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An Overview: Somatic Embryogenesis Through Thin Cell Layer (TCL) Technique

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Abstract – Somatic embryogenesis (SE) has been described as the most efficient pathway to obtaining plants. Somatic embryogenesis can be divided into direct and indirect embryogenesis. Direct SE occurs when embryos are started directly from explant tissues (preembryogenic cells) creating an identical clone, while indirect SE occurs from unorganized tissues (calli) which are further developed into embryos. While different explants have been tested, e.g., shoot tips, adventitious buds, leaf primordia, zygotic embryos, inflorescences, and transverse thin cell layers (TCLs) (consisting of a few cell layers, usually 0.5–2.0 mm thick). TCLs have been excised from different plant organs and successfully used as explants for SE. Thin cell layers (TCLs) can be prepared from almost any plant organ. These, when cut longitudinally, are referred to as lTCLs, and when cut transversally, are referred to as tTCLs. This article provides an overview of the concept of TCL as applied to induce SE in different plant species and remarks the factor that affecting this technique. This review will certainly revitalize this important technology so that it can be used effectively for successful mass propagation.

Keywords – embryo somatic; in vitro; micropopagation;TCL

I. INTRODUCTION

Tissue culture techniques are considered to be the most likely strategy for efficient clonal plantlet regeneration. Among the available techniques for in vitro plant generation, somatic embryogenesis (SE) offers advantages such as large scale automated production and genetic stability of the regenerated plantlets. Somatic embryogenesis has been considered the preferred in vitro regenerative pathway because of the larger number of regenerated plantlets that can be produced compared to organogenesis. [1]. Somatic embryogenesis permits creation of cycling cultures through the use of cell suspensions [2] or through secondary somatic embryogenesis [3]. The production of somatic embryos capitalizes upon the totipotency of plant cells and involves the development of bipolar structures resembling zygotic embryos [4]. This morphogenetic route is influenced by several factors imposed by in vitro conditions.

Somatic embryogenesis can be divided into direct and indirect embryogenesis. Direct somatic embryogenesis occurs when embryos are started directly from explant tissues (preembryogenic cells) creating an identical clone, while indirect somatic embryogenesis occurs from unorganized tissues (calli) which are further developed into embryos [5]. Formation of embryo through direct embryogenesis is preferred than the indirect one because it can avoid a problem in forming seed in the somatic germination stage [6] and limit the occurrence of somalonal variation. However, the number of somaclones produced through direct embryogenesis is usually limited and not uniform; therefore indirect somatic embryogenesis is used for improvement and propagation of crops.

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Successful induction of SE has already been reported from different tissues, such as leaf primordia from adult plants, shoots and leaf primordia from in vitro- grown plantlets, immature inflorescences, mature zygotic embryos, and immature zygotic embryos. The choice of the explant source depends also upon the aim pursued and explant availability. Somatic embryogenesis has been induced from different explants, SE has also been reported from bulb scales as explants e.g. L. Longiflorum [7], Fritillaria imperialis [8]. Among these, the explant source and the developmental stage are considered key elements that alter cellular competence. In addition, the size of the explants greatly influences their morphogenetic capacity, probably due to the establishment of a symplast domain that maintains the coordinated development of the cells and tissues. The symplast domain as a continuum between the cells of a specific tissue domain, allowing molecular movement through the plasmodesmata, and maintaining and coordinating morphogenetic activity in the tissue [9].

The thin cell layer (TCL) technique utilizes very small explants and was first described in Nicotiana tabacum. Regeneration and reprogramming of an organ or embryo under in vitro conditions is possible if some layers of differentiated cells were isolated from the initial organ or tissue. TCL technology is based on a multicellular system using very small explants from different plant parts or organs (stems, leaves, roots, floral organs, internodes, hypocotyls, apical zones, or embryos) [10]. This technique uses small explants about 0.5–2 mm in thickness. The TCL system allows the isolation of a precise cell or tissue layer that enables the in vitro induction of a specific morphogenetic program depending on factors such as genetic state, age, size and shape, pH of the medium, and synchrony with tightly controlled growth conditions such as light, media additives, temperature, plant growth regulator (PGR) concentration, etc. [11]. Through the use of TCLs, the regeneration of specific organs may be effectively manipulated and, together with specific controlled in vitro conditions and exogenously applied PGRs, many problems hindering the improvement in in vitro plant systems are potentially removed, as has been reviewed [10].

It has been successfully used in the large-scale propagation of vegetables, legumes, and plants.Thin explants have shown higher response rates to in vitro conditions as compared to more voluminous ones, probably due to a larger surface area in contact with the surrounding environment and, thus, better perceiving chemical and physical [12]. The TCL technique promotes culture proliferation with enhanced productivity and reduced time, which is a key factor in plant cell and tissue differentiation. Based on the pattern of explant excision, two types of TCLs are distinguished: transverse TCLs (tTCLs) and longitudinal TCLs (lTCLs) [13]. This technique has been used for the production of somatic embryos of several plant species [14]. This procedure gave enhanced results for numerous in vitro culture systems [15], including Pelargonium x Hortorum bailey [16], Elaeis guineensis [17], Gladiolus hort [18], Rosa hybrid [19], Digitaria sanguinalis [20], Cocos nucifera [21], Oryza sativa [22], L. Longiflorum [23], Bactris gasipaes [24], Elaeis guineensis [14], Agave fourcroydes [25], Ceropegia bulbosa [26], and Stevia rebaudina [27].

Explant size has an important role in plant in vitro response, as in our preliminary experiments low callus induction was observed from explants that were 1 cm thick [28]. Small explants (TCL) also presented higher morphogenetic capacity. Larger explants maintain normal tissue interactions, and such interactions may inhibit cell division through maintenance of symplast domains [29]. Additionally, explants with reduced size showed synthesis of new cell wall components, such as oligosaccharides, that can act as signals to the cell to re-enter the mitotic cycle [15]. Small explants also present higher surface contact with the culture medium, and can be considered to be more stressed, increasing the cell's metabolism [30]. Among the type of TCL, the tTCL explants gave promising response, which produced good quality callus and then highest number of shoots with better growth on full strength solid MS medium supplemented with PGRs. In the present investigations, it was noticed that tTCLs system produced good quality callus. TCL technology is also a solution to many of the issues currently hindering the efficient progress, since it resolves problems at the first stage, i.e., regeneration by using the most basic developmental building blocks, cells, and tissues [31].

II. Result and Discussion

Numerous studies about somatic embryogenesis through the thin cell layer (TCL) method in different plant cultures as follow:

2.1. Type of ES Induction

Several studies have been conducted regarding the response both of the ES type through TCL technique to the propagation of several plant species (Table 1).

TABLE I. THE RESPONSE OF TWO TYPE SOMATIC EMBRYOGENESIS OF SEVERAL PLANT SPECIES THROUGH THIN CELL LAYER (TCL) TECHNIQUE

Plants species	Type of SE	Media	PGRs	Type of explant	Responses	References
Cymbidium aloifolium	Direct	MS	Zeatin ribosid e (ZR)	Seedlings	Produced 28.2 PLBs per explant	[32]
Lilium ledebourii	Direct	MS	NAA	Young bulblet roots	Produced 65.55% of embryogenesis persentage	[33]
Agave fourcroydes	Direct	MS	Piclora m	Stems	The best embryogenic response	[25]
Dendrobium aqueum	Direct	MS	2iP	Stems	Produced 42.66 globular SE	[34]
Dendrobium nobile	Direct	MS	BA	PLB	was Produced 34.0 PLBs per explant	[35]
Dendrobium candidum	Indirect	½ MS	NAA and BA	Nodes	Optimal for shoot regeneration	[36]
Saccharum species hybrids	Indirect	MS	2.4-D	Young leafwhorls	Produced 100% callus	[31]
Bactris gasipaes	Indirect	MS	Piclora m	Shoot meristem	Produced 43 % embryogenic callus	[37]
Lilium longiflorum	Indirect	MS	NAA or TDZ	Pseudob ulblet	Induced yellow friable calluses	[23]
Dendrobium aqueum	Indirect	MS	Zeatin	Stems	Produced 41.42 %embryogenic callus	[34]

Two different types of somatic embryogenic routes are generally involved in plants: direct somatic embryogenesis and indirect somatic embryogenesis [38]. In direct SE, there is no dedifferentiation stage, and embryonic cell formation can be completed directly from the surface of explants, in which minimal genetic reprogramming is involved; in contrast, indirect SE is a multistep regeneration process including somatic embryo formation, maturation, and conversion that requires major reprogramming [39]. Compared to direct SE, indirect SE has a higher propagation efficiency and is applied for a longer period of frozen storage technology, which can lay a solid foundation for industrialized seedling culture by somatic embryo induction. In species such as *Dendrobium aqueum* [34], two types of somatic embryogenetic pathways have been successfully established. Also, on Table. I, the DSE pathway was established in *Cymbidium aloifolium* [35], *Lilium ledebourii* [33], *Agave fourcroydes* [25], *Dendrobium nobile* [35], and the ISE pathway was established in *Dendrobium candidum* [36], *Saccharum species hybrids* [31], *Bactris gasipaes* [37], and *Lilium longiflorum* [23].

Embryogenesis somatic affected by the addition of PGRs. Each species have specialized PGRs that suitable to their metabolism. Auxin is one of the suitable PGRs to induce SE in several plants. This auxin analogue has been successfully used for somatic embryogenesis induction in numerous plant species [40]. The auxin 2,4-D is an important hormone for callus induction when compared to other auxins. Efficient callogenesis on MS medium with 2,4-D and the highest tendency and ability for regeneration on MS with NAA have been reported [41]. Different concentration of auxins used in the culture media for callogenesis showed variable regeneration capacity and proliferation [42]. However, other reports covered that picloram is better than 2,4-D for callus initiation and proliferation in the sugarcane cultivars [43]. Combination of auxin with other PGRs was the best treatment to induce SE. Cytokinin in combination with auxin has been used to regenerate shoots in sugarcane [44]. One of cytokinin groups that combine with auxin was TDZ. Although TDZ has been effectively used to induce shoot regeneration in different explants of various species [45], its use has not been reported for *Lilium ledebourii*. TDZ is also a potent PGR in in vitro morphogenesis and is considerably more effective than other cytokinins for inducing shoot regeneration. It is a powerful inducer of adventitious shoots and somatic embryo formation in manyornamental plants [46].

2.2. Type of TCL

Several studies have been conducted regarding the response of ES through both of TCL type technique to the propagation of several plant species (Table II).

TABLE II. THE RESPONSE OF SOMATIC EMBRYOGENESIS OF SEVERAL PLANT SPECIES THROUGH TWO TYPE THIN CELL LAYER (TCL) TECHNIQUE

Plants species	Type of TCL	Media	PGRs	Type of explant	Responses	References
Citrus limon	tTCL	MS	Malt extract and BA	Stigma	Produced 42.4% embryo formation	[47]
Brasilidium forbesii	tTCL	MS	BA	Protocorms	Produced 9.3 PLBs per explant	[48]
Brasilidium forbesii	lTCL	MS	BA	Protocorms	Produced the highest percentage of new protocorms (77%) with a total of 22.7 PLBs per explant.	[48]
Actinidia chinensis	tTCL	MS	NAA and TDZ	Leaf main vein	Produced the highest SE (98.67%)	[49]
Actinidia chinensis	lTCL	MS	NAA and TDZ	Leaf main vein	Produced 32.00 embryos per explant	[49]
Phalaenopsis amabilis cv. *Jinan*	lTCL	½ MS	TDZ	leaf	Produced the highest number of somatic embryos (SEs) (21.37 embryos per explant)	[50]
Phalaenopsis amabilis cv. *Jinan*	tTCL	½ MS	TDZ	flower stalk nodes	Produced 3.84 embryos per explant	[50]
Pinus patula	tTCL	WPM	2.4-D and BAP	immature embryos	Produced 90.0 % embryogenic callus	[27]
Pinus patula	lTCL	WPM	2.4-D and BAP	immature embryos	Produced 88.7 % embryogenic callus	[27]
Panax vietnamensis var. *langbianensis*	lTCL	MS	2.4-D	leaf	Produced 100% SE and 51.80 embryos	[51]

The TCL system employs various small-sized explants from different plant organs excised either longitudinally [longitudinal thin cell layer (lTCL)] or transversally [transverse thin cell layer (tTCL)] [15]. TCL culture systems are promising and efficient with regard to the total output of orchid plantlets compared to other conventional in vitro methods for rapid regeneration of orchids. However, these culture systems have not yet been completely exploited for propagating commercially important plants [52]. In some species of orchid, tTCl has been successfully employed for protocorm-like bodies (PLBs) of *Cymbidium aloifolium* and *Dendrobium nobile* [35] and *Cymbidium Sleeping Nymph* [53], and lTCL has been used to produce PLBs of the hybrid *Cymbidium Twilight Moon* 'Day Light' [54].

The highest SE number was obtained through lTCL explants. It seems that fewer lignified cells, and the least latex and phenol components in the leaves assist them to be much more preferable explant for SE formation [55]. lTCL explants cultured on 1/2 MS medium supplemented with 3 mgL–1 TDZ, produced the highest number of SE, the lowest yellowish SE, the highest plantlet regeneration, and the highest survival rate [50]. lTCL segments were more efficient than tTCL in the generation of

somatic embryos. Orientation influences the morphogenetic response. The lTCL segments have a better morphogenetic response because they present several types of tissues (epidermis, endodermis, xylem, phloem) and tTCLs only one or two types [56]. However, there are few reports of the evaluation of the two types of TCL in the morpho- genesis of different species of economic interest [19].

III. Conclusion

Many studies have been performed on embryogenesis somatic through TCL technique, which proves that the combination of ES and TCL techniques is the most important tools that allow us to understand processes such as micropropagation, morphogenesis, and regeneration. Embryogenesis somatic was a high-efficiency inducted system for direct SE and indirect SE in some species. Both type of SE can induce embryo by lTCL or tTCL. In process of it, response of plants also affected by addition of PGRs, explant, and the in vitro media. Based on the results reviewed, we conclude that this technique can be a reference for the multiplication of various plants in the future. This technique can also be a solution to various plant propagation problems of production capability.

References

[1] D. A. Steinmacher, A. S. Heringer, V. M. Jiménez, M. G. G. Quoirin, and M. P. Guerra, "Somatic embryogenesis in peach-palm (Bactris gasipaes) using different explant sources," *Methods Mol. Biol.*, vol. 1359, no. January, pp. 279–288, 2016, doi: 10.1007/978-1-4939-3061-6_12.

[2] D. Sané *et al.*, "Histocytological analysis of callogenesis and somatic embryogenesis from cell suspensions of date palm (Phoenix dactylifera)," *Ann. Bot.*, vol. 98, no. 2, 2006, doi: 10.1093/aob/mcl104.

[3] M. T. Pérez-Núñez, J. L. Chan, L. Sáenz, T. González, J. L. Verdeil, and C. Oropeza, "Improved somatic embryogenesis from Cocos nucifera (L.) plumule explants," *Vitr. Cell. Dev. Biol. - Plant*, vol. 42, no. 1, 2006, doi: 10.1079/IVP2005722.

[4] V. L. Dodeman, G. Ducreux, and M. Kreis, "Zygotic embryogenesis versus somatic embryogenesis," *Journal of Experimental Botany*, vol. 48, no. 313. 1997. doi: 10.1093/jxb/48.8.1493.

[5] T. Taniguchi, E. Maeda, and T. Nakamura, "Studies on Somatic Embryogenesis of Coffee by Scanning Electron Microscope," *Japanese J. Crop Sci.*, vol. 61, no. 3, 1992, doi: 10.1626/jcs.61.476.

[6] V. Ravishankar Rai and J. McComb, "Direct somatic embryogenesis from mature embryos of sandalwood," *Plant Cell. Tissue Organ Cult.*, vol. 69, no. 1, 2002, doi: 10.1023/A:1015037920529.

[7] A. M. Arzate-Fernández, T. Nakazaki, Y. Okumoto, and T. Tanisaka, "Efficient callus induction and plant regeneration from filaments with anther in lily (Lilium longiflorum Thunb.)," *Plant Cell Rep.*, vol. 16, no. 12, 1997, doi: 10.1007/s002990050330.

[8] M. Mohammadi-Dehcheshmeh, A. Khalighi, R. Naderi, M. Sardari, and E. Ebrahimie, "Petal: A reliable explant for direct bulblet regeneration of endangered wild populations of Fritillaria imperialis L.," *Acta Physiol. Plant.*, vol. 30, no. 3, 2008, doi: 10.1007/s11738-007-0126-2.

[9] A. G. Roberts and K. J. Oparka, "Plasmodesmata and the control of symplastic transport," *Plant, Cell and Environment*, vol. 26, no. 1. 2003. doi: 10.1046/j.1365-3040.2003.00950.x.

[10] J. A. Teixeira da Silva and J. Dobránszki, "Plant thin cell layers: update and perspectives," *Folia Hortic.*, vol. 27, no. 2, 2015, doi: 10.1515/fhort-2015-0029.

[11] D. T. Nhut, B. V. Le, J. A. T. Da Silva, and C. R. Aswath, "Thin cell layer culture system in Lilium: Regeneration and transformation perspectives," *In Vitro Cellular and Developmental Biology - Plant*, vol. 37, no. 5. 2001. doi: 10.1007/s11627-001-0090-2.

[12] J. A. Teixeira Da Silva and J. Dobránszki, "Recent advances and novelties in the thin cell layer-based plant biotechnology - a mini-review," *Biotechnologia*, vol. 100, no. 1, 2019, doi: 10.5114/bta.2019.83215.

[13] J. A. Teixeira da Silva and J. Dobránszki, "Plant Thin Cell Layers: A 40-Year Celebration," *Journal of Plant Growth Regulation*, vol. 32, no. 4. 2013. doi: 10.1007/s00344-013-9336-6.

[14] J. E. Scherwinski-Pereira, R. S. da Guedes, P. C. P. Fermino, T. L. Silva, and F. H. S. Costa, "Somatic embryogenesis and plant regeneration in oil palm using the thin cell layer technique," *Vitr. Cell. Dev. Biol. - Plant*, vol. 46, no. 4, 2010, doi: 10.1007/s11627-010-9279-6.

[15] K. Trân Thanh Vân and B. Van Lê, "Current status of Thin Cell Layer method for the induction of organogenesis or somatic embryogenesis," 2000. doi: 10.1007/978-94-017-3030-3_3.

[16] R. Gill, J. M. Gerrath, and P. K. Saxena, " High-frequency direct somatic embryogenesis in thin layer cultures of hybrid seed geranium (Pelargonium × hortorum) ," *Can. J. Bot.*, vol. 71, no. 3, 1993, doi: 10.1139/b93-045.

[17] J. B. Teixeira, M. R. Söndahl, and E. G. Kirby, "Somatic embryogenesis from immature inflorescences of oil palm," *Plant Cell Rep.*, vol. 13, no. 5, 1994, doi: 10.1007/BF00233313.

[18] B. Stefaniak, "Somatic embryogenesis and plant regeneration of Gladiolus (Gladiolus hort.)," *Plant Cell Rep.*, vol. 13, no. 7, 1994, doi: 10.1007/BF00234143.

[19] C. N. Hsia and S. S. Korban, "Factors affecting in vitro establishment and shoot proliferation of Rosa hybrida L. and Rosa chinensis minima," *Vitr. Cell. Dev. Biol. - Plant*, vol. 32, no. 4, 1996, doi: 10.1007/BF02822690.

[20] B. Van Le, D. M. N. Thao, C. Gendy, J. Vidal, and K. Tran Thanh Van, "Somatic embryogenesis on thin cell layers of a C4 species, Digitaria sanguinalis (L.) Scop," *Plant Cell. Tissue Organ Cult.*, vol. 49, no. 3, 1997, doi: 10.1023/A:1005816126824.

[21] Y. M. S. Samosir, I. D. Godwin, and S. W. Adkins, "An improved protocol for somatic embryogenesis in coconut (Cocos nucifera L.)," in *Acta Horticulturae*, 1998, vol. 461. doi: 10.17660/actahortic.1998.461.55.

[22] D. T. Nhut, B. Van Le, and K. T. Thanh, "Somatic embryogenesis and direct shoot regeneration of rice (Oryza sativa L.) using thin cell layer culture of apical meristematic tissue," *J. Plant Physiol.*, vol. 157, no. 5, 2000, doi: 10.1016/S0176-1617(00)80112-1.

[23] D. T. Nhut *et al.*, "Somatic embryogenesis through pseudo-bulblet transverse thin cell layer of Lilium longiflorum," *Plant Growth Regul.*, vol. 37, no. 2, 2002, doi: 10.1023/A:1020532511081.

[24] D. A. Steinmacher, N. G. Krohn, A. C. M. Dantas, V. M. Stefenon, C. R. Clement, and M. P. Guerra, "Somatic embryogenesis in peach palm using the thin cell layer technique: Induction, morpho-histological aspects and AFLP analysis of somaclonal variation," *Ann. Bot.*, vol. 100, no. 4, pp. 699–709, 2007, doi: 10.1093/aob/mcm153.

[25] K. M. Monja-Mio and M. L. Robert, "Direct somatic embryogenesis of Agave fourcroydes Lem. through thin cell layer culture," *Vitr. Cell. Dev. Biol. - Plant*, vol. 49, no. 5, 2013, doi: 10.1007/s11627-013-9535-7.

[26] R. Dhir and G. S. Shekhawat, "In Vitro Propagation Using Transverse Thin Cell Layer Culture and Homogeneity Assessment in Ceropegia bulbosa Roxb.," *J. Plant Growth Regul.*, vol. 33, no. 4, 2014, doi: 10.1007/s00344-014-9432-2.

[27] M. A. Ramírez-Mosqueda *et al.*, "Effect of the thin cell layer technique in the induction of somatic embryos in Pinus patula Schl. et Cham," *J. For. Res.*, vol. 30, no. 4, pp. 1535–1539, 2019, doi: 10.1007/s11676-018-0663-0.

[28] D. A. Steinmacher, G. C. Cangahuala-Inocente, C. R. Clement, and M. P. Guerra, "Somatic embryogenesis from peach palm zygotic embryos," *Vitr. Cell. Dev. Biol. - Plant*, vol. 43, no. 2, 2007, doi: 10.1007/s11627-007-9032-y.

[29] H. Benkirane, K. Sabounji, A. Chlyah, and H. Chlyah, "Somatic embryogenesis and plant regeneration from fragments of immature inflorescences and coleoptiles of durum wheat," *Plant Cell. Tissue Organ Cult.*, vol. 61, no. 2, 2000, doi: 10.1023/A:1006464208686.

[30] A. Fehér, T. P. Pasternak, and D. Dudits, "Transition of somatic plant cells to an embryogenic state," *Plant Cell, Tissue and Organ Culture*, vol. 74, no. 3. 2003. doi: 10.1023/A:1024033216561.

[31] A. Iqbal *et al.*, "Efficient Regeneration in Sugarcane Using Thin Cell Layer (TCL) Culture System," *Sugar Tech*, no. May, 2022, doi: 10.1007/s12355-022-01162-y.

[32] L. Pipino, L. Braglia, A. Giovannini, G. Fascella, and A. Mercuri, *Protocols for In Vitro Propagation of Ornamental Plants*,

vol. 589, no. 1. 2010. doi: 10.1007/978-1-60327-114-1.

[33] M. Bakhshaie, M. Babalar, M. Mirmasoumi, and A. Khalighi, "Somatic embryogenesis and plant regeneration of Lilium ledebourii (Baker) Boiss., an endangered species," *Plant Cell. Tissue Organ Cult.*, vol. 102, no. 2, pp. 229–235, Aug. 2010, doi: 10.1007/s11240-010-9726-4.

[34] S. Parthibhan, M. V. Rao, J. A. Teixeira da Silva, and T. Senthil Kumar, "Somatic embryogenesis from stem thin cell layers of Dendrobium aqueum," *Biol. Plant.*, vol. 62, no. 3, 2018, doi: 10.1007/s10535-018-0769-4.

[35] N. R. Nayak, S. Sahoo, and S. Patnaik, "Establishment of thin cross section (TCS) culture method for rapid micropropagation of Cymbidium aloifolium (L.) Sw. and Dendrobium nobile Lindl. (Orchidaceae)," *Sci. Hortic. (Amsterdam).*, vol. 94, no. 1–2, 2002, doi: 10.1016/S0304-4238(01)00372-7.

[36] P. Zhao, W. Wang, F. S. Feng, F. Wu, Z. Q. Yang, and W. J. Wang, "High-frequency shoot regeneration through transverse thin cell layer culture in Dendrobium Candidum Wall Ex Lindl.," *Plant Cell. Tissue Organ Cult.*, vol. 90, no. 2, pp. 131–139, 2007, doi: 10.1007/s11240-006-9181-4.

[37] S. Campos-Boza *et al.*, "Somatic embryogenesis and plant regeneration from transverse thin cell layers of adult peach palm (Bactris gasipaes) lateral offshoots," *Front. Plant Sci.*, vol. 13, no. September, pp. 1–14, 2022, doi: 10.3389/fpls.2022.995307.

[38] W. R. Sharp, M. R. Sondahl, L. S. Caldas, and S. B. Maraffa, "The Physiology of in Vitro Asexual Embryogenesis," in *Horticultural Reviews*, 2011. doi: 10.1002/9781118060759.ch6.

[39] S. Von Arnold, I. Sabala, P. Bozhkov, J. Dyachok, and L. Filonova, "Developmental pathways of somatic embryogenesis," *Plant Cell, Tissue and Organ Culture*, vol. 69, no. 3. 2002. doi: 10.1023/A:1015673200621.

[40] P. Kaur and S. L. Kothari, "In vitro culture of kodo millet: Influence of 2,4-D and picloram in combination with kinetin on callus initiation and regeneration," *Plant Cell. Tissue Organ Cult.*, vol. 77, no. 1, 2004, doi: 10.1023/B:TICU.0000016505.20448.44.

[41] . R. B., . R. A., . M. Z. K., . S. A. M., . B. P. P., and . A. B. M. M. R., "Effect of Auxin, Sucrose and pH Level on in vitro Rooting of Callus Induced Micro Shoots of Sugarcane (Saccharum officinarum)," *J. Biol. Sci.*, vol. 3, no. 10, 2003, doi: 10.3923/jbs.2003.915.920.

[42] S. Geetha and D. Padmanabhan, "Effect of hormones on direct somatic embryogenesis in sugarcane," *Sugar Tech*, vol. 3, no. 3, 2001, doi: 10.1007/bf03014576.

[43] I. A. Khan, M. U. Dahot, N. Seema, S. Bibi, and A. Khatri, "Genetic variability in plantlets derived from callus culture in sugarcane," *Pakistan J. Bot.*, vol. 40, no. 2, 2008.

[44] Arjun and R. Srinath, "Callus Induction and Organogenesis in Sugarcane (*Saccharum officinarum* L.) var 93v297," *Int. Lett. Nat. Sci.*, vol. 48, 2015, doi: 10.18052/www.scipress.com/ilns.48.14.

[45] X. LingFei, M. FengWang, and L. Dong, "Plant regeneration from in vitro cultured leaves of Lanzhou lily (Lilium davidii var. unicolor)," *Sci. Hortic. (Amsterdam).*, vol. 119, no. 4, 2009, doi: 10.1016/j.scienta.2008.08.026.

[46] M. Sanikhani, S. Frello, and M. Serek, "TDZ induces shoot regeneration in various Kalanchoë blossfeldiana Poelln. cultivars in the absence of auxin," *Plant Cell. Tissue Organ Cult.*, vol. 85, no. 1, 2006, doi: 10.1007/s11240-005-9050-6.

[47] F. Carimi, F. De Pasquale, and F. G. Crescimanno, "Somatic embryogenesis and plant regeneration from pistil thin cell layers of citrus," *Plant Cell Rep.*, vol. 18, no. 11, pp. 935–940, 1999, doi: 10.1007/s002990050687.

[48] L. R. P. Gomes, C. do R. B. Franceschi, and L. L. F. Ribas, "Micropropagation of Brasilidium forbesii (Orchidaceae) through transverse and longitudinal thin cell layer culture," *Acta Sci. - Biol. Sci.*, vol. 37, no. 2, pp. 143–149, 2015, doi: 10.4025/actascibiolsci.v37i2.27276.

[49] N. T. M. Hanh *et al.*, "Efficient somatic embryogenesis and regeneration from leaf main vein and petiole of Actinidia chinensis planch. via thin cell layer culture technology," *Sci. Hortic. (Amsterdam).*, vol. 298, May 2022, doi:

10.1016/j.scienta.2022.110986.

[50] R. Ghahremani, S. D. Daylami, M. Mirmasoumi, N. Askari, and K. Vahdati, "Refining a protocol for somatic embryogenesis and plant regeneration of phalaenopsis amabilis cv. Jinan from mature tissues," *Turkish J. Agric. For.*, vol. 45, no. 3, 2021, doi: 10.3906/tar-2004-107.

[51] T. T. L. Anh *et al.*, "Micropropagation of Lang Bian ginseng: an endemic medicinal plant," *Plant Cell. Tissue Organ Cult.*, vol. 151, no. 3, 2022, doi: 10.1007/s11240-022-02372-8.

[52] M. M. Hossain, R. Kant, P. T. Van, B. Winarto, S. Zeng, and J. A. Teixeira da Silva, "The Application of Biotechnology to Orchids," *Critical Reviews in Plant Sciences*, vol. 32, no. 2. 2013. doi: 10.1080/07352689.2012.715984.

[53] S. Vyas, S. Guha, P. Kapoor, and I. U. Rao, "Micropropagation of Cymbidium Sleeping Nymph through protocorm-like bodies production by thin cell layer culture," *Sci. Hortic. (Amsterdam).*, vol. 123, no. 4, pp. 551–557, 2010, doi: 10.1016/j.scienta.2009.11.020.

[54] J. A. Teixeira Da Silva and M. Tanaka, "Multiple regeneration pathways via thin cell layers in hybrid Cymbidium (Orchidaceae)," *J. Plant Growth Regul.*, vol. 25, no. 3, 2006, doi: 10.1007/s00344-005-0104-0.

[55] M. O. Adero, E. D. Syombua, L. Kwamboka Asande, N. O. Amugune, E. S. Mulanda, and G. Macharia, "Somatic embryogenesis and regeneration of Kenyan wheat (Triticum aestivum L.) genotypes from mature embryo explants," *African J. Biotechnol.*, vol. 18, no. 27, 2019.

[56] J. A. Teixeira da Silva and J. Dobránszki, "Dissecting the Concept of the Thin Cell Layer: Theoretical Basis and Practical Application of the Plant Growth Correction Factor to Apple, Cymbidium and Chrysanthemum," *Journal of Plant Growth Regulation*, vol. 33, no. 4. 2014. doi: 10.1007/s00344-014-9437-x.