PLBs organogenesis in orchid

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A protocorm-like structure those are generated from the vegetative explant in vitro, are known as protocorm like body (PLB). For the mass propagation of plant, PLBs regeneration is one of the key focusing. Orchid is the largest genus of the flowering plants and they have number of commercially important genus. Orchids are difficult to propagate by seeds and vegetative propagation methods. In vitro propagation is the most efficient technique for the orchid propagation. Induction and proliferation of PLBs can accelerate their propagation by reduce the time and costs. It is possible to propagate numerous numbers of plants within short period of time with low costs from PLBs. Researchers are trying to develop efficient PLBs induction and proliferation techniques using different plant growth regulators, carbon sources, and light emitting diodes. Here, we are discussing about the progress of the PLBs organogenesis in orchids.

Orchids | Mass propagation | protocorm like bodies

Introduction

Orchids have a huge demand in flower market as cut and pot flower, it belongs to Orchidaceae family with approximately 900 genera, about 27,800 accepted species and over 100,000 hybrids[1][2]. Orchidaceae family is enriched with more than 10 new genera on an average in each year[1] whereas only few genera are popular, and these are *Cattleya*, *Cymbidium*, *Dendrobium*, *Oncidium*, *Phalaenopsis* and *Vanda.* Only these few genera are considered as the most important genus for commercial cut and pot flower, most of the research are focused on these genera while rest of the genera are still underestimated. It can be propagated sexually by seeds or, asexually by vegetative propagation. Though orchids can produce a huge quantity of seeds but more than 99.7% does not have any functional endosperm[3], while vegetative propagation require a long period and the quality of the flower from vegetatively propagated seedling deteriorate. So, like other flowering plants, orchids are also not easy to regenerate by seeds or from their vegetative propagation. In vitro culture is an effective technique to propagate such plants[4]. Proliferation of the protocorm like bodies (PLBs) is one of most efficient techniques for orchid micropropagation. PLBs are triggered from explants or, calluses or, both[5]. Callus cells form compact regions during PLBs development (meristemoids[6]), and this compact region initiates polarized growth starts from the surface cells. PLBs then generate anterior smaller cells (shoot pole which generates first leaf) and posterior larger cell by continuous cell division[6][7]. PLBs organogenesis is also considered as somatic embryogenesis[6][8], and for that reason, PLBs regeneration is a routine practice for commercial orchid production to produce clonal plantlets[9]. Though several studies have already been conducted for the PLBs organogenesis of the dissimilar orchid genus, nonetheless it needs more studies for the effective PLBs organogenesis of orchids.

Protocorm like bodies (PLBs) and their organogenesis

A protocorm (embryo derived) alike structure those are generated from the vegetative explant in vitro, and these

are known as protocorm like bodies[5]. A compact region composed with meristemoids is firstly form during PLB organogenesis, thereafter, it starts the polarized growth from the surface cell of each compact pool of meristemoids; and then cell division creates the shoot pole of a PLB, and a posterior larger and vacuolated cells is also formed at the base of PLB[6]. Shoot pole leads to the leaf development and base of the PLB leads for the root development. However, root initiation can also be occurred from the middle or bottom of the PLB[7]. From single explant, numerous numbers of PLBs can be generated which can be further multiplied by separation, and each single PLB would be lead to the development of a single plantlet.

It can be generated from different plant parts such as shoot tips, root tips, buds of the flower stalks, nodal segments, leaf segments etc. PLBs organogenesis is aim for the mass propagation of plants in vitro. PLBs proliferation that can be influenced by several factors such as growing media, growing conditions, genetic materials etc.[10][11]. Murashige and Skoog (MS) is commonly used medium for in vitro. Plant regeneration or PLBs proliferation can be improved by the properly modified MS medium and culture condition; and this manipulation is plant specific[12][13]. This can fasten the micro-propagation process and improve the quality of regenerated plantlets as well. Medium can be modified by several ways such as alteration of the carbon sources, addition of the growth regulators or, elicitor. The requirement of plant growth regulators varies from species to species and explant to explant.

PLBs proliferation by dissimilar carbon sources

Carbon source is the basic ingredient for the in vitro culture media, where sucrose is widely used as the universal carbon sources irrespective to plant species for the plantlet generation and PLBs organogenesis as well. Besides, there are some efficient carbon sources such as glucose, fructose, maltose, sorbitol, manitol etc. are found as effective for the PLBs organogenesis in orchids; however, the efficiency of the carbon sources for the PLB organogenesis is highly plant specific[14][15]. We found that sucrose and trehalose both are equally acted as efficient carbon sources for the PLBs proliferation of *Phalaenopsis*[15].

Growth regulators and elicitors for PLBs organogenesis

In general, two main groups of plant growth hormones, cytokinins and auxins, are commonly used in orchid culture media, their types, concentration, and combinations are critically important. Plant growth regulators (PGRs) such as indol-3-acetic acid (IAA), indol-3-butyric acid (IBA), 1-naphthylacetic acid (NAA), 6-Benzyladenine (BA), 6-Benzylaminopurin (BAP), thidiazuron (TDZ) etc. are widely used in vitro micro-propagation, and their role for the in vitro plant growth and development has already been identified in various plants[16]. However, auxins, especially NAA affect the process of regeneration in monopodial epiphytic species; they act synergistically on the formation of PLBs[9]. On the other hand, a lower NAA and BA ratio in the culture medium was induced the formation of PLBs on rhizomes of *Cymbidium kanran*[17], and their higher ratio was effective in *Spathoglottis plicata*[18], However, lower NAA and BA ratio can results the slow development of plantlets from PLBs[17]. TDZ is comparatively less used chemicals for the manipulation of basal MS medium, and it was found as an effective plant growth media modifier to induce PLBs and their subsequent proliferation *Dendrobium*, *Vanda, Oncidium* and *Phalaenopsis*[19][20][21][22], it is considered as a diversified growth regulator[23]. However, the use of higher concentration of TDZ is sometimes associated with morphological abnormalities as has been reported in several species[24][25]. Chitosan also plays

singnificant role for the in vitro PLBs organogenesis[26]. Hyaluronic acid (HA), a polymer of disaccharides, could be a potential growth regulator for the PLBs proliferation in vitro, and it has diverse functions according to their molecular weight such as HA9, HA12, HA20 etc. However, PLB organogenesis of different orchid genus or even species would have independent response against each forms of HA[27][28][29]. 5-aminolevulinic acid (ALA) is known plant growth regulator for the increasing plant growth and yield that generally increase the carbon and nitroge assimilation, chlorophyll content, photosynthesis process by the exogenous application[30][31][32]. Recently, it has been used for the PLBs organogenesis in orchid and found as an effective plant growth regulator for PLBs proliferation in orchids[33].

Growth retardants like chlorocholine chloride (CCC: (2-chloroethyl) trimethyl-ammonium chloride) can be used for the *in vitro* plant micro-propagation, and it can influence on plant growth and development[15][34] by inhibiting the biosynthesis of gibberellic acid[35][36]. Lysozymes (N-acetylmuramoylhydrolases) has found in many organisms that has the capability to defend plant against infection. It can act as an elicitor on *in vitro* propagation[37][38][39]. Similar to lysozyme, methyl jasmonate (MeJa) is associated with plant defense[40] and growth inhibition rather than promotion[41]. It can alter physiological responses in plants thus make a plant in more organized form by reducing the growth unwanted parts. It is an elicitor for secondary metabolite production *in vitro* micro-propagation[42][43], and can improve PLBs formation[38][44]. Lysozyme is considered a “natural” antibiotic[45][46], is an elicitor; and it could be an important factor to initiate PLBs. PLBs of orchids can well proliferated by dipping 30 minutes into lysozyme aqueous solution[47]. MeJA is a volatile derivative of jasmonic acid[48], MeJA or JA acts as an elicitor in the culture media to enhance the anthocyanin production[49]. MeJA has successfully used as an elicitor in other plant species for enhancing the production of secondary metabolites like anthocyanin in cell cultures[50][51][52][53]. MeJA (@1 µM) stimulates protocorm like body and shoot formation in epiphytic and terrestrial *Cymbidum*[54], while it stimulated PLB formation from half-moon PLBs and PLB TCLs in a hybrid Cymbidium (@1 mg/l)[44].

PLB proliferation under light emitting diodes (LEDs)

Efficiency of PLBs production and shorten the culture period is an important task in recent year. Culture condition can manipulate by altering the light color and intensity, and that can significantly fasten the culture period and PLBs proliferation as well. However, It has been reported different culture media and growth conditions manipulation for PLBs organogenesis in orchids[15][55][56][57][58][59]. Plant can respond well to a wide spectrum of light in terms of plant growth and development with wavelengths of <400 nm (UV radiation), 400-700 nm (visible) and 700-800 nm (far-red)[60]. White fluorescent light with spectral emission of 350-750 nm wavelengths generally used as a light source in plant *In vitro* culture. Now a days, several studies reported that LEDs are the most efficient over white fluorescent light[61]. Plants do not need the all spectral of light for their growth and development, and their absorption is highly plant specific. LED provides the extra advantage by emitting narrower wavelength than the traditional light, that can be selected for the plant specific requirements[62][63]. In general, blue and red light spectrum are essential for plant growth and development[64][65][66][67], and most of the studies focuses on these two light while efficiency of other light spectrum can not be underestimated.

Red and blue LEDs combinations was reported as effective for the growth and development of PLBs in *Cymbidium*, *Doritaenopsis*, *Phalaenopsis*, and *Calanthe*[61][64]. Blue LED increase shoot formation of PLB cultures in *D. officinale* and *D. kingianum*[67][68]. Red LED shows the lowest differentiation rate vise versa in blue LED for

the in vitro PLBs proliferation of orchids[69][67], and it can be beneficial for the accumulation of more carbohydrate during PLB proliferation[70]. A mixture of red plus blue light, and red LED alone, enhanced both plant growth and development by increasing the net photosynthesis[71]. This is because the spectral energy distribution of red and blue light coincided with that of chlorophyll absorption[72]. In contrast, there is very little information for effect of monochromatic yellow and green LED on growth and environmental factors of plants. Yellow LED has also the potentiality for the early PLBs proliferation[73], and the green LEDs would have similar potentiality for the PLB proliferation of orchids.

Combination of more than one factors

Combination of the different PLBs organogenesis showed the better potentiality for their proliferation and fresh weight in orchids. Recent years, researchers are sorted out the combination of carbon sources, growth regulators, growth retardants, elicitors, and light emitting diodes. Recently, we have studied LEDs combined with different carbon sources for the PLBs proliferation of *Phalaenopsis*, we compared the PLBs proliferation under monochromatic lights those were combined with different carbon sources[15]. We found three different combinations for the efficient PLBs proliferation of Phalaenopsis, and these are Red-White-sucrose (PLBs were cultured first half of the culture period under red LED and last half of the culture period under white LED, and the culture media was supplied with sucrose as carbon sources), Blue-White-trehalose (PLBs were cultured first half of the culture period under blueLED and last half of the culture period under white LED, and the culture media was supplied with trehalose as carbon sources), Red-Blue-White-trehalose (PLBs were cultured first 1/3 of the culture period under red LED, then 1/3 of the culture period under blue LED and last 1/3 of the culture period under white LED; and the culture media was supplied with trehalose as carbon sources)[15]. Our results notified that, LEDs for the PLBs proliferation not only plant specific, but also the growing stage specific. Red-White-sucrose, Blue-White-trehalose and Red-Blue-White-trehalose combinations notified that Phalaenopsis need red and/or blue LED at the primary period of their PLBs culture and thereafter it needs the white LED. However, Red-White-sucrose and Blue-White-trehalose combinations suggested that sucrose respond well as carbon sources under red light, while trehalose can supplied more carbon for the PLBs proliferation under blue LED.

Finally, PLBs propagation is the best way for the mass propagation of orchids, however, the efficient PLBs propagation techniques need to disclose. From the previous studies, it was clear that PLBs proliferation in orchids is plant specific, and each orchid species respond differently to the carbon sources, plant growth regulators, plant growth retardants, elicitors, and light emitting diodes.

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