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Micropropagation of Bulbophyllum Orchids

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Abstract— Bulbophyllum (Orchidaceae) is recognized as the largest genus of orchids in the world. Bulbophyllum is a popular orchid by its unique flowers and its benefits. Although Bulbophyllum is a large genus, research on in vitro propagation of orchids from this genus is still limited. This article aims to examine the micropropagation of several Bulbophyllum species to determine the affecting factor for the propagation of Bulbophyllum orchids. A systematic Literature Review (SLR) uses in this paper. The micropropagation of Bulbophyllum have studied in B.auricomum, B. fascinator, B. macrantum, B. phalaenopsis, B. lasianthum B. odoratissimum B. niphondii, B. dhaninivatii, B. lilacinum, B. echinolabium, B. putidum and B. plumatum. The use of appropriate media can increase the growth of orchids. The addition of growth regulators and/or organic compounds to a suitable basal medium can be a way to find a suitable medium for orchid growth. Acclimatization should be conducted on plantlets with healthy roots. The appropriate acclimatization media can increase the survival rate of orchid seedlings.

Keywords—Bulbophyllum; Micropropagation; Germination; Multiplication; Acclimatization.

I. INTRODUCTION

Bulbophyllum (Orchidaceae) is recognized as the largest genus of orchids with around 2000 species from all over the world. This genus is naturally distributed in several countries but the tropical forests of Asia are considered the main center of their diversity, with at least 1600 species [1][2][3]. The Bulbophyllum is derived from the term Bulbus, meaning Bulb-like, and Phyllon meaning leaf, referring to the pseudobulb on the apex of the growing leaf. This genus Bulbophyllum generally grows as an epiphyte but is also often found as a lithophyte. The pseudobulb in this orchid plays a role in photosynthesis. The inflorescences generally have a racemose or umbrella pattern with one or many flowers and the flower bracts are typically small [4][5].

Bulbophyllum is one of the economic value orchids by its unique flowers and benefits. Several Bulbophyllum are interesting because of their uniqueness, such as *B.auricomum [6], B. fascinator [7], B. macrantum*, and *B. phalaenopsis* [8]. Several of them are useful as medicine because they contain secondary metabolites such as *B. odoratissimum* which is known to contain *bulbophythrins* that show cytotoxic activity against several human cells cancers such as leukemia, hepatoma, lung adenocarcinoma and stomach cell cancer [9] and several listed as rare orchids such as *B. niphondii* [10], *B. dhaninivatii* [11], *B. auricomum* [12]. Based on CITES, Bulbophyllum is included in the Appendix II category [13]. Several orchid species lead decreased due to their over-exploited, limited population size, symbiont requirements, complex reproductive methods, unique habitat requirements, and scarcity as a result of logging or natural habitat damage by agriculture and the development of human settlements [14].

The conventional propagation is too slow to proceed with the worldwide demand for orchids. Therefore, alternatives in orchid propagation are needed, one of which is through *in vitro* culture. *In vitro* culture is a method of plant propagation using media

whose composition is adjusted according to the requirements of the plant, so this propagation method is mostly used for endemic or endangered plants that require conservation efforts. The condition of orchid seeds that do not have endosperm as nutrition for seed germination, the germination naturally in nature is difficult. Propagation through in vitro culture can produce large quantities of orchids in a relatively short time and have the same characteristics as their parents [15]. Although Bulbophyllum is a large genus, research on in vitro propagation of orchids from this genus is still limited. Therefore, this article examines the micropropagation of several Bulbophyllum species to determine the affecting factor for the propagation of Bulbophyllum orchids.

II. Research Methods

In this article, a systematic literature review (SLR) is used. SLR is a technique that aims to find, examine, assess, and interpret data in journals in a systematic manner in accordance. The collected data are related to a topic about the micropropagation of Bulbophyllum. Data were analyzed descriptively and tabulated into tables to summarize all the data obtained.

III. Result and Discussion

On the micropropagation of Bulbophyllum orchids, several studies have been conducted. Bulbophyllum has been propagated using micropropagation in:

A. Germination

Orchid seeds generally do not have an endosperm. Limited nutrition sources in orchid seeds cause orchids need symbiosis with endophytic fungi to help absorb nutrients for germination under natural conditions. Carbohydrates, nitrogen, minerals, and vitamins are nutrients provided by fungi during orchid germination in nature [31]. In asymbiotic germination, the required nutrition for orchid seeds obtains through artificial media [33]. Considering the constraints of establishing a symbiotic culture, the asymbiotic germination procedure has advantages in the easier cultivation process, and large-scale and rapid production of in vitro plantlets [17][34][35]. The several studies have been conducted to determine the asymbiotic germination of several Bulbophyllum orchid (Table 1).

TABLE I. The Asymbiotic Germination of Several Bulbophyllum Orchids

Type of orchid	Seed age	Media	Response	References
Bulbophyllum auricomum	± 3 month	KC and MS	MS medium was found to be the most effective culture medium for seed germination, and a high rate of protocorms formation, multiplication, and differentiation into seedlings.	[6]
Bulbophyllum fascinator	± 5 month	½ MS, ¼ MS and $^{1}/_{10}$ MS	Seeds were germinated in all media, the media ¼ MS resulted the highest germinate percentage (92%).	[7]
Bulbophyllum niphondii	**1.1.1.1 12 month**	**1.1.1.2 ¼ MS ; ½ MS; MS; VW; ½ VW**	VW resulted the highest germinated media (91.1%) and formed the stage 5 of seed development up to 34.4%.	[10]
Bulbophyllum plumatum	-	¼ MS, CG, KC, MM	Germination had more success on CG (supplemented 50 $mL.L^{-1}$ almond milk), ¼ MS and MM but the protocorms development into stage 5, emergence of first leaf, was observed only on 1/4MS and MM	[29]
Bulbophyllum putidum	3 month	¼ MS, ½ MS and MS	½ MS medium was found accelerating the germination percentage and protocorm formation to stage 3.	[54]
Bulbophyllum lilacinum	-	PM, MS, MVP	Seed germinated on all three medium, and PM medium was proved to be most effective	[56]

Note: MS: Murashige & Skoog's Medium (1962), KC: Knudson C Orchid's Medium (1922), VW: Vacin & Went's Medium (1949), MM: Malmgren Modified medium – Malmgren (1996), CG: Calevo & Giovannini (2020),

Orchid seeds are known as dust seeds because the size of the seeds is very small and produced in abundance by each flower. The small seeds are adapted for wind dispersal [16][17]. The number of seeds per capsule ranges from 4,000,000 to 20,000,000 seeds [18]. Orchid seeds contain an embryo, have a small reserve of nutrients, and an air space covered by the testa [17][18]. The testa originates from the integumentary tissue that protects the embryo after leaving the capsule. Various accumulations of hydrophobic materials in the testa lead to differences in asymbiotic seed germination in these species. In terrestrial species that are difficult to germinate, the accumulation of hydrophobic materials such as cuticle substances, phenolic compounds, and lignin in the integumentary tissue usually forms a dense cover covering the embryo which serves as a barrier to nutrient absorption when the seed matures [7][19][20].

Seed maturity is closely related to the initiation of suspensors and embryo development. The suspensor is an embryonic organ that plays an important role in embryo development in flowering plants [21]. Orchid embryo development has been studied in *Bulbophyllum fascinators*. The study showed that most of the ovules have been fertilized and embryonic development at 60 day after pollination (DAP). At 100 DAP, a distinct protoderm layer of the embryo had formed. At this stage, numerous starch granules were observed in the cytoplasm of the embryo. As the embryo develops, the single-celled suspensor begins to enlarge and elongates towards the micropyle. In maturity (160 DAP) embryos were covered with shriveled seed coats. The thick radial wall of the outermost layer of the seed coat is blue-green with TBO staining, indicating the presence of phenol. In this final stage of seed maturation, the suspensors degenerate. The mature testa consists only of the outer integument and is about two cells thick. In the globular embryo stage, the cells of the inner integument gradually degenerate, and their cell contents was presumably absorbed by the embryo. in maturity, testa cells dehydrate and are compressed into a thin layer [7].

In *B. fascinator* it was found that there was no germination on fruit aged before 80 DAP. The seed germination was poor at 100 DAP but thereafter increased gradually to 80% at 120 DAP. Optimum germination of around 90% was found when seeds were collected by 140 DAP [7]. In *B. niphondii*, fruit was harvested 12 months after pollination and stored at 10^0C before use [10], in *B. auricomum* fruit was used 3-4 months after pollination [6][12].

Several germination media have been tested on Bulbophyllum orchid germination including MS, KC, VW, and their modifications. Murashige & Skoog (MS) media is a plant basal medium primarily used in laboratories for *in vitro* propagation. This medium is the most widely used culture medium because most plant cell cultures react well to this medium. This medium contains a high salt medium compared to other media formulations with high levels of nitrogen, potassium, and several micronutrients (boron and manganese). Knudson C orchid medium was the first medium specially formulated for *in vitro* orchid cultures. Vacin and Went medium is a modified medium used for orchid culture [23]. Modifying the concentration of minerals in media can be an alternative protocol to find suitable media to increase plant growth. Nutrient concentrations that are too high or too low can inhibit plant growth and increase propagation costs [27].

The nutritional requirements of specific orchid plants depend on the type [22]. MS media increase germination most effectively than KC in *B. auricomum* [6]. In the *B. fascinator*, reducing the strength of the MS medium to ¼ MS resulted in a higher germination percentage than ½ MS and 1/10 MS [7]. In *B. nipondhii*, VW media was more effective in increasing the germination percentage than MS [10]. Calevo & Giovanini (CG) medium and Malmgren Modified (MM) medium have been tested on *B. plumatum*. CG media is a newly formulated medium that contains low salt and is enriched with the addition of casein. MM media is a modified medium for terrestrial orchids that contain lower mineral salts. The use of CG media supplemented with 50 $mL.L^{-1}$ almond milk increased germination in *B. plumatum* but protocorm development to form stage 5 (appearance of first leaves) was only observed in ¼ MS media and MM media [29].

The addition of organic compounds and growth regulators can increase the germination of some orchids. Several organic materials such as potato extract (PE), banana extract (BE) and coconut water (CW) have been used in asymbiotic germination of Bulbophyllum. The VW media supplemented 20 $g.L^{-1}$ BE + 20 $mg.L^{-1}$ NAA has been used in the germination of *B. macranthum, B. lasianthum, and B. phalaenopsis* [8]. The supplemented 10 $mg.L^{-1}$ PE and 150 $mL.L^{-1}$ CW on VW medium resulted in a high germination percentage (91.1%) of *B. nipondhii* [10]. Apart from using PE, BE, and CW, the use of organic compounds such as peptones increases germination in several orchids. Peptone is a source of organic nitrogen that plays a role in accelerating seed germination, PLB formation, and seedling formation [39][45][46][47]. Nitrogen is the main component in nucleic acids and

proteins and plays a role in plant morphogenesis. This morphogenic action is dependent on the ratio of NO3− and NH4+, which determines the overall nitrogen content. Nitrogen also has a role in the reproduction of zygotic embryos and their development [23][48]. In general, the main forms of nitrogen taken up by plants are nitrate and ammonium [25][32]. However, peptone application studies on Bulbophyllum germination have not been evaluated.

Germination Stage

Bulbophyllum orchid seeds are fusiform in shape with the embryo in the center. Testa on the outside of the seed protects the embryo. The suspensor part of the testa is a way for water to enter the seed [26]. Testa which is permeable will facilitate the process of water absorption. The absorption of water by seeds causes the growing size of the embryo [37]. While water absorption, the absorption of nutrients also occurs to encourage germination in orchids [28]. Observation of germination stages in the genus Bulbophyllum has been described on *B. niphondii*. The germination of this orchid was observed to consist of five stages: stage 0, no development seed; stage, 1 swollen embryo; stage 2, Embryo enlarge and testa rupture (germination stage); stage 3, protocorm with acute apex and rhizoids; stage 4, emergent of first leaf ; stage 5, appearance of second leaf [10].

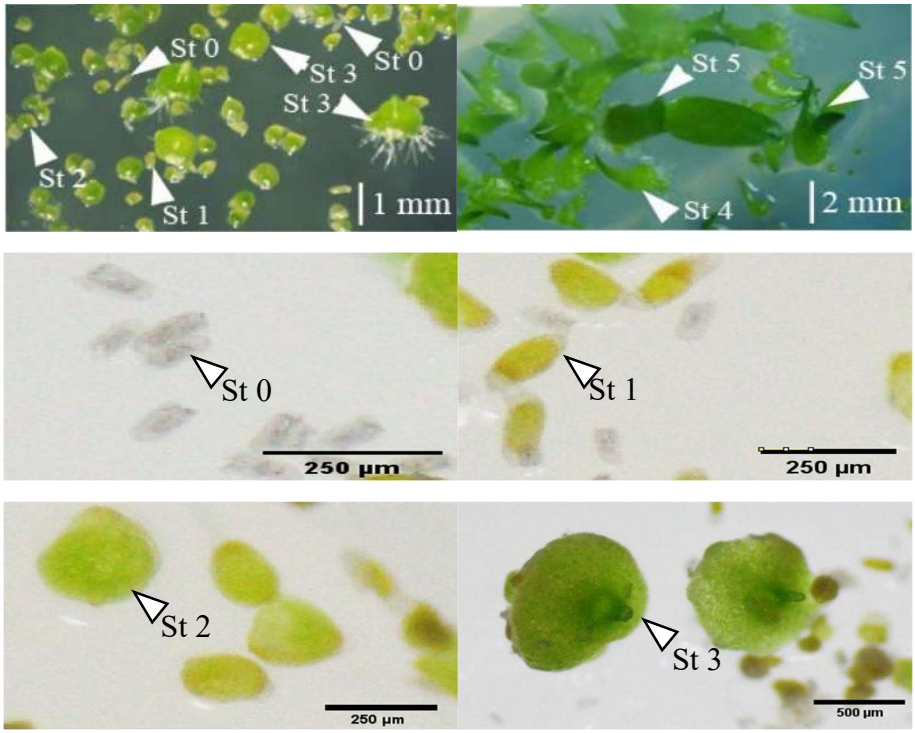

Fig. 1. Developmental stage of asymbiotic germination. a-b *Bulbophyllum nipondhii* [10], c-f *Bulbophyllum putidum* [54]. Stage 0 (St 0), no development of seed; stage 1 (St 1), swollen embryo; stage 2 (St 2), embryo enlarges and testa rupture (germination stage); stage 3 (St 3), protocorm with acute apex and rhizoids. Stage 4 (St 4), emergence of first leaf; Stage 5 (St 5), appearance of second leaf.

In germination, the orchid seeds will develop from protocorm to plantlet. Protocorm is the initial germination stage. The main goal in protocorm development is the formation of shoot apical meristem (SAM). SAM develops leading to a cell fate during embryogenesis. After being placed in a suitable medium, the embryo inside the seed coat will swell due to the absorption of water and nutrients. Nutrients absorbed by the seeds are stored and distributed to each cell to support mitotic activity. Mitotic activity at the apical pole of the protocorm forms SAM and continues to undergo division resulting in the enlargement of the SAM [49]. The protocorm will also produce rhizoids on the parts that come into contact with the culture media [50]. The first leaf that develops from the protocorm formed after the SAM development [49].

Apical and basal polarity, cell differentiation, and tissue specification in the protocorm will lead to shoot formation [51]. The green color of the protocorm is due to the presence of chlorophyll. Fe (Iron), Mg (Magnesium), and N (Nitrogen) are some of the

main nutritional elements in the synthesis of chlorophyll [52]. During shoot formation, the genes involved in photosynthesis are significantly regulated. Photosynthesis is the main process for the survival and development of protocorms into plantlets. Photosynthetic antenna proteins, which are protein pigment complexes, play a role in capturing energy from sunlight and participating in the initial steps of photosynthesis [53]. This indicates that the change in the color of the protocorm to green is due to the fact that the explants have obtained a source of energy and nutrients from photosynthesis [51].

The protocorm forms plantlets through the formation of SAM. Protocorm consists of compact meristematic cells on the surface of the explant. The ability of the protocorm to divide rapidly makes it an ideal explant for micropropagation and transformation research. The meristem mass mainly consists of large cell aggregates without intercellular spaces and clusters of smaller cells within. These cells may become centers of division and contribute to the continuous proliferation of the meristem cell mass through active cell division. Division of the compact meristem cells results in elongation and an increase in the size of the protrusions on the surface of the protocorm mass. This division will form a mature protocorm (spheroid) and show different growth polarities and cell size gradients. The protocorm remains polar and continues to elongate until it differentiates into an apex. Primordial leaves formed from protocorm apical cells will transform the protocorm into plantlets [51].

B. Multiplication

In multiplication through *in vitro* techniques, the medium is a major factor. The basal media in multiplication contains the nutrients required for plant growth. The nutritional requirements of orchids are specific to each species [22]. In Bulbophyllum, the use of MS, VW, and KC media has been widely used (Tabel 2). In the multiplication of several Bulbophyllum, the growth regulators and some organic extracts have been evaluated (Tabel 2).

TABLE II. THE MULTIPLICATION OF SEVERAL BULBOPHYLLUM ORCHIDS

Type of orchid	Media	Plant growth regulator	Organic extract	Response	References
Bulbophyllum auricomum	MS	a) 1 mg.L^{-1} BAP + 0.5 mg.L^{-1} NAA b) 1 mg.L^{-1} KIN + 2 mg.L^{-1} NAA c) 2 mg.L^{-1} BAP + 1 mg.L^{-1} NAA d) 2 mg.L^{-1} NAA + 1 mg.L^{-1} BAP e) 2 mg.L^{-1} BAP + 1 mg.L^{-1} BAP	e) 150 mL.L^{-1} CW f) 150 mL.L^{-1} CW + 30 g.L^{-1} BE + 20 g.L^{-1} PE	a) Resulted the highest multiple shoot b) Showed the optimal rooting c) Resulted the highest percentage of *in vitro* flowering (50%) with 10 weeks time of flowering d) PLBs developed into pseudobulb explants with friable callus at the base of pseudobulb explants e) Resulted the maximum plantlet regeneration from callus and PLBs f) Obtained a large amount of callus and PLBs induction	[6][12]
Bulbophyllum niphondii	**1.1.1.3 VW**	-	75 g.L^{-1} PE + 100 mL.L^{-1} CW	The combination of regeneration media resulted the highest number of new pseudobulb per explant, percentage of explant showing PLBs, number and length of leaves, number and length of root.	[10]

Bulbophyllum dhaninivati **1.1.1.4**	VW	-	100 and 150 $mL.L^{-1}$ CW + 50 $g.L^{-1}$ BH + 50 $g.L^{-1}$ PE	The highest shoot number (6.92 shoots) could observe on the medium with 150 $mL.L^{-1}$ coconut water while the highest leaf and root number could obtain when cultured on the medium supplemented with 100 $mL.L^{-1}$ coconut water.	[11]
Bulbophyllum odoratissimum	MS	4 $mg.L^{-1}$ BA + 0.5 $mg.L^{-1}$ IBA	-	The used of BA alone resulted the highest shoot induction (77%). The combination of BA and IBA result the highest number and length of shoot formation. The used of IBA alone showed the better growth of root.	[9]
Bulbophyllum macrantum	Growmore 1.5 g/l	2 $mg.L^{-1}$ NAA	100 $g.L^{-1}$ BE	Resulted the highest number of leaves and shoots. The increased NAA to 6 mg/l showed the increased of height of explants, but response to shoot formation was low.	[8]
Bulbophyllum phalaenopsis	½ MS	10 $mg.L^{-1}$ NAA	100 $g.L^{-1}$ BE	Resulted increased leaves and shoot of explants	[8]
Bulbophyllum lasianthum	½ MS	10 $mg.L^{-1}$ NAA	100 $g.L^{-1}$ BE	Showed better growth (height, number of shoot and leaves)	[8]
Bulbophyllum lilacinum	MS	a) 2 $mg.L^{-1}$ BAP + 1 $mg.L^{-1}$ IAA b) 0.5 Pic + 2 $mg.L^{-1}$ BAP	-	a) Result the highest elongation of shoot. b) Result the highest multiple shoot bud from pseudobulb segment lower part.	[56]

Note: MS: Murashige & Skoog's Medium (1962), KC: Knudson C Orchid's Medium (1922), VW:Vacin & Went's Medium (1949), MM: Malmgren Modified medium – Malmgren (1996), CG: Calevo & Giovannini (2020), BAP: Benzyl Amino Purine, KIN: Kinetin, NAA: Naphthalene Acetic Acid, IBA: Indole Butyric Acid, Pic: Picloram, BE:Banana Ekstract, CW: Coconut Water, PLB: Protocorm Like Bodies

Plant growth regulators (PGRs) are chemical substances in the plant tissue culture medium for their specific functions. These PGRs play a role in organogenesis. There are different types of PGRs available in nature that have a specific function, and there are synthetic PGRs that are added to the artificial plant tissue culture medium for organogenesis [23]. Cytokinin and auxin group regulatory substances are widely used in multiplication. Cytokinins are derivatives of adenine that have a role in promoting cell division, stimulating initiation and growth of shoots *in vitro*. Higher concentrations of cytokinins lead to the inhibition of root formation and promote adventitious shoot formation. Cytokinins are divided into two main classes: natural (trans-zeatin, cis-zeatin, iP, dihydrozeatin, and zeatin riboside) and synthetic cytokinins. Organic supplements such as yeast extract or coconut milk are rich sources of natural cytokinins. Synthetic cytokinins are further divided into two classes namely (1) purines (N6-substituted adenine derivatives and several other less structurally related compounds such as 4-alkylaminopteridine and 6-benzyloxypurine) and (2) phenylurea (1,3-diphenylurea and thidiazuron) [24]. Auxins have diversified physiological activities such as cell

elongation, differentiation, somatic embryogenesis, root, shoot initiation, and apical dominance. Many synthetic auxins are substitutes for IAA and are added to artificial plant tissue culture medium. 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), 4-amino-3,5,6-trichloropicolinic acid (picloram), 3,6-dichloro-o-anisic acid (Dicamba), and naphthoxyacetic acid are some of the synthetic auxins which are used in plant tissue culture medium [23][30]. The auxin/cytokinin ratio plays a major role in morphogenesis. A high auxins ratio causes embryogenesis and root initiation while a low ratio causes axillary bud and shoot proliferation, and a balance ratio for callus formation [24].

In several multiplication Bulbophyllum species, PGRs have been used. The cytokinin and auxin were reported to have increased the growth of shoots and leaves in *B. auricomum* [12], *B. odoratissimum* [9], *B. macrantum* [8] and also induces *in vitro* flowering in *B. auricomum* [6]. The addition of 1 $mg.L^{-1}$ BAP with 0.5 $mg.L^{-1}$ NAA was most responsive for shoot formation while 1 $mg.L^{-1}$ KIN with 2 $mg.L^{-1}$ NAA was the optimum concentration in root formation of *B. auricomum* [6]. The addition of 2 $mg.L^{-1}$ NAA to Growmore media which was supplemented with peptone and PE increased the number of leaves and shoots, while increasing NAA to 6 $mg.L^{-1}$ increased plantlet height in *B. macranthum* [8]. The addition of 4 $mg.L^{-1}$ BAP alone on MS media increased shoot induction in *B. odoratissimum.* The combination of 4 $mg.L^{-1}$ BAP with 0.5 $mg.L^{-1}$ IBA resulted in the highest shoot formation while root induction was optimum in media with 0.5 $mg.L^{-1}$ IBA alone [9].

As artificial media give different results depending on the target species, screening media and supplements would be helpful to determine the best nutrient formulation that maximizes the growth of orchids [34][35][36]. The addition of organic nutrients to media is considered to have a major role in the growth of orchids through *in vitro* techniques. The addition of organic matter composition can increase the germination rate of orchid embryos, support the development of PLB (Protocorm Like Body) regeneration, initiate the formation of shoots and roots, and provide sufficient additional nutrition for plantlet development [37][38].

The effect of organic extracts in increasing the growth of the orchids has been evaluated on several Bulbophyllum orchids. Various organic ingredients have been studied in Bulbophyllum including coconut water, potato extract, and banana extract. Coconut water added to the media can be a source of amino acids, organic acids, vitamins, a source of sugar, and also hormones, both auxins and cytokinins [39][40]. The complex content of coconut water, especially its auxin and cytokinin hormones, has a major influence on the growth of explants *in vitro* [41]. Potatoes are known to contain many nutrients that can support the growth of orchids. Carbohydrates are one of the high content in potatoes. Carbohydrates have the main factors for supporting the primordial development of shoots and roots. Carbohydrates in potatoes are a basic source of energy-producing energy to grow and develop. Bananas contain many beneficial compounds to plant growth, including protein, fat, carbohydrates, fiber, minerals such as calcium, phosphorus, and ferrous, as well as vitamin C, vitamin B6, vitamin A, riboflavin, and niacin. In addition, bananas contain thiamine which can accelerate cell division in tissue culture [41][42]. The addition of 100 $mL.L^{-1}$ coconut water and 25 $g.L^{-1}$ potato extract increased the formation of new pseudobulbs and the total number of leaves in *B. nipondhii*. Whereas the increase in potato extract to 50 $g.L^{-1}$ increases leaf length and root length [10]. In *B. auricomum* orchids, MS medium supplemented with 150 $mL.L^{-1}$ CW, 2 $mg.L^{-1}$ bap, and 1 $mg.L^{-1}$ NAA increased shoot induction and plantlets growth [6].

C. Acclimatization

The study on acclimatization to Bulbophyllum revealed *B. odorratisimum*. Acclimatization is carried out on plantlets that already have healthy roots. To reduce the microbial infection, the cleaned plantlets were treated in a fungicide solution for a few minutes. Several acclimatization media on Bulbophyllum have been tested [9]. The main characteristics of an appropriate potting mixture are water holding capacity, aiding aeration, and draining out of excess water for proper plantlet growth [43][44]. Media contains small brick chips with charcoal pieces and coco peat in a ratio of 1: 1: 1 result the highest survival percentage (91.66%) in *B. odoratissimum*. While the fern root with charcoal pieces media in ratio 3:1 result the survival percentage to 44,4% [55]. Using suitable acclimatization media can provide a higher water retention capacity and better aeration for transplanted orchids. [9].

TABLE III. THE MICROPROPAGATION OF SEVERAL BULBOPHYLLUM ORCHIDS

Type of orchid	Media	Ratio	Survival Percentage	References
Bulbophyllum odoratissimum	Small brick chips, charcoal pieces and cocopeat	1:1:1	91.66%	[9]
Bulbophyllum echinolabium	Fern root, charcoal pieces	3:1	44,4%	[55]

The survival rate of the explant in acclimatization is influenced by the right protocol to bring out the explant. Several things considered in acclimatization include explants must be free from the culture media because it can cause contamination, and the larger size of the plantlets can increase the survival rate. In maintenance, environment and nutrition conditioning is an important factor in increasing the percentage of life. This factor affects the overall physiological process, be it anabolism or catabolism [55]. In *B. odorratisimum*, seedlings are maintained in a greenhouse environment (i.e. 25 ^{0}C, RH: 90%) [9].

IV. CONCLUSION

In this review, we present the micropropagation of several Bulbophyllum orchids. *In vitro* propagation of this genus has been carried out on *B. auricomum, B. fascinator, B. nipondhii, B. dhaninivatii, B. odiratissimum, B. phalaenopsis, B. macranthum, B. lasianthum*, *B. lilacinum, B. echinolabium, B. putidum* and *B. plumatum*. The use of appropriate media can increase the growth of orchids. The addition of growth regulators and/or organic compounds to a suitable basal medium can be a way to find a suitable medium for orchid growth. Acclimatization should be conducted on plantlets with healthy roots. The appropriate acclimatization media and protocols can increase the survival rate of orchid seedlings.

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