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The Application of Plant Growth Regulators in Propagation of Dendrobium Orchid with Thin Cell Layer (TCL) Technique: A Review

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Abstract—Plant growth regulators is one of the success factors for in vitro propagation. Plant 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. The most used plant growth regulators in tissue culture are auxins, cytokinins and gibberellins. One of technique for tissue culture is the thin cell layer technique. 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 plant growth regulators in propagation of Dendrobium orchid with thin cell layer (tcl) technique. A systematic Literature Review (SLR) uses in this paper. The growth regulators commonly used in Dendrobium orchid propagation using the TCL technique are auxins and cytokinins and the type of media that is often used in Dendrobium orchid propagation is Murashige and Skoog (MS). Protocorm Like Bodies (PLB) is the most potential explant in Dendrobium orchid propagation. The TCL technique both transverse and longitudinal has its own specificity with each type of explant used. TCL explants proved to be more effective than large conventional explants.

Keywords— Dendrobium; plant growth regulators; propagation; thin cell layer.

I. INTRODUCTION

Orchids 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], one of them is *Dendrobium. Dendrobium* is one of the largest orchid genera in the Orchidaceae family, and includes more than 2,000 species. *Dendrobium* has high potential as a medicinal plant, cut flower and potted plant so that it is widely developed and propagated in Indonesia because the climate in Indonesia is suitable for orchid propagation [2]. Most types of *Dendrobium* orchids are increasingly difficult to find in their natural habitat due to land conversion and illegal hunting [3]. 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 [4]. One of the efforts to propagate orchids is through tissue culture techniques or *in vitro* propagation.

One of the success factors for *in vitro* propagation is plant growth regulators. Plant 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. The most used plant growth regulators in tissue culture are auxins, cytokinins and gibberellins [5]. One of technique for 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, 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 [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]. 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]. Several studies using plant growth regulators in propagation of *Dendrobium* orchid with thin cell layer technique are *Dendrobium aduncum [8], Dendrobium aqueum* [9], *Dendrobium aphyllum* [10], *Dendrobium* Sabin Blue [11], *Dendorbium gratiosissimum* [12], *Dendrobium pulchellum* [13], *Dendrobium draconis* [14], *Dendrobium candidum* [15], *Dendrobium* species, D16 and D36 [16], and *Dendrobium nobile* [17]. This literature review aims to gather information related to the application of plant growth regulators in propagation of *Dendrobium* orchid with thin cell layer (tcl) technique.

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 plant growth regulators in propagation of *Dendrobium* orchid with thin cell layer (tcl) technique.

III. Result and Discussion

Several studies have been conducted to see the application of plant growth regulators in propagation of *Dendrobium* orchid with thin cell layer (tcl) technique. The success of orchid propagation with the addition of growth regulators using the thin cell layer technique is influenced by several factors:

1. Plant Growth Regulators

Several studies have been conducted to determine the type of plant growth regulators used in the propagation of several types of *Dendrobium* orchids using the TCL technique (Table 1).

TABLE I. PLANT GROWTH REGULATORS USED IN THE PROPAGATION OF SEVERAL TYPES OF *DENDROBIUM* ORCHIDS USING THE TCL TECHNIQUE

Type of Orchids	Plant Growth Regulators	References
Dendrobium aduncum	BAP, NAA, Kinetin	[8]
Dendrobium aqueum	IBA	[9]
Dendrobium aphyllum	TDZ	[10]
Dendrobium Sabin Blue	NAA, BAP	[11]
Dendorbium gratiosissimum	Kinetin	[12]
Dendrobium pulchellum	BA	[13]
Dendrobium draconis	BA, Kinetin, NAA	[14]
Dendrobium candidum	NAA, BA, Kinetin	[15]
Dendrobium species, D16 and D36	BA, NAA	[16]
Dendrobium nobile	BA, Kinetin, NAA	[17]

Combination of auxin and cytokinin was used on 6 species of *Dendrobium* orchids namely Dendrobium aduncum, *Dendrobium* Sabin Blue, *Dendorbium draconis*, *Dendrobium candidum*, *Dendrobium* species, D16 and D36, and *Dendrobium nobile*. While auxin and single cytokinin PGRs were used on 4 species of *Dendrobium* orchids namely *Dendrobium aqueum*, *Dendrobium aphyllum*, *Dendorbium gratiosissimum*, and *Dendrobium pulchellum*. It can be seen that the growth regulators that are often used in orchid propagation are auxins and cytokinins (Table 1). 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. The most used plant growth regulators in tissue culture are auxins, cytokinins and gibberellins [5].

Cytokinins are adenine derivative 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 BA, BAP, kinetin, and thidiazuron [19]. The function of auxin are 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 [20].

2. Media

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

TABLE II. THE TYPE OF MEDIA IN THE PROPAGATION OF SEVERAL TYPE OF *DENDROBIUM* ORCHIDS USING THE TCL TECHNIQUE

Type of Orchids	**Media**	**References**
Dendrobium aduncum	½ MS	[8]
Dendrobium aqueum	½ MS	[9]
Dendrobium aphyllum	MS	[10]
Dendrobium Sabin Blue	MS	[11]
Dendorbium gratiosissimum	MS	[12]
Dendrobium pulchellum	MS	[13]
Dendrobium draconis	MS	[14]
Dendrobium candidum	½ MS	[15]
Dendrobium species, D16 and D36	MS	[16]
Dendrobium nobile	MS	[17]

MS media was used on 10 species of *Dendrobium* orchids: *Dendrobium aduncum, Dendrobium aqueum*, *Dendrobium aphyllum*, *Dendrobium* Sabin Blue, *Dendorbium gratiosissimum, Dendrobium pulchellum, Dendrobium draconis*, *Dendrobium candidum*, *Dendrobium* species, D16 and D36, and *Dendrobium nobile.* It can be seen that the media that is often used in *Dendrobium* orchid propagation is Murashige and Skoog (MS) media (Table 2). 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 [21].

MS media has the most complete nutritional composition when compared to growth media for most other orchids such as VW and Knudson C, because of that MS media is the best medium for in vitro propagation of epiphytic and terrestrial orchid species [22]. 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. 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 [23].

The use of MS media with different concentrations can have different effects on the growth of several types of *Dendrobium* orchids. Research conducted by [8], ½ MS gave the highest number of PLB, shoot regeneration, and root growth for *Dendrobium aduncum*. ½ MS effective for shoot regeneration in propagation of *Dendrobium candidum* [15]. 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 [24].

3. Explants

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

TABLE III. THE TYPE OF EXPLANTS IN THE PROPAGATION OF SEVERAL TYPE OF *DENDROBIUM* ORCHIDS USING THE TCL TECHNIQUE

Type of Orchids	**Explants**	**References**
Dendrobium aduncum	Protocorm Like Bodies (PLB)	[8]
Dendrobium aqueum	Shoots	[9]
Dendrobium aphyllum	Protocorm Like Bodies (PLB)	[10]
Dendrobium Sabin Blue	Protocorm Like Bodies (PLB)	[11]
Dendorbium gratiosissimum	Protocorm Like Bodies (PLB)	[12]
Dendrobium pulchellum	Protocorm Like Bodies (PLB)	[13]
Dendrobium draconis	Protocorm Like Bodies (PLB)	[14]
Dendrobium candidum	Nodes	[15]
Dendrobium species, D16 and D36	Protocorm Like Bodies (PLB)	[16]
Dendrobium nobile	Shoots	[17]

The type of explants used in *Dendrobium aduncum, Dendrobium aphyllum, Dendrobium* Sabin Blue, *Dendorbium gratiosissimum, Dendrobium pulchellum, Dendrobium draconis,* and *Dendrobium* species, D16 and D36 are Protocorm Like Bodies (PLB). While *Dendrobium aqueum and Dendrobium nobile,* and *Dendrobium candidum* orchids, explant types used shoots and nodes. It can be seen that the types of explants that are often used in *Dendrobium* orchid propagation are Protocorm Like Bodies (PLB) (Table 3). 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 [25].

According to [8], protocorm like bodies were able to produce the highest number of PLB, shoot regeneration, and root growth in *Dendrobium aduncum*. Research conducted by [9], effective shoot explants for the induction of somatic embryogenesis in *Dendrobium aqueum* orchids. Protocorm-like bodies (PLBs) of *Dendorbium gratiosissimum* were successfully induced percentage of PLBs and number of PLBs [12].

4. Incision Type

Several studies have been conducted to determine the types of incisions and the response of the TCL technique to the propagation of several types of *Dendrobium* orchids (Table 4).

TABLE IV. THE TYPES OF INCISIONS AND THE RESPONSE OF TCL TECHNIQUE TO THE PROPAGATION OF SEVERAL TYPE OF *DENDROBIUM* ORCHIDS

Type of Orchids	**Incision Type (tTCL/lTCL)**	**Response**	**References**
Dendrobium aduncum	tTCL	The highest number of PLB, shoot regeneration, and root growth	[8]
Dendrobium aqueum	tTCL	Effective for induction of somatic embryogenesis	[9]
Dendrobium aphyllum	tTCL	The highest rooting frequency and shooting frequency	[10]
Dendrobium Sabin Blue	lTCL and tTCL	The highest number of PLB and number of shoot	[11]
Dendorbium gratiosissimum	lTCL and tTCL	The highest percentage of PLBs and number of PLBs	[12]
Dendrobium pulchellum	lTCL and tTCL	Effective for regeneration plant	[13]
Dendrobium draconis	lTCL and tTCL	Effective for induce PLBs	[14]
Dendrobium candidum	tTCL	Effective for shoot regeneration	[15]
Dendrobium species, D16 and D36	lTCL and tTCL	The highest response of shoots	[16]
Dendrobium nobile	lTCL and tTCL	The highest number of PLBs and shoots	[17]

The tTCL incision type was used on 10 species of Dendrobium orchids: *Dendrobium aduncum,* Dendrobium aqueum, Dendrobium aphyllum, Dendrobium Sabin Blue, Dendorbium gratiosissimum, Dendrobium pulchellum, Dendrobium draconis, Dendrobium candidum, Dendrobium species, D16 and D36, and Dendrobium nobile. While both lTCL and tTCL incision type was used on 6 species of Dendrobium orchids: Dendrobium Sabin Blue, Dendorbium gratiosissimum, Dendrobium pulchellum, Dendrobium draconis, Dendrobium species, D16 and D36, and Dendrobium nobile. The type of incision that is often used in orchid propagation is the transverse thin cell layer (TCL) (Table 4). 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). 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 [6].

According to [17], both TCL techniques in *Dendrobium nobile* were effective for PLB and shoots and effective for regeneration plant in *Dendrobium pulchellum* [13]. tTCL more effective for PLB, shoot regeneration, and root growth than lTCL in *Dendrobium aduncum* [8].

IV. CONCLUSIONS

Within these reviews, we show the application of plant growth regulators in propagation of *Dendrobium* orchid with thin cell layer (tcl) technique. The plant growth regulators with TCL technique has been used in the propagation of several species of *Dendrobium* orchid including *Dendrobium aduncum, Dendrobium aqueum*, *Dendrobium aphyllum*, *Dendrobium* Sabin Blue, *Dendorbium gratiosissimum, Dendrobium pulchellum, Dendrobium draconis*, *Dendrobium candidum*, *Dendrobium* species, D16 and D36, and *Dendrobium nobile.* The growth regulators commonly used in *Dendrobium* orchid propagation using the TCL technique are auxins and cytokinins and the type of media that is often used in *Dendrobium* orchid propagation is Murashige and Skoog (MS). Protocorm Like Bodies (PLB) is the most potential explant in *Dendrobium* orchid propagation. The TCL technique both transverse and longitudinal has its own specificity with each type of explant used. TCL explants proved to be more effective than large conventional explants.

REFERENCES

[1] Nurcahyani, E., M.L. Lande., & R.A. Noviantia. "Induced Resistance of Moon Orchid Planlet (*Phalaenopsis amabilis* (L,) as Result of The In Vitro Salicylic Acid Selection Toward to *Fusarium oxysporum". Jurnal Penelitian Pertanian Terapan,* vol. 17(2), pp. 132-137, 2017.

[2] Agustini, V., I. Rahayu, L.A. Numberi, dan Z. Ni'mah. "Peran Chitosan Sebagai Pemacu Pertumbuhan Kultur Anggrek *Dendrobium lasianthera* J.J.Sm. secara *In Vitro". Jurnal Biologi Papua*, vol. 12(1), pp. 43-49, 2020.

[3] Isda, M. N & S. Fatonah. "Induksi Akar pada Eksplan Tunas Anggrek *Grammatophylum scriptum var. citrinum* secara *In Vitro* pada Media MS dengan Penambahan NAA Dan BAP". *Al-Kauniyah Jurnal Biologi*, vol. 7(2), pp. 53-57, 2014.

[4] Rahayu, T. "*Modul Praktek Kultur Jaringan Tanaman".* Universitas Muhammadiyah Surakarta. Surakarta, 2016.

[5] Deli, R.N., Z.A. Noli., & Suwirmen. "Respon Pertumbuhan Nodus Artemesia vulgaris L. Pada Medium Murashige-Skoog dengan Penambahan Beberapa Zat Pengatur Tumbuh Secara *In Vitro". Jurnal Biologi Universitas Andalas (J. Bio. UA),* vol. 4(3), pp. 162-168, 2015.

[6] Teixeira-da-Silva, J.A & Dobranszki, J. "Plant thin cell layers: A 40-year celebration". *Journal Plant Growth Regulator*, vol. 43(32), pp. 922, 2013.

[7] Agisimanto, D. "Thin Cell Layer mempercepat pembuatan populasi genotip unggul hortikultura". *Iptek Hortikultura*, vol. 11, pp. 67-72, 2015.

[8] Thanh, T.N., L.V. Diep., N.M. Trung., & T.T.B. Phoung. "Application of Thin Cell Layer Culture Method in Micropropagation on *Dendrobium aduncum". Journal of Biotechnology,* vol. 8, pp. 361-367, 2010.

[9] Parthibhan, S., M.V. Rao., J.A. Teixeira Da Silva, & T.S. Kumar. "Somatic Embryogenesis from Stem Thin Cell Layers of *Dendrobium aqueum". Biologia Plantarum,* vol. 62(3), pp. 439-450, 2018.

[10] Bhattacharyya, P., P. Paul., S. Kumaria., & P. Tandon. "Transverse thin cell layer (tTCL) mediated improvised micropropagation protocol for endangered medicinal orchid *Dendrobium aphyllum* Roxb: an integrated phytomoleculer approach". *Acta Physiologiae Plantarum*, vol. 40(137), 2018.

[11] Malik, A.N.B.A. "Proliferation and Elicitation of Protocorm Like Bodies od *Dendrobium* Sabin Blue for *In Vitro* Production of Anthocyanin". *Thesis.* USM Malaysia, 2019. Serliana, Mukarlina, & R. Linda. "Pertumbuhan Anggrek Hitam (*Coelogyne* pandurata Lindl.) secara *in vitro* dengan Penambahan Ekstrak Tomat dan *benzylaminopurine* (BAP)". *Probiont,* vol. 6(3), pp. 310-315, 2017.

[12] Jaiphet, C & N. Rangsayatorn. "Micropropagation of a Rare Orchid *Dendrobium gratiosissimum* Using Thin Cell Layers". *ISHS Acta Horticulture,* vol. 878(21), pp. 185-189, 2010.

[13] Jaiphet, C. "*In Vitro* Conservation and Micropropagation of *Dendrobium* spp. and *Eulophia spectabilis*". *Thesis*. University of Phayao Thailand, 2012.

[14] Rangsayatorn, N. "Micropropagation of *Dendrobium draconis* Rchb. f. from Thin Cross-Section Culture". *Sci Hortic*, vol. 122, pp. 662-665, 2009.

[15] Zhao, P., W. Wang., F.S. Feng., F. Wu., Z.Q. Yang., & 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, pp. 131-139, 2007.

[16] Nhat, N.T., & T.T. Dung. "*In Vitro* Propagation of *Dendrobium* orchid through Thin Stem Section Culture". *In: Proceedings of International Workshop on Biotechnology in Agriculture,* Nong Lam University, Ho Chi Minh City, October 20-21, 2006.

[17] Malabadi, R.B., G.S. Mulgund., & N. Kallappa. "Micropropagation of *Dendrobium nobile* from Shoot Tip Sections". *J. Plant Physiology,* vol. 162, pp. 473-478, 2005.

[18] Triandini, E., S. Jayanatha., A. Indrawan., G.W. Putra., & B. Iswara. "Metode Systematic Literature Review untuk Identifikasi Platform dan Metode Pengembangan Sistem Informasi di Indonesia". *Indonesian Journal of Information Systems,* vol. 1(2), pp. 63-77, 2019.

[19] Serliana, Mukarlina, & R. Linda. "Pertumbuhan Anggrek Hitam (*Coelogyne* pandurata Lindl.) secara *in vitro* dengan Penambahan Ekstrak Tomat dan *benzylaminopurine* (BAP)". *Probiont,* vol. 6(3), pp. 310-315, 2017.

[20] Nikmah, Z.C., W. Slamet, & B.A. Kristanto. "Aplikasi Silika dan NAA terhadap Pertumbuhan Anggrek Bulan (*Phalaenopsis amabilis* L.) pada Tahap Aklimatisasi". *J. Agro Complex,* vol. 1(3), pp. 101-110, 2017.

[21] Tuhuteru, S., M.L. Hehanussa, & S.H.T. Raharjo. "Pertumbuhan dan Perkembangan Anggrek *Dendrobium anosmum* pada Media Kultur *In Vitro* dengan Beberapa Konsentrasi Air Kelapa". *Agrologia,* vol. 1(1), pp. 1-12, 2012.

[22] Jualang, A.G., D. Devina, M. Hartinie, J.S. Sharon & J. Roslina. "Asymbiotic seeds germination and seedlings development of *Vanda dearei"*. *Malaysian Applied Biology,* vol. 43(2), pp. 25-33, 2014.

[23] Kartikaningrum, S., P. Dewi, D. Minangsari, S. Rudy, & M.P. Yufdy. "Konservasi Anggrek Spesies Alam Menggunakan Eksplan Biji pada Media *Vacin & Went"*. *Plasma Nutfah,* vol. 23(2), pp. 109-118, 2017.

[24] Fithriyandini, A., M.D. Maghfoer, & T. Wardiyati. "Pengaruh 6-Benzylaminopurine (BAP) terhadap Pertumbuhan dan Perkembangan Nodus Tangkai Bunga Anggrek Bulan (Phalaenopsis amabilis) dalam Perbanyakan secara *In Vitro*". *Produksi Tanaman*, vol. 3, pp. 39-43, 2015.

[25] Steinmacher, D.A., N.G. Krohn, A.C.M. Danras, V.M. Stefenon, C.R. Clement, & M.P. Guerra. "Somatic embryogenesis in peach palm using the thin cell layer technique: induction, morpho-histological aspects and AFLP analysis of somaclonal variation". *Annals of Botany*, vol. (9)100, pp. 699, 2012.