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THE EFFECT OF DIFFERENT GROWTH MEDIA, CARBON SOURCE AND PGRs ON *DENDROBIUM* BROGA GIANT ORCHID'S PROTOCORM-LIKE BODIES (PLBs) PROLIFERATION SUPPORTED WITH SEM AND TEM ANALYSIS

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Abstract

Dendrobium Broga Giant (*Dendrobium* Bobby Messina × *Dendrobium* Superbiens) is a new orchid hybrid in Malaysia. The aim of this study was to investigate the effect of different growth media, carbon sources and PGRs on *Dendrobium* Broga Giant orchid's protocorm-like bodies (PLBs) proliferation supported with SEM and TEM analysis. The PLBs were cultured on different strength of semi-solid and liquid MS medium and the proliferation rate was recorded based on the fresh weight basis. The maximum PLBs proliferation (12.31% ± 0.57) was obtained in ½ MS semi-solid medium. PLBs were cultured on ½ MS semi-solid media supplemented with different sucrose concentrations (0, 10, 20, 30 & 40 $g.L^{-1}$). The highest proliferation rate of PLBs (15.48% ± 1.20) was recorded from ½ MS supplemented with 20 $g.L^{-1}$ sucrose. Different concentrations of BAP (0, 0.5, 1.0, 2.0 $mg.L^{-1}$) and NAA (0, 0.5, 1.0, 2.0 mgL^{-1}) were added to ½ MS semi-solid medium. Combinations of 1.0 $mg.L^{-1}$ BAP and 0.5 $mg.L^{-1}$ NAA produced higher PLBs proliferation. Micromorphological studies by SEM and TEM displayed trichome, leaf primordial, stomata, various sizes of mitochondria, vacuoles, different shapes of chloroplast were found in PLBs which ameliorated the slow proliferation nature of plantlets.

Key words: *Dendrobium* Broga Giant, PLBs, PGRs, SEM, and TEM.

Introduction

Orchid is an important group of ornamental plants comprising of several thousand species and hybrid. Orchids are valuable ornamentals and have become the second largest cut flowers and potted floricultural crop (Hossain *et al.,* 2010). *Dendrobiu*m is the second largest orchid genus in the world after *Bulbophyllum* (Puchooa, 2004). *Dendrobium* species are either epiphytic or occasionally lithophytic and is commonly abbreviated as 'Den' in horticulture science. The orchids especially *Dendrobium* occupies the foremost position in floriculture industry especially in ornamental flower business due to its beautiful colours, its ability to produce flowers continuously and a prolonged post-harvest life in comparison to other species. They have high potential to be used as cut flowers, potted plants and for landscaping purposes (Sugapriya *et al.,* 2012).

The success of plant tissue culture is highly influenced by the nutrition supplied in the media, carbon sources and growth regulators. The media used for tissue culture of orchids is mainly high in salt, minerals, vitamins, growth regulators and water (Murdad *et al.,* 2010). Protocorm-like bodies (PLBs), callus, cells suspension, immature embryo, pollen, shoot apex, *in planta*, floral and seeds are the commonly used target materials for application of tissue culture techniques. The PLBs regenerate into a new plant and are the most suitable target explants for the establishment of orchid cultures. PLBs or thin cell layers (TCLs) of PLBs are the most frequently used as a target tissue for successful genetic transformation study in *Dendrobium* orchids (Men *et al.,* 2003a).

Carbon source is another essential component for plant tissue culture as the energy source to the plants particularly during the early stage of tissue culture when plantlets are not able to photosynthesize their own food (Al-Khateeb, 2008). The most preferred carbon source in plant tissue culture is sucrose. Sucrose is a major carbohydrate source which supply energy to culture cells for transportation as well as its higher efficiency of across the plasma membrane (Kumaraswami *et al.,* 2010). Plant growth regulators (PGRs) such as auxins, gibberellins, cytokinins and abscisic acid have been successfully used in the orchid cut flower industry. PGRs help to induce PLBs or shoots and flower initiation of orchids. Different types of explants, concentrations and combination of plant growth regulators play a remarkable role during *In vitro* propagation of *Cymbidium* orchid (Tao *et al.,* 2011).

SEM and TEM analysis were determined in order to justify that PLBs is a meristematic tissue and contains actively dividing cells that is suitable as a target material for *In vitro* system work. Both SEM and TEM analysis have allowed understanding the formation and the development of PLBs. Dense cytoplasm, large nuclei with prominent nucleoli, small vacuoles and the presence of starch grains were in embryogenic cells which displayed an intense synthesis of RNA and extensive metabolic activity analyzed by TEM (Aslam *et al.,* 2011). As callus differentiation progressed, the globular somatic embryos composed of cells with dense cytoplasm was discovered to developed from the inside or surface of the callus prior development to PLBs (Zhao *et al.,* 2008).

Thus, the aim of this study was to investigate the effects of media, carbon sources and PGRs on the performance of *In vitro* cultures supported with SEM and TEM analysis based on the PLBs proliferation of *Dendrobium* Broga Giant orchid. This study has provided important information on the improvement for a higher proliferation rate of *Dendrobium* Broga Giant orchid plantlets via *In vitro* culture system.

Materials and Methods

Plant materials: *Dendrobium* Broga Giant orchid's protocom-like bodies (PLBs) were cultured by aseptically culturing seeds of the hybrid in half-strength semi-solid MS medium supplemented with 2% sucrose, 2.75 gl^{-1} gelrite^{TM} (DUCHEFA, Netherlands) and 1 $mg.ml^{-1}$ 6-benzylaminopurine (BAP; DUCHEFA, Netherlands). The ensuing cultures were chopped into clumps of two to three PLBs and subcultured every four weeks. For experimental purpose, One (1) - 2 mm sized PLBs were cultured on different strengths of MS media such as half-strength, full-strength and double-strength MS semi-solid and liquid media. The pH of the media was adjusted to 5.80 with 1 N NaOH or HCl prior to autoclaving for 15 min at 121^{o}C. All cultures were incubated at 25±2^{o}C and under cool-white fluorescent light of 30µmol$m^{-2}s^{-1}$ for 16 hours per day. One (1) gram of PLBs marked as the initial fresh weight was cultivated into a 250 mL culture jars as a treatment. Each treatment consisted of six replicates and the PLBs growth responses were calculated based on the fresh weight of PLBs after four weeks. This experiment was repeated 3 (three) times. Proliferation rate (%) was calculated as below:

$$\text{Proliferation rate (\%)} = \frac{\text{Final fresh weight (g) - Initial fresh weight (g)}}{\text{Initial fresh weight (g)} \times \text{Days of inoculation}} \text{ X } 100$$

Carbon sources: Sucrose was used in this study as a carbon source. Different concentrations of sucrose such as 0, 10, 20, 30 and 40$g.L^{-1}$ were used in ½ MS semi-solid and liquid media to identify the optimal sucrose concentration produced the highest proliferations of PLBs. One (1) - 2 mm sized one gram sixty-days-old PLBs were selected for this experiment. The PLBs proliferation was considered based on the increasing fresh weight after four weeks of culture. Proliferation rate of PLBs was calculated following the same formula as for proliferation rate calculation of media and culture conditions. This experiment were repeated 3 (three) times.

Effect of PGRs on PLBs proliferation of *Dendrobium* Broga Giant orchid: Half strength of MS semi-solid media supplemented with various concentrations of NAA (0.5, 1.0, 2.0 $mg.L^{-1}$) and BAP (0.5, 1.0, 2.0 $mg.L^{-1}$) with 20 $g.L^{-1}$ sucrose were used for determining the effects of PGRs on PLBs proliferation of D*endrobium* Broga Giant orchid in this study. One (1) - 2 mm sized and sixty-days-old PLBs were used for the experiment. Data were recorded after four weeks in culture. This experiment were repeated 3 (three) times.

Scanning electron microscopy observation: Protocom-like bodies (PLBs) were used for scanning electron microscopy (SEM) followed by freeze drying method. The freeze drying method involved vapour fixing of the samples with 1% osmium tetroxide for an hour, followed by freeze drying (Emitech k 750 X freeze dryer) of the samples in liquid nitrogen (-210^{o}C) slush and coated with 5- 10 nm of gold sputter (Polaron SC515 sputter Coater, Fison Instruments, VG Microtech, Susses, UK). The analysis were conducted using a scanning electron microscope (Leo Supra 50 VP Field Emission SEM, Carl-Ziess SMT, oberkochen, Germany) and all images were processed digitally by using Oxford INCA 400 energy dispersive X-ray microanalysis system software.

Transmission electron microscopy observation: Protocorm-like bodies (PLBs) were used for transmission electron microscopy (TEM) observation through the Spurr's resin method. Fixed PLBs sample were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer, dehydrated with an increasing ethanol series and infiltrate in spurr's resin for embedding. Ultrathin sections (<0.1µm) were prepared by ultramicrotome (PoweTome XL-RMC Products, Boeckeler Instruments Inc, Arizona, USA) and contrasted with uranyl acetate and lead citrate (Rocha *et al.,* 2012). The sections were examined and photographed on transmission electron microscope (EFTEM Libra 120, Carl Zeiss, Germany) at 120 kV by using Olympus SIS iTEM version 5.0 software.

Experimental designs and data analysis: The experiment was designed in a completely Randomized Design (CRD). All data were analyzed by one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) Software version 20.0 (SPSS Inc., Chicago, IL, USA). Comparisons of the mean and standard errors were determined by Duncan's multiple range tests at $p \leq 0.05$ level of significance.

Results and Discussion

The newly induced protocorm-like bodies (PLBs) formed a distinct shape and underwent fully developed PLB and PLB masses after four weeks. The significant effects on proliferation rate of *Dendrobium* Broga Giant orchid's PLBs were obtained from different strength of MS media (Table 1). The maximum proliferation rate of PLBs (12.31%) were recorded from half strength MS in semi-solid media. Increasing the strength of both MS semi-solid and liquid media to full and double strength significantly ($p \leq 0.05$) decreases the proliferation rate of PLBs and the lowest proliferation rate of PLBs (0.84%) were recorded from double MS liquid media (Table 1). *Dendrobium* Broga Giant orchid PLBs proliferation rate was observed higher in the half strength of MS semi-solid compared to half strength of MS liquid medium. However, increasing the strengths of MS media to full and double strength significantly decreases the proliferation rate of PLBs. Similar result was obtained on *Dendrobium* sonia-28 orchid by reducing MS media concentrations into half which significantly increased PLBs production within 4 weeks (Advina, 2012). Cardoso & Ono (2011) observed that reducing reducing the strength of MS media could enhance the *In vitro* growth of *Brassocattleya* orchid. They suggested that reducing the strength of MS media to lower concentration of nitrogen and potassium could increase orchid biomass based on fresh weight. They also suggested that high accumulation of nitrogen and potassium inhibit the maximum capacity of cell growth. Naing *et al.* (2011) reported that half strength of MS media produced the highest survival rate and growth of wild medicinal orchid, *Coelogyne cristata*'s PLBs. Groll *et al.* (2002) suggested that Most of the embryo cell could tolerate only to full strength MS level and thereby increasing concentration could negatively affect cell growth in the embryo of cassava.

Table 1. Effect of different strengths MS media on the proliferation rate (%) of *Dendrobium* Broga Giant orchid PLBs.

Strength of MS media	Proliferation rate of PLBs (%)
½ MS semi-solid	12.31 ± 1.14^{a}
½ MS liquid	8.28 ± 2.32^{b}
Full MS semi-solid	6.22 ± 0.99^{c}
Full MS liquid	2.77 ± 0.95^{d}
Double MS semi-solid	0.88 ± 0.30^{e}
Double MS liquid	0.84 ± 0.20^{e}

The data represent the mean values ± standard error. Different letter (s) corresponds to significant differences at p≤0.05 by Duncan's multiple range tests

Table 2. Effect of different concentrations of sucrose on the ½ MS media for the proliferation rate (%) of *Dendrobium* Broga Giant orchid PLBs.

Growth media	Sucrose concentrations ($g.L^{-1}$)	Proliferation rate of PLBs (%)
½ MS semi-solid media	0	3.18 ± 0.11^{f}
	10	9.18 ± 0.85^{c}
	20	15.48 ± 1.20^{a}
	30	13.65 ± 0.37^{b}
	40	5.32 ± 0.21^{e}
½ MS liquid media	0	1.12 ± 0.12g
	10	6.04 ± 0.16^{de}
	20	7.07 ± 0.54d
	30	5.57 ± 0.21de
	40	1.33 ± 0.27^{g}

The data represent the mean values ± standard error. Different letter (s) corresponds to significant differences at p≤0.05 by Duncan's multiple range tests

Dendrobium Broga Giant orchid PLBs were cultured in half strength MS semi-solid and liquid media supplemented with different sucrose concentrations (0, 10, 20, 30 and 40 gL^{-1}). Sucrose concentrations significantly (p≤0.05) affected PLBs proliferation in both forms of media (Table 2). The maximum proliferation rate (15.48%) of PLBs was recorded from half strength of MS semi-solid media supplemented with 20 gL^{-1} sucrose concentration and the minimum (1.12%) proliferation rate was in 0 gL^{-1} sucrose concentration in half MS liquid media (Table 2).

The proliferation rate of PLBs was significantly higher in semi-solid compared to liquid media. Based on the proliferation rate of PLBs, 20 gL^{-1} sucrose is the optimum for *Dendrobium* Broga Giant orchid PLBs proliferation in ½ MS semi-solid culture media. Absence of sucrose in MS media significantly reduced PLBs growth rate (Table 2). *Dendrobium* Broga Giant orchid PLBs proliferation was observed under different sucrose concentrations. Sucrose significantly increases the growth of *Grammatophyllum speciosum* Blume protocorms. Pimsen & Kanchanapoom, (2011) reported that 2% (w/v) sucrose response better growth of *Grammatophyllum speciosum* Blume protocorms. They also suggested that higher concentrations of sucrose had an inhibitory effect on *Grammatophyllum speciosum* Blume protocorms. Zha *et al.* (2007) investigated that 35 gL^{-1} sucrose produced maximum production of PLBs of *Dendrobium* huoshanense orchid. Farra *et al.* (2000) reported that sucrose has a significant regulatory and integrative function in plant development and that changes in sucrose content are transduced into changes in gene expression. They also observed that sucrose not only provide energy for proliferation but also play a regulatory role in plant. Sucrose is specially needed in plant embryo to increase cell division by cell encouraging cell expansion and reserve accumulation. When increases the sucrose concentration over the threshold level than negatively affected the PLBs growth and morphogenesis in *Vandofinetia* orchid (Kishi *et al.,* 1997).

PLBs were cultured on half strength of MS medium with addition of different concentrations of BAP and NAA. Different concentration of BAP significantly (p≤0.05) affected on PLBs proliferation of *Dendrobium* Broga Giant orchid (Fig. 1). The maximum PLBs proliferation (5.67%) was recorded in 1.0 mgL^{-1} of BAP and the minimum (2.71%) was in control (0 mgL^{-1} of BAP). In the other hand, NAA also significantly (p≤0.05) influenced PLBs proliferation (Fig. 2). The highest PLBs proliferation (5.31%) was observed in half strength of MS media supplemented with 0.5 mgL^{-1} of NAA whereas the lowest Proliferation (2.48%) was recorded in control (0 mgL^{-1} of NAA) medium. BAP and NAA with their combinations also significantly (p≤0.05) affected in proliferation of PLBs (Table 3). The percentage of PLBs induced was the highest on half strength MS semi-solid medium supplemented with 1.0 mgL^{-1} BAP and 0.5 mgL^{-1} NAA. The highest proliferation rate at 8.7% of PLBs was obtained in 1.0 mgL^{-1} BAP and 0.5 mgL^{-1} NAA. However, the lowest proliferation rate (1.21%) of PLBs was obtained on half strength MS semi-solid medium with in the absence of plant growth regulators (Table 3). In addition, highest survival rate (100%) of PLBs was found in half strength MS semi-solid medium with 1.0 $mg.L^{-1}$ BAP and 0.5 mgL^{-1} NAA, 0.5 $mg.L^{-1}$ BAP and 1.0 mgL^{-1} NAA and 1.0 $mg.L^{-1}$ BAP and 1.0 mgL^{-1} NAA. However, lowest percentage (70%) of survival rate of PLBs was obtained in half strength MS semi-solid medium with 0 mgL^{-1} BAP and 0 $mg.L^{-1}$ NAA (Table 4). BAP and NAA plant growth regulators induction the shoots of *Dendrobium* orchids (Martin & Madassery, 2006). The highest percentage of explants producing shoots was induced on the ½ MS media supplemented with 1.0 $mg.L^{-1}$ NAA and 0.5$mg.L^{-1}$ BA (Naing *et al.,* 2011). The maximum response of PLBs (86.6%) was obtained in medium supplemented with NAA at 30 µM, meanwhile the maximum number of shoots (4.42) and maximum bud-forming capacity (3.51) were observed in medium containing 15 µM BAP and 5 µM NAA in combination. The maximum number of explants forming PLBs (41.48%) was obtained in medium containing 15 µM BAP and 15 µM 2,4-D (Dohling *et al.,* 2012). The number of secondary protocorms that developed from primary protocorms was increased by the addition of 5.0 µM BAP and 2.5 µM NAA in *Dendrobium nobile* Lindl (Mohanty *et al.,* 2012).

PLBs at four weeks of culture were used in this experiment. The PLB surface rich in stomata could be seen clearly under the scanning electron microscope (SEM) (Fig. 3a). Leaf primordial which eventually develops into first leaf was found in Fig. 3b. PLB surface displayed the randomized projections of branched trichome as been shown in Fig. 3c. SEM observation also shows such cone-shaped projections on the surface of PLBs (Fig. 3d). Continuous cell divisions of the meristematic cells lead to the development of vertically elongated cone-shaped meristematic dome. Cultured plants with non-functional stomata, weak root systems and poorly developed cuticles caused mortality upon the transfer to *ex vitro* conditions (Mathur *et al.,* 2008). Presence of stomata contributes to the capacity of *Dendrobium* Broga Giant orchid PLBs to control its water relations and to gain carbon similar to a mature plant. Trichomes are bush-like appendages on the surface of plant tissues, which range in size from a few microns to several centimeters (Tissier, 2012). Theoretically, trichomes may serve as absorption or conduction tube since they are known to accommodate fluids inside.

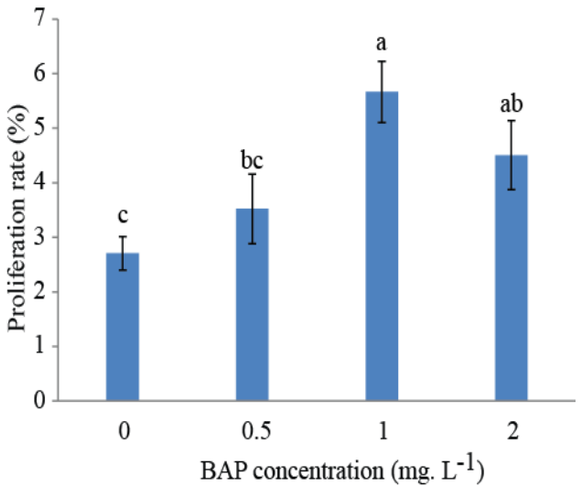

Fig. 1 Effect of different concentration of BAP on proliferation rate of *Dendrobium* Broga Giant orchid PLBs. The data represent the mean values ± standard error. Different letter (s) corresponds to significant differences at p≤0.05 by Duncan's multiple range tests.

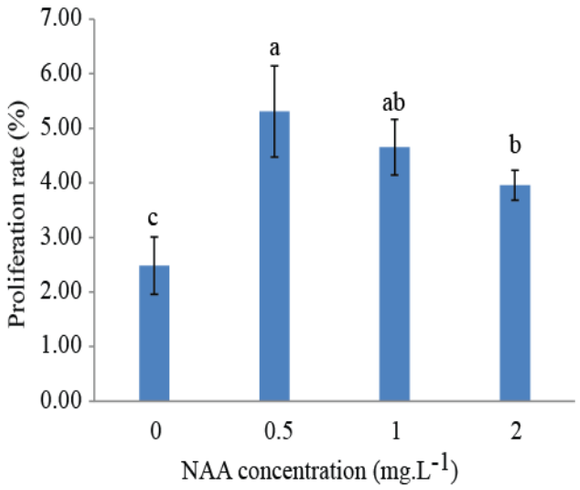

Fig. 2 Effect of different concentration of NAA on proliferation rate of *Dendrobium* Broga Giant orchid PLBs. The data represent the mean values ± standard error. Different letter (s) corresponds to significant differences at p≤0.05 by Duncan's multiple range tests.

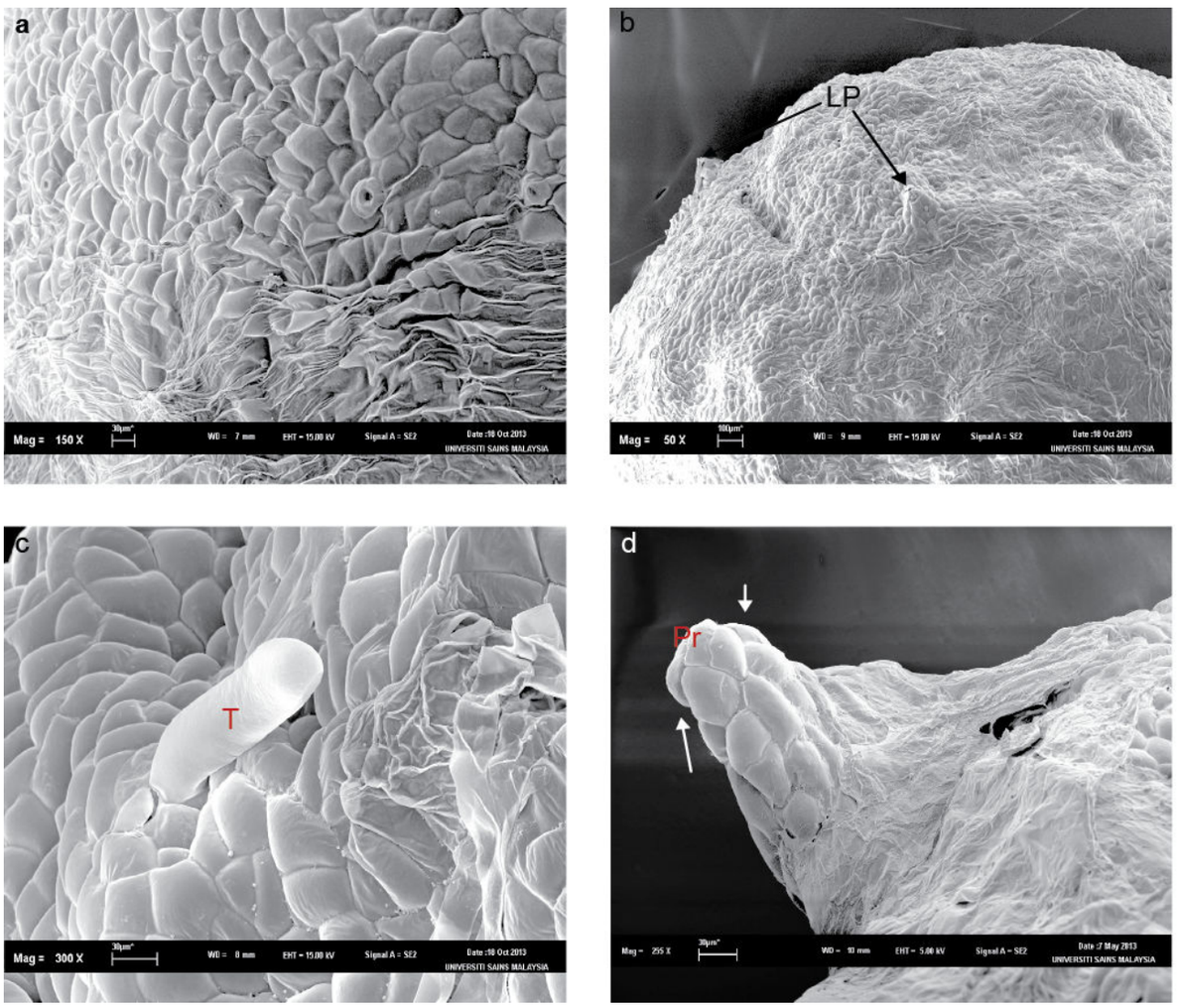

Fig. 3. Scanning electron micrographs of *Dendrobium* Broga Giant orchid PLBs. (**a)** Stomata randomly distributed on the PLB surface, (**b)** Enlarged view apical meristems of PLB, (**c)** trichome and (**d)** Meristematic dome, LP Leaf primordial, T, trichome, Pr Promeristem. In a, c,d = 30 µm and b = 100 µm *bars*.

Table 3. Effect of different concentrations of BAP and NAA on the proliferation rate (%) of *Dendrobium* Broga Giant orchid PLBs.

Treatments		Final fresh weight of PLBs (g)	Initial fresh weight of PLBs (g)	Proliferation rate of PLBs (%)
BAP (mg.L^{-1})	NAA (mg.L^{-1})			
0	0	1.34	1.00	1.21 ± 0.33^{f}
0	0.5	1.46	1.00	1.63 ± 0.05^{ef}
0	1.0	2.32	1.00	4.7 ± 0.59^{cd}
0	2.0	1.92	1.00	3.3 ± 0.26^{de}
0.5	0	1.62	1.00	2.23 ± 0.46^{e}
0.5	0.5	2.39	1.00	4.97 ± 1.31^{c}
0.5	1.0	1.91	1.00	3.25 ± 0.61^{de}
0.5	2.0	2.02	1.00	3.64 ± 0.17^{d}
1.0	0	1.96	1.00	3.42 ± 0.77^{de}
1.0	0.5	3.46	1.00	8.77 ± 0.90^{a}
1.0	1.0	2.59	1.00	5.69 ± 0.35^{bc}
1.0	2.0	2.34	1.00	4.78 ± 0.22^{cd}
2.0	0	1.86	1.00	3.07 ± 0.54^{de}
2.0	0.5	2.64	1.00	5.87 ± 1.07^{b}
2.0	1.0	2.39	1.00	4.97 ± 0.48^{c}
2.0	2.0	2.15	1.00	4.11 ± 0.44^{cd}

The data represent the mean values ± standard error. Different letter (s) corresponds to significant differences at p≤0.05 by Duncan's multiple range tests

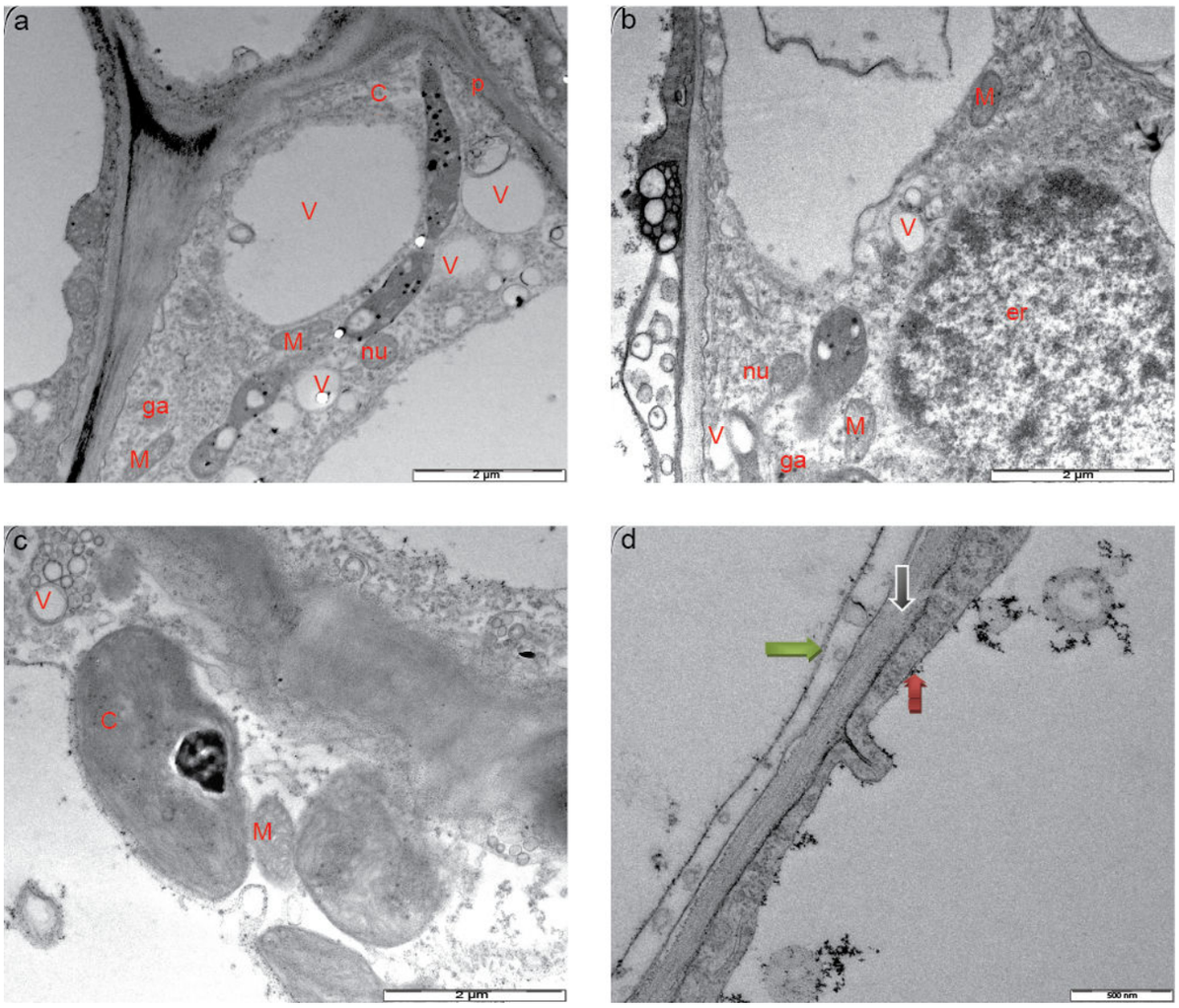

Fig. 4. Transmission electron microscopy of *Dendrobium* Broga Giant orchid PLBs. **a-c** Thin cell walls, Cells with dens cytoplasm, vacuoles, mitochondria, nucleus with prominent nucleoli, plasmodesmata, rough endoplasmic reticulum, chloroplast and Golgi apparatus **d** three layers of the outer periclinal walls was clearly visible: the pecto-celulosic wall (green arrow), the pectin lamelle (black arrow) and a cuticle (red arrow). C chloroplast, ga Golgi apparatus, M mitochondria, nu nucleolus, er rough endoplasmic reticulum, V vacuole. In a-c *bars* = 2 µm and d *bars* = 500 nm.

Table 4. Effect of different combination of BAP and NAA concentrations on the survival rate of *Dendrobium* Broga Giant orchid PLBs.

Treatments		Survival rate of PLBs (%)
BAP (mg.L^{-1})	NAA (mg.L^{-1})	
0	0	70.00 ± 5.77^{d}
0	0.5	80.00 ± 5.77^{bcd}
0	1.0	86.67 ± 5.77^{abc}
0	2.0	90.00 ± 0.00^{ab}
0.5	0	86.67 ± 5.77^{abc}
0.5	0.5	90.00 ± 6.67^{ab}
0.5	1.0	100.00 ± 3.33^{a}
0.5	2.0	90.00 ± 8.82^{ab}
1.0	0	90.00 ± 5.77^{ab}
1.0	0.5	100.00 ± 0.00^{a}
1.0	1.0	100.00 ± 0.00^{a}
1.0	2.0	93.33 ± 0.00^{ab}
2.0	0	80.00 ± 5.77^{bcd}
2.0	0.5	80.00 ± 5.77^{bcd}
2.0	1.0	73.00 ± 3.33^{cd}
2.0	2.0	71.67 ± 1.67^{cd}

The data represent the mean values ± standard error. Different letter (s) corresponds to significant differences at p≤0.05 by Duncan's multiple range tests

TEM analysis was performed by cross section of PLB at higher magnification. The TEM analysis indicated the presence of some cells with thin walls, dens cytoplasm, small vacuoles, mitochondria, nucleus with prominent nucleoli, plasmodesmata, rough endoplasmic reticulum, chloroplast and Golgi apparatus Fig. 4(a-c). Three layers of the outer periclinal walls were clearly visible in Fig. 4(d). The dividing cells in developing tissues and organs were smaller and dispensed all cell organs in cytoplasm. The mechanical strength of the cell wall is the main source of structural strength and rigidity for the organs. The majority of plant tissues rely on turgor pressure of the vacuole sap maintaining the cell wall in tension to achieve rigidity. Cuticle is functionally significant for the exchange of water, solutes, gases and the deposition of different substances (Kerstiens, 2006). Cuticles protect the plants against UV radiation, mechanical damage, pathogens and insect. It also provides mechanical strength and contributes to the viscoelastic properties of the cell wall (Reina-Pinto & Yephremov, 2009). A large number of mitochondria indicate a high level of energy utilization by these cells and is characteristic of tissues undergoing differentiation (Rocha *et al.,* 2012). The frequency of plasmodesmata has been reported to increase in meristimatic cells because these conections are essential for the intercellular transport of signaling molecules involved in controlling the differentiation pathway of these cells (Appezato-da-Gloria & Machado, 2004). The increased number of Golgi apparatus typically involved in the secretion of substances into apoplast, was associated with an accelerated synthesis of cell wall components in *Glycine max* meristemoids (Steinmacher *et al.,* 2012). Konieczna *et al.* (2008) reported that in embryogenic callus, organelles such as mitochondria and rough endoplasmic reticulum occupied a peripheral position since it affected by large vacuoles. They also demonstrated that round or oval shape mitochondria, microbodies, plastids, dicyosomes and numerous ribosomes could be observed in the callus culture of kiwifruit based on TEM analysis.

Conclusions

In conclusion, the best PLBs proliferation rate was observed in ½ MS semi-solid media with 20 gL^{-1} sucrose and 1.0 mgL^{-1} BAP + 0.5 mgL^{-1} NAA plant growth regulators combination. SEM and TEM analysis can significantly contribute to our understanding of the formation of an organ or embryo from a single cell. The analysis system also showed that PLBs contained stomata, trichome, mitochondria, chloroplast, vacuoles and cytoplasm which were important and influences morphological and physiological growth of *Dendrobium* Broga Giant orchid.

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