International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

Vol. 39 No. 2 July 2023, pp. 340-345

An Overview: Effect of Plant Growth Regulatory on Orchid Propagation through The Thin Cell Layer technique

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Abstract—Thin Cell Layer (TCL) is a micropropagation method using thin-sized explants. A TCL can be prepared from any explant source and the thichness of explant less than 5 mm. TCL is more efficient in producing total plantlet output than conventional in vitro methods. TCL have been applied to the in vitro culture of orchids, field, vegetable crops and medicinal plants The successful in vitro orchid propagation is influenced by many factors, such as plant genotype and media composition. Additions of plant growth regulator (PGR) in media culture is essential factor. The formation of complete plant depent on concentration and type of plant growth regulator. TCL explants require growth regulators to form an embryonic callus and zygotic embryos. Explant in medium without supplemented of growth regulators resulted in browning and failed to grow. The most commonly plant growth regulator in tissue culture are thidiazuron (TDZ), 6-benzylaminopurine (BAP) 2,4 dichlorophenoxyacetic acid (2,4-D), α-naphthaleneacetic acid (NAA), and meta-Topolin. Recently, microalgae can also be used as an alternative source of hormones to increase plant growth through in vitro culture techniques

Keywords—In Vitro; Micropopagation; Pgrs;TCL

I. INTRODUCTION

The Orchidaceae is one of the largest families of flowering plant, consisting of about 28,000 accepted species and distributed in about 763 genera [1]. Indonesia as a mega-biodiversity country in the tropical equator, consists of 5000 species of orchids [2]. Orchids are distributed in almost every ecosystem and habitats except Antarctica and deserts, however it is estimated that over 55% of the species globally are under IUCN Red List [3]. People interest to collect the orchid from nature to cultivated at home or nursery due to a high value of flower for commercial use. This is causing the scarcity of orchids in nature, decreasing its population and endangering its existance. Therefore an effort for the conservation of orchid is needed [4]. One of the conservation efforts that can be done is in vitro micropropagation.

Thin Cell Layer (TCL) is a micropropagation method using thin-sized explants. The thickness of the tcl explants isapproximately 0,1- 0,5 cm [5]. The TCL is more efficient in producing total plantlet output than conventional in vitro methods [6]. This is due to the thin size of the explants which facilitates the process of diffusion of nutrients contained in the media into the tissue [7]. TCL consists of several cells that can form somatic embryos at a higher frequency than thick explants [8]. TCL technology is more prospective in clonal propagation [9]. Shoot proliferation was faster using the TCL technique [5]. TCL hava been succesfully for micropropagation several orchid such as *Vanilla planifolia* [10], *Brasilidium forbesii* [11], *Epidendrum secundum* [12], *Paphiopedillum callosum* [13], *Dendrobium aqueum* [14], *Dendrobium aphyllum* [15], and *Phalaenopsis hybrid* [16].

TCL can be prepared from almost any plant organ. Cutting explants using the TCL technique can be done longitudinally (lTCL) and transversely (tTCL). In transverse sectioning (tTCL), the explant consists of a number of cells originating from various tissues such as epidermis, cortical, cambium, parenchyma, perivascular and medullary. In longitudinal sectioning (lTCL), the explants consist of only one tissue like a monolayer of epidermal cells [17]. Histological analysis of explants propagated through TCL showed that there was no difference between transverse and longitudinal sections [10]. The percentage of explant responses to form somatic embryos did not have a significant difference between tTCl and lTCL [18].

The successful of TCL technique is influenced by many factors such as genotype, light, age of the explant source, media pH, thickness and plant growth regulators (PGRs)[17]. The composition of media culture have significant effect on the growth and development ef explant. Additions of PGRs in media culture is essential factor. The formation of complete plant depent on concentration and type of PGRs [19]. PGRs are organic compund but not nutrients when applied in low concentration give effect physiological process [20]. TCL explants require growth regulators (PGR) to form an embryonic callus and zygotic embryos (14). The addition of individual cytokinins to TCL culture has been reported to encourage the frequency of EC and SE formation in many orchids [6]. Based on the several research that has been done, each species has different types and concentrations of growth regulators for its development.Explant in medium without supplemented of growth regulators resulted in browning and failed to grow. Culture media are supplemented with cytokinins that promote cell division, induction adventitious shoots and induce somatic embryogenesis. The most commonly used cytokinins in tissue culture are thidiazuron (TDZ), 6-benzylaminopurine (BAP) and which may be combined with auxins such as 2,4 dichlorophenoxyacetic acid (2,4-D) and α-naphthaleneacetic acid (NAA) [17]. Recently, *meta*-topolin have emerged as an effective alternative to conventional cytokinins has been applied in various plant species including orchids [15].

II. Result And Discussion

Table I. Effect Of Plant Growth Regulator On Orchid Propagation Through The Thin Cell Layer Technique

Orchid	PGRs	Response	Reference
Dendrobium draconis	NAA & BA	The combination of 2 mg/L BA and 1 mg/L NAA showed the highest percentage of forming somatic embryos (68%) than individual treatment.	[20]
Malaxis wallichii	*meta*-topolin & NAA	The combination of *meta*-topolin 1 mg/L and 0,5 mg/L NAA showed the highest regeneration frequency, number and lenght of shoot	[22]
Dendrobium aphyllum	*meta*-topolin	15 µM *meta*-topolin showed the highest percentage of shoot proliferation (79,43%).	[15]
Dendrobium aqueum	BA &2iP	Combination 0,5 BA and 1,5 2iP showed highest somatic embrio (33,67%) than individual treatment.	[14]
Paphiopedillum callosum	TDZ	VW medium supplemented with 1.0 mg/L TDZ (46,67%) showed the highest percentage of PLB induction and number of PLBs per explant were obtained on	[13]
Phalaenopsis amabilis	TDZ	lTCL explants cultured on medium supplemented with 3 mg/L TDZ produced the highest number of somatic embryos (95,7)	[18]
Cattleya labiate	PGRs from Microalga	Higher percentages of PLB regeneration were obtained with BM (*Messastrum gracile* biomassa) and EM (*Messastrum gracile* ekstrack)	[23]

Based on the research media without the addition of growth regulators resulted in browning and failed to grow. Hormones accelerate the transport of proteins to the nucleus. Thus it can shorten the time needed by cells to complete the cell cycle. Giving a stimulator like hormones increases the rate of cell division. This resulted in the initiation of the cell division cycle occurring

earlier than the control treatment. Cytokinins can increase the number of cells by binding to protein receptors in the plasma membrane of target cells [24]. Cytokinins activate the phosphatase enzyme which will release phosphate from CDK protein (cyclin dependent kinase). The CDK protein which contains only one phosphate will become active and induce cells to enter the mitotic phase [25].

Each orchid species propagated through the TCL technique responds to PGRs at different times. On *Cattleya labiata* , PLBs were observed on the surface of the TCL explants after 4 weeks in all treatments (*Messastrum gracile* extract and biomass, *Chlorella vulgaris* extract and biomass, BAP, TDZ, and ZEA) [23]. On *Phalaenopsis amabilis*, globular shaped protuberances was formed after 6 weeks culture in media containing 2 and 3 mg/L TDZ [18]. Explant TCL of *Dendrobium aqueum* cultured on the medium supplemented with cytokinins induced embryonic callus after 5 weeks culture [14]. In the presence of individual cytokinins, PLBs of *dendrobium draconis* formed directly from the TCS explants after 2–3 weeks of culture, but the percentage of forming somatic embryos was lower [21]. In *Dendrobium aphyllum*, *t*-TCL explants were cultured in MS medium supplemented with meta-Topolin induced of adventitious buds within 2 weeks of culture and the highest response of shoot proliferation was found in medium supplemented 15 µm *meta*-topolin [15]

Based on several study about micropropagation orchid through thin cell layer technique, *Dendrobium* gave a faster response than *Phalaenopsis* and *Cattleya*. Explant TCL of *Dendrobium aphyllum* in media supplemented cytokinin alone (*meta*-Topolin*)* showed better respon than *Dendrobium aqueum* and *Dendrobium draconis. meta*-topolin is a cytokinin that play a role for in vitro shoot induction, multiplication and in vitro rhizogenesis in plants [26]. Medium supplemented *meta*-Topolin was better to increase numbers and lengths of shoots, roots of *D. chrysanthum* Wall. ex Lindl. than BAP [27]. *Meta*-Topolin (6-(3-hydroxylbenzylamino) purine) is aromatic cytokinin that isolated from leaves of a poplar and so it is considered a natural [28]. meta-Topolin increases the levels of basic organic compounds, such as chlorophyll, free amino acids and soluble proteins. Photosynthetic pigments play a key role in morphogenesis of plant [29]. Media supplemented *meta*-Topolin showed the highest levels of proteins and free amino acid. Addition of *meta*-Topolin resulted in a better regeneration of shoots compare medium suppplemented plant growth regulator. This may be due to the role of amino acids for the formation of new structures [30].

TDZ has a better effect compared to BA. This is possible due to the ability of TDZ to counteract cytokinin oxidation [31], providing a appropriate internal balance between cytokinins and auxins [32] and increases the synthesis of adenine-type cytokinins[33]. Browning is a common problem of plant propagation using the TCL technique. TDZ showed a higher accumulation of serotonin and melatonin. These compounds play an important role in counteracting free radicals, therefor increasing the percentage of plantlet survival [34]. TDZ plays a role in stimulating the production of endogenous cytokinins and has a role as an inhibitor of cytokinin oxidase which is an enzyme that eliminates the activity of free adenine-type cytokinins. Therefore TDZ increase the function of other cytokinins, both exogenous and endogenous cytokinins [35]. Thidiazuron is needed by plants in relatively low concentrations, the high concentrations, of thidiazuroninhibit plant growth and tends to have no effect [36]

Morphologically the growth of developing globular EC of *Dendrobium aqueum* was better on medium supplemented BA than to other cytokinins (TDZ, ZEA, 2 iP, and KN). Green, pale green, and yellowish-green callus formed SEs whereas yellow and yellowish-green callus turned brown [14]. BAP inhibit chlorophyll degradation and regulate embryonic [37]. BAP is a hormone that can stimulate cell division and induced shoot multiplication [38]. BAP is effectively used in tissue culture because it has stable and does not easily decompose during the heating process [39]. BAP has a benzyl ring which makes it more stable and stronger than other cytokinins such as zeatin and kinetin [40].

Combination cytokinin and auxin increase the response of explant which propagated through TCL technique. The balance between auxin and cytokinin induced morfogenesis of plant.The high concentration of cytokinin induced shoots [41]. When the concentration of auxin is higher than that of cytokinins will induced callus formation. When cytokinin levels increase in plants, auxin levels will decrease. Addition of exogenous cytokinins will increased endogenous cytokinin levels thereby suppressing endogenous auxin levels [42]. Cells generally contain sufficient or almost sufficient auxin to elongate normally.Auxin plays a role in increasing the permeability of cell walls to increasing the diffusion of water into cells. NAA is an auxin which plays a role in increasing cell enlargement. Cell enlargement occurs due to increased sugar levels in the cell vacuoles so that osmotic pressure increases [43].

Recently the addition of microalgae in a culture medium is a new option as a source of PGRs[44]. Extracts of microalgae may have cytokinin and auxin actions [45]. That is essential for the growth and development of plants. Recently used *Messastrum gracile* and *Chlorella vulgarisssa* for propagation *Cattleya labiata* using thin cell layer technique. The type cytokinin compound biochemical composition in the extracts and biomass of *Chlorella* vulgaris and *Messastrum gracile* is zeatin. The highest level of zeatin was found in the *Messastrum gracile*. When *Cattleya labiata* culture in medium supplemented biomassa of *Messastrum gracilea* showed a high percentage of plant formation compared ZEA, which is avery expensive cytokinin [23].

III. CONCLUSION

TCL is a micropropagation method using thin-sized explants. Additions PGR in media culture is essential factor. The formation of complete plant depent on concentration and type of plant growth regulator. TCL explants require growth regulators to form an embryonic callus and zygotic embryos. Most research showed the explant TCL in medium culture without PGR failded to growth. The PGRs commonly used in plant propagation through TCL are cytokinin, auxin and combination cytokinin and auxin. Each orchid requires a different type and concentration of PGRs for its growth. Recently the addition of microalgae in a culture medium is a new option as a source PGRs.

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