biol Sci. 2003;3(11):1058-10562.

129. Tamuli PS, Talukdar MC, Talukdar P. Extent of genetic variation in Anthurium cultivars for growth, flowers and physiological characters under soil-less culture. J Agri Vet Sci. 2015;8(3):07-10.

130. TANAKA M, HASEGAWA A, GOI M. Studies on the clonal propagation of monopodial orchids by tissue culture I. Formation of protocorm-like bodies from leaf tissue in Phalaenopsis and Vanda. J Jpn Soc Hortic Sci. 1975;44(1):47-58.

131. Tanaka M, Kumura M, Goi M. Optimal conditions for shoot production from *Phalaenopsis* flower-stalk cuttings cultured *in vitro*. Sci Hortic. 1988 1;35(2):117-126.

132. Tanaka M, Sakanishi Y. Clonal propagation of *Phalaenopsis* by leaf culture. Am Orchid Soc Bull. 1977;46:733–737.

133. Tanaka M, Sakanishi Y. Factors affecting the growth of *in vitro* cultured lateral buds from *Phalaenopsis* flower stalks. Scientia Horticulturae. 1978;8(2):169-178.

134. Tanaka M, Sakanishi Y. Clonal propagation of *Phalaenopsis* through tissue culture. InProc. 9th World Orchid Conference, Bangkok 1980:215-221.

135. Myint KT, Kim CK, Chung MY, Park JS, Lim KB, Chung JD. Somatic embryogenesis and plant regeneration from thin-sectioned leaf explants of *Phalaenopsis hybrid*. Hortic Environ Biotechnol. 2006;47(6):344-8.

136. Thomas B, Lekharani C. Assessment of floral characters in commercial varieties of monopodial orchids. J Ornam Hortic. 2008;11(1):15-20.

137. Tippit B. Colour variation in an orchid hybrid. Orchid Dig. 1997;61:28-31.

138. Tokuhara K, Mii M. Induction of embryogenic callus and cell suspension culture from shoot tips excised from flower stalk buds of Phalaenopsis (Orchidaceae). *In Vitro* Cell Dev Biol Plant. 2001;37:457-461.

139. Vacin EF, Went FW. Some pH changes in nutrient solution. Botan Gaz. 1949;110: 605–617.

140. Vendrame WA, Maguire I, Carvalho VS. *In vitro* propagation and plantlet regeneration from *Doritaenopsis* purple gem 'Ching Hua'flower explants. Hort Science. 2007;42(5):1256-1258.

141. Wei QL, Jian W, Littong X, Da CH. A highly effective propagation technology for *Phalaenopsis. Guangxi*. Agric Sci. 2009;40(12):1523.

142. Wimber DE. Additional observations on clonal multiplication of Cymbidiums through culture of shoot meristems. Cymbidium Soc. News. 1965;20:7–10.

143. Withner CL. Nutrition experiments with orchid seedlings. Amer Orch Soc Bull. 1942;11: 112-114.

144. Yeh CM, Chung K, Liang CK, Tsai WC. New Insights into the Symbiotic Relationship between Orchids and Fungi. Appl Sci. 2019;9(3):585.

145. Young PS, Murthy HN, Yoeup PK. Mass multiplication of protocorm-like bodies using bioreactor system and subsequent plant regeneration in Phalaenopsis. Plant Cell Tissue Organ Cult. 2000;63:67–72.

146. Yuan SC, Lekawatana S, Amore TD, Chen FC, Chin SW, Vega DM, et al. The Global Orchid Market. In The Orchid Genome. Springer. 2021;1-28.

147. Zhang QX, Fang YM. Tissue culture and *in vitro* seedling and protocorm like body examination of Dendrobium candidum. Acta Bot Sin. 2005;25:1761–1765.

148. Zhao P, Wu F, Feng FS, Wang WJ. Protocorm-like body (PLB) formation and plant regeneration from the callus culture of Dendrobium candidum Wall ex Lindl. *In vitro* Cell Dev Biol Plant. 2008;44(3):178-185.