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Systematic Literature Review: The Aplication Of Thin Cell Layer (TCL) Technique In Orchid Plant Propagation

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Abstract – Thin Cell Layer (TCL) is a technique using small thin sections produced from organ pieces. TCL explants more effective than large conventional explants. Thin explants facilitate contact and diffusion of media into the tissue, better than thick explant slices. This literature review aims to gather information related to the application of the thin cell layer technique in orchid plant propagation. **A systematic Literature Review (SLR) uses in this paper.** **TCL explants proved to be more effective than large conventional explants. Thin explants facilitate contact and diffusion of media into the tissue, better than thick explant slices. The TCL technique has been successfully used in the propagation of several orchid species including Paphiopedilum callosum, Cattleya forbesii, Dendrobium aduncum, Phalaenopsis hybrid, Brasilidium forbesii, Dendrobium aqueum, and Hadrolaelia grandis. The TCL technique both transversely and longitudinally has its own specificity with each type of explant used. In the TCL technique, protocorm is the most potential explant in orchid propagation. The development of explants using the TCL technique requires media supplemented with growth regulators. In general, the growth regulators commonly used in orchid propagation using the TCL technique are auxins and cytokinins.**

Keywords – Orchid; propagation; thin cell layer

I. INTRODUCTION

Orchids (Family Orchidaceae) are ornamental plants that have the potential to be developed due to their economic value with a variety of shapes, colors, sizes, distinctive aromas, long-lasting quality of flowers, in addition to their potential as medicinal plants, cut flowers and potted plants. Orchids consist of 800 genera and 25,000 species [1], but most types of orchids are increasingly difficult to find in their natural habitat due to land conversion and illegal hunting [2]. In an effort to maintain the existence of the types of orchids need to be multiplied. Propagation can be done in two ways, generative and vegetative propagation. Vegetative propagation requires a longer time, while generative propagation is constrained by orchid seeds not having endosperm to store food reserves making it difficult to germinate naturally [3]. One of the efforts to propagate orchids is through tissue culture techniques or *in vitro* propagation.

In vitro propagation is a propagation technique used to grow plant parts under aseptic conditions. Propagation in vitro has several advantages: it does not require a large area, can be used for difficult or slow propagation, can be done throughout the year, produces healthier and more uniform plants, and plants can be stored for a long time [4]. Many orchid plants have been propagated *in vitro*, including *Phalaenopsis* Hybrid, *Hadrolaelia grandis, Paphiopedilum callosum, Cattleya forbesii, Dendrobium aduncum, Brasilidium forbesii*, and *Dendrobium aqueum.*

The success of *in vitro* culture is influenced by several factors: media, plant growth regulators, and explant sources. Media is a place to grow plants in vitro [5]. Growth regulators are organic compounds that are not nutrients and, in small amounts,

promote, inhibit, or regulate physiological processes in plants. The function of growth regulators is to stimulate the growth of morphogenesis in cell, tissue and organ cultures. Explants are cells, tissues or organ slices grown in vitro on artificial media. Explants can be protocorms, flowers, nodes, Protocorm Like Bodies (PLB), leaves and shoots [4].

One of technique for taking explants in tissue culture is the thin cell layer technique. Thin Cell Layer (TCL) is a technique using thin slices produced from organ pieces. The selected organs are embryonic organs such as stems (epicotyl/hypocotyl), roots, leaves, floral organs (stigma, style, ovary), cotyledons, and embryos, which are planted whole in conventional multiplication preparation. Based on the direction of cut, TCL can be divided into 2 types, transverse Thin Cell Layer (tTCL) and longitudinal Thin Cell Layer (lTCL). In tTCL slices (0.2/0.5 or a few mm of thickness), the explants will consist of a number of cells originating from various tissues, namely the epidermis, cortical, cambium, parenchyma, perivascular and medullary. While lTCL (1 mm × 0.5 or 10 mm) contains only one type of tissue, such as a monolayer of epidermal cells. TCL also called TTL (thin tissue layer), tTTL = transverse thin tissue layer (symmetrical cross-section through a donor explant tissue) = 5-10 mm in length and diameter, maximum 1 mm in thickness; lTTL = longitudinal thin tissue layer (longitudinal section through a donor explant tissue) = 5-10 mm in length and diameter, maximum 1 mm in thickness; µtTTL/µlTTL = TTLs prepared with a microtome under aseptic conditions = 5-10 mm in length and diameter, 10-100 µm in thickness. In conclusion, size does matter, but more importantly, so do area and volume [6].

TCL explants more effective than large conventional explants. Thin explants facilitate contact and diffusion of media into the tissue, better than thick explant slices. In terms of the number of plantlets produced, the TCL technique is more effective than planting large explants. This is because explants with thin slices facilitate the process of media diffusion into the tissue [7]. In the right growing environment (media) will encourage and control morphogenesis (organogenesis and somatic embryogenesis) and regeneration of shoots or somatic embryos with a higher frequency and faster. The speed of cell growth without passing through callus formation minimizes the opportunity for diversity to occur due to chimeras with a small size of 0.1-0.5 mm providing high competition for nutrients against microorganisms that may live in tissues thus allowing elimination of contaminants [8].

This technique has wide applications in the field of in vitro propagation of ornamental, horticultural, and medicinal plants. TCL is an effective model system to study the control morphogenesis mechanism and transformation and has been proved as an efficient system in some monocotyledonous and dicotyledonous species. TCLs, very small explants derived from a limited cell number of uniform tissue, are useful for reducing the time period and can produce high number of shoots with more competence than primary in vitro culture techniques. The thin cell layer system is a highly organized (and considered to be an essential) system to study the growth and development of plants. The actual regeneration capacity of TCL explants is often much higher than thicker conventional explants partly due to having a higher ratio of morphogenic cells and better transport between the medium and these cells [9].

TCL culture system could be used for the large scale production required for plant conservation. For instance, protocorm-like bodies (PLBs) of *Dendrobium malones* 'Victory' and *Xenikophyton smeeanum* (Reichb.f.) were successfully induced from thin sections of leaf and shoot tips, respectively, in a short period of time. The secondary PLBs were induced from tTCL of primary PLBs of *Cymbidium* Sleeping Nymph [10]. TCL technique also contributes to mass propagation of plants which are used in genetic transformation, micropropagation or bioreactors. Over 49 years since the birth of the TCL concept, morphogenesis in many different plant species or hybrids including many horticultural, ornamental, orchid species, and a few crop plants were successfully reported using TCLs as the explant [11].

The benefits of using TCL explants can be maximized if factors that affect the growth and regeneration of the TCL system such as hormones, media, type of incision, age of the explant, and the level of thickness of the cut, and the position of the organs are identified and controlled properly. TCL technology has been widely applied across dozens of plant families to induce clear and successful regeneration protocols, superior to when larger size, more conventional explants are used. TCLs are also applied in genetic engineering, in vitro flowering, establishment of cultures for standardized secondary metabolite production, and the use of these small explants in studying genetics, differentiation and biochemical events. TCL system can reprogram differentiated cells into multi-programmable patterns with a specific spatial/temporal sequence to its original. Therefore, TCL is more effective than conventional explants [6]. Studies using the TCL technique in orchid propagation include *Paphiopedilum callosum* [12], *Phalaenopsis* hybrid [13], *Dendrobium gratiosissimum* [14], *Phalaenopsis* hybrid [15], *Brasilidium forbesii* [16], and *Epidendrum secundum* [17]. This literature review aims to gather information related to the application of the thin cell layer technique in orchid plant propagation.

II. Research Methods

A systematic Literature Review (SLR) uses in this paper. SLR is a method that aims to identify, review, evaluate and interpret data in journals systematically according to the steps specified [18]. In searching for and collecting data related to a topic about the application of thin cell layer technique in orchid plant propagation. Data were analyzed descriptively and tabulated into tables to summarize all the data obtained.

III. Result And Discussion

Several studies have been conducted to see the application of the thin cell layer (TCL) technique in orchid plant propagation. The success of the TCL technique is influenced by several factors including:

1. Incision Type

Several studies have been conducted regarding the response of the TCL technique to the propagation of several types of orchids (Table 1).

TABLE I. THE RESPONSE OF THE TCL TECHNIQUE TO THE PROPAGATION OF SEVERAL TYPES OF ORCHIDS

Type of Orchids	Incision Type (tTCL/lTCL)	Response	References
Paphiopedilum callosum	tTCL	The highest percentage of PLB regeneration, shoot and root induction	[12]
Cattleya forbesii	tTCL	The highest percentage of PLB and number of shoots	[19]
Dendrobium aduncum	tTCL	The highest number of PLB, shoot regeneration, and root growth	[20]
Phalaenopsis Hybrid	tTCL	Highest shoot growth	[13]
Brasilidium forbesii	lTCL	The highest PLB induction and shoot regeneration	[16]
Dendrobium aqueum	tTCL	Effective for induction of somatic embryogenesis	[21]
Hadrolaelia grandis	lTCL	Highest PLB regeneration	[22]

The tTCL incision type was used on 5 types of orchids: *Paphiopedilum callosum, Cattleya forbesii, Dendrobium aduncum, Phalaenopsis* Hybrid, and *Dendrobium aqueum*. While the LTCL incision type was used on 2 types of orchids: *Brasilidium forbesii* and *Hadrolaelia grandis.* The type of incision that is often used in orchid propagation is the transverse thin cell layer (TCL) (Table 1). Both types of incisions have been shown to provide the best response for the propagation of several types of orchids.

Based on the direction of cut, TCL can be divided into 2 types, transverse Thin Cell Layer (tTCL) and longitudinal Thin Cell Layer (lTCL). The lTCLs (1 mm × 0.5 or 10 mm) include only one tissue-type for example a monolayer of epidermal cells (which could be peeled off the organs) or several (3-6) layers of cortical cells whereas the tTCLs (0.2/0.5 or a few mm of thickness) include a small number of cells of different tissue-types (epidermal, cortical, cambium, perivascular and medullar tissue as well as parenchyma cells). TCL also called TTL (thin tissue layer), tTTL = transverse thin tissue layer (symmetrical cross-section through a donor explant tissue) = 5-10 mm in length and diameter, maximum 1 mm in thickness; lTTL = longitudinal thin tissue layer (longitudinal section through a donor explant tissue) = 5-10 mm in length and diameter, maximum 1 mm in thickness; µtTTL/µlTTL = TTLs prepared with a microtome under aseptic conditions = 5-10 mm in length and diameter, 10-100 µm in thickness. In conclusion, size does matter, but more importantly, so do area and volume [6]. According to [16], both TCL techniques in *Brasilidium forbesii* micropropagation and lTCL were more effective than tTCL for PLB formation, and subculture using the same BA concentration increased the frequency of formation and the total number of PLB.

Approximately 30% of the tTCL explants and 60% of the lTCL sections produced 14.9 – 19.5 PLBs per responsive explant within eight to 16 weeks of culturing on growth regulator-free WPM. Protocorms of *Esmeralda clarkei* responded readily on Murashige and Skoog (MS) medium supplemented with or without growth regulators. In contrast to the present study, for TCLs of some orchid species, death occurred after two to three weeks on a medium without growth regulators [23]. According to [22], Culture of lTCL protocorm in medium without growth regulators showed a better PLB regeneration response compared to the tTCL technique (98.3% and 80.0% from two and three months of age, respectively).

2. Type of explants

Several studies have been conducted to determine the type of explants in the propagation of several types of orchids using the TCL technique (Table 2).

TABLE II. THE TYPE OF EXPLANTS IN THE PROPAGATION OF SEVERAL TYPES OF ORCHIDS

Type of Orchids	Type of Explants	References
Paphiopedilum callosum	Flower	[12]
Cattleya forbesii	Nodes	[19]
Dendrobium aduncum	Protocorm Like Bodies (PLB)	[20]
Phalaenopsis Hybrid	Protocorms	[13]
Brasilidium forbesii	Seed dan Protocorm Like Bodies (PLB)	[16]
Dendrobium aqueum	Shoots	[21]
Hadrolaelia grandis	Protocorms	[22]

The type of explants used in *Phalaenopsis* Hybrid and *Hadrolaelia grandis* are protocorms. While *Paphiopedilum callosum*, *Cattleya forbesii*, *Dendrobium aduncum, Brasilidium forbesii,* and *Dendrobium aqueum* orchids, explant types used flowers, nodes, Protocorm Like Bodies (PLB), seeds and PLB, and shoots respectively. It can be seen that the types of explants that are often used in orchid propagation are protocorms (Table 2). Explants are cells, tissues or organ slices grown in vitro on artificial media. Thin explants proved to be more effective than large conventional explants.

Thin explants facilitate contact and diffusion of media into the tissue, better than thick explant slices. In the right growing environment (media) will encourage and control morphogenesis (organogenesis and somatic embryogenesis) and regeneration of somatic shoots/embryos with a higher frequency and faster. The speed of cell growth without passing through callus formation minimizes the opportunity for diversity to occur due to chimeras with a small size of 0.1-0.5 mm providing high competition for nutrients against microorganisms that may live in tissues thus allowing elimination of contaminants [8].

According to [13], protocorm explants were able to produce the highest shoot growth in *Phalaenopsis* Hybrid orchids. Explants from seeds and protocorm like bodies were able to produce the highest PLB induction and shoot regeneration in *Brasilidium forbesii* [16]. Research conducted by [21], effective shoot explants for the induction of somatic embryogenesis in *Dendrobium* aqueum orchids. Protocorm-like bodies (PLBs) of *Dendrobium malones* 'Victory' and *Xenikophyton smeeanum* (Reichb.f.) were successfully induced from thin sections of leaf and shoot tips, respectively, in a short period of time. The secondary PLBs were induced from tTCL of primary PLBs of *Cymbidium* Sleeping Nymph [10].

3. Media

Several studies have been conducted to determine the media used in the propagation of several types of orchids using the TCL technique (Table 3).

TABLE III. TYPE OF MEDIA IN THE PROPAGATION OF SEVERAL TYPES OF ORCHIDS USING THE TCL TECHNIQUE

Type of Orchids	Type of Media	References
Paphiopedilum callosum	½ MS	[12]
Dendrobium aduncum	½ MS	[20]
Phalaenopsis Hybrid	MS	[13]
Brasilidium forbesii	WPM	[16]
Dendrobium aqueum	½ MS	[21]
Hadrolaelia grandis	WPM	[22]

MS media was used on 4 types of orchids: *Paphiopedilum callosum, Dendrobium aduncum, Phalaenopsis Hybrid,* and *Dendrobium aqueum*. While the WPM media was used on 2 types: *Brasilidium forbesii* and *Hadrolaelia grandis*. It can be seen that the media that is often used in orchid propagation is Murashige and Skoog (MS) media (Table 3). Media is the main factor in propagation with tissue culture. The success of plant propagation and propagation by tissue culture methods in general is highly dependent on the type of media. The growing media in tissue culture has a very large influence on the growth and development of explants and the seeds they produce [24].

MS media is the best medium for in vitro propagation of epiphytic and terrestrial orchid species [25]. This is because MS media is a medium with the most complete nutritional composition when compared to growth media for most other orchids such as VW and Knudson C. MS media in tissue culture consists of macro stock and micro stock which have high concentrations of mineral salts. The content of macro and micro nutrients in this media is more complex when compared to other media. This medium has a high concentration of mineral salts and N compounds in the form of NO_3^- and NH_4^+. MS media is a medium that is rich in nitrogen elements in the process of cell division and enlargement as well as the preparation of amino acids [26].

The use of MS media with different concentrations can have different effects on the growth of several types of orchids. Research conducted by [27], a decrease in MS media composition of 25-50% resulted in better growth of all observed parameters. ½ MS media is MS media in which the concentration of macronutrients is reduced to ½ of the commonly used concentration [28]. Reducing the composition of the MS media up to ½ MS is still able to support plant growth in vitro due to the presence of endogenous hormones in the explants which support the availability of macro and micro nutrients in the reduced composition of the media so that it continues to affect plant growth [29].

4. Plant Growth Regulators

Several studies have been conducted to determine the plant growth regulators used in the propagation of several types of orchids using the TCL technique (Table 4).

TABLE IV. PLANT GROWTH REGULATORS USED IN THE PROPAGATION OF SEVERAL TYPES OF ORCHIDS

Type of Orchids	Plant Growth Regulators	References
Paphiopedilum callosum	BAP, TDZ	[12]
Dendrobium aduncum	BAP, NAA, Kinetin	[20]
Phalaenopsis Hybrid	BAP, NAA	[13]
Brasilidium forbesii	BA	[16]
Dendrobium aqueum	IBA	[21]
Hadrolaelia grandis	BA, IBA, BAP	[22]

Combination of auxin and cytokinin was used on 3 types of orchids namely Dendrobium aduncum, Phalaenopsis Hybrid, and Hadrolaelia grandis. While auxin and single cytokinin PGRs were used on 3 types of orchids namely Paphiopedilum callosum, Brasilidium forbesii, and Dendrobium aqueum. It can be seen that the growth regulators that are often used in orchid propagation are auxins and cytokinins (Table 4). Plant Growth Regulators are organic compounds that are not nutrients, and in small amounts encourage, inhibit, or regulate physiological processes in plants. The function of growth regulators is to stimulate the growth of morphogenesis in cell, tissue and organ cultures. There are five types of growth regulators, namely auxin, gibberellins, cytokinins, ethylene and abscisic acid. The success of a tissue culture technique depends on the use of plant growth regulators. In general, the commonly used growth regulators are the auxins, cytokinins, and gibberellins [30].

Auxin can stimulate division, enlargement, cell differentiation and flow of protoplasm in vegetative growth of plants including root organs. Auxin has several types including NAA, IAA, IBA, 2,4-D, and others [31]. Cytokinins are adenine replacement compounds that increase cell division and growth regulation functions. Cytokinins are thought to be produced in roots and transported to shoots, because they are found in xylem solution, but cytokinins are found in large quantities in fruit and seed tissues. The role of cytokinins in plants is as (a) regulating cell division (b) organ formation, enlargement of cells and organs (c) preventing damage to chlorophyll, forming chloroplasts (d) delaying senescens, opening and closing of stomata (e) development of shoots. Cytokinins have various types, such as BAP, kinetin, and thidiazuron [5].

IV. CONCLUSIONS

Within these reviews, we show the application of thin cell layer technique in orchid plant propagation. The TCL technique has been used in the propagation of several orchid species including *Paphiopedilum callosum, Cattleya forbesii, Dendrobium aduncum, Phalaenopsis* hybrid, Brasilidium forbesii, and *Hadrolaelia grandis*. The TCL technique both transverse and longitudinal has its own specificity with each type of explant used. Protocorm is the most potential explant in orchid propagation in TCL technique. The development of explants using the TCL technique requires media supplemented with growth regulators. In general, the plant growth regulators commonly used in orchid propagation using the TCL technique are auxins and cytokinins.

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