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Enhance Efficient Plant Regeneration of *Dendrobium pulchellum* with Polyamine

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Dendrobium pulchellum is a resident orchid that is in Dendrobium genus and ORCHIDACEAE classified in subfamily. There can be weather-resistant and growing commercially for improved breeding. But nowadays, orchids are reduced in number and risk of extinction. The purpose of research project to effects of various of kinds and concentrations of polyamine (spermidine, spermine and putrescine) on plant growth and regeneration of *D. pulchellum*. Protocorm-like bodies (plbs; full-plbs and half-plbs) were cultured on Murashige and Skoog (MS) medium supplemented with kinds (spermidine, spermine and putrescine) and various concentrations (0, 0.2, 0.4, 0.8 and 1 mM) of polyamine. 3% sucrose was added to all culture media, after that, 0.8% agar was added to solidify it. The cultures were kept at 25 2 °C in a light setting. Half-plbs cultured on MS medium with 0.4 mM spermidine had the best survival rate of 93.33 % after a month of culture. While full-plbs were cultured on 1 mM spermidine gave the highest survival rate at 83.33%. For effect of various of kinds and concentrations of polyamine on plbs proliferations, the result found that half-plbs were cultured on MS medium added with 1 mM spermidine gave the highest plbs induction (80 %), average number of plbs (4.11 plbs/explant) and shoot induction (1 shoot/explant) when compared with other treatments after 3 months of culture. Full-plbs were cultured on 1 mM spermidine gave the highest shoot formation (1.67 shoots/explant), average plant height (1.31 cm.) and average number of leaves (3.63 leaves/explant), significant difference ($p \leq 0.05$). Plbs were grown on MS and ½ MS media supplemented with or without 8% agar and 0.2% phytagel obtained from plbs proliferation. After culturing for 3 months, MS medium supplanted with 0.2 % phytagel gave the highest average fresh weight at 351.76 mg and average number of shoot at 27.50 shoots/explant, significant difference ($p \leq 0.05$). For root induction, The maximum root induction was achieved using MS medium added with 0.2% activated charcoal (100%) gave the highest root induction (100%), average number of root (7.13 roots/explant), average root length (5.07 cm) and average plant height (5.07 cm.) when compared with other treatments. Complete plantlets were successfully acclimatised to the greenhouse environmental. Plantlets potted in survival rate of 100% after 1 month of transfer. Moreover, after transferred complete plantlets to acclimatise in spathe, charcoal smash and spathe: charcoal (1:1) gave the highest survival rate at 100% while plastic pot gave survival rate at 90 for 1 month.

Keywords: plant regeneration; protocorm-like body; polyamine

I. INTRODUCTION

Dendrobium is a genus with about 1100 species. It can be found all across the world and is frequently farme (Puchooa, 2004). It is the most popular orchid all over the world, also in Bangladesh. Different characteristics of *dendrobium* such as beautiful flowers, grow well and are in demand by the market (Talukder *et al.,* 2003). *Dendrobium pulchellum* Roxb.ex Lindl is in the sub-genus ORCHIDACEAE and is an orchid in the genus Dendrobium. *D. pulchellum* can be easily grown and have beautiful flowers. It is suitable for constructing a pot for a potted orchid. Nowadays found that *D. pulchellum* has

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been reduced. It is usually propagated vegetatively, which is a laborious and time-consuming process. Normally there are a lot of orchid seeds in the orchid pods. It contained naked embryos around 80-100 cells without endosperm. However, orchid seeds have very little ability to naturally germinate because the seeds do not receive sufficient moisture or receive other inappropriate environments such as too high or low temperatures. Including the seeds there is very little food accumulating inside not enough for seed germination. Therefore, tissue culture is one technique that is currently popular to use to help quickly grow a lot of plants. Important factors affecting plant tissue culture include culture media, plant hormones and characteristic of protocorms have been necessary. Successful plant tissue culture technique can be cultured from nodal segment in D.sonia-28 (Advina *et al.,* 2014), shoot tip in *Dendrobium fimbriatum* Lindl. (Roy & Banerjee, 2003), seed in *Dendrobium peguanum Lindl* (Sagaya *et al.,* 2015). For plant tissue culture, there are still other additives that can be used in a variety of tissue cultures. To induce plants to meet their objectives and needs, such as organic polyamine groups and have the ability to induce plants such as spermidine, spermine and putrescine. Polyamine (PA) is an organic substance that has two or more groups of amines. Polyamines are engaged in the phenomena because they are involved in both plant cell ageing and differentiation (Kaur-Sawhney *et al.,* 1982). Recent research, on the other hand, reveal that PA plays a function in the differentiating of many in vitro dicot plant embryos, specifically carrot somatic embryos (Montaguec, 1978) and tobacco tissue cultures in thin layer, as well as the differentiation between vegetative and floral buds (Flores & Protacio, 1990; Kaur-Sawhney *et. al.,* 1988; Tiburcio *et. al.,* 1987; Torrigiani *et al.,* 1987) and roots (Tiburcio et al., 1989). Polyamine was shown to be responsible for the transmission of hormone actions in plant cells. This molecule functions as a second messenger in cell division, interacting with plant development regulators (Zaliyatun *et al.,* 2015). However, research on the cultivation of *D. pulchellum* is still rare. The researcher is interested and studied about the enhancement of the development of a new plant of *D. pulchellum* with polyamine. In order to increase the number of orchids. It is also a conservation and preservation of extinction in sterile conditions.

II. MATERIALS AND METHOD

A. Plant Material

Six-month-old green pod of *D. pulchellum* was clean thoroughly under tap water. After that they were placed on a sterile petridish and were cut longitudinally with sterilised surgical blade. The yellowish seeds powdery were cultured on MS medium-free added 0.8% agar.

B. Effects of Type and Difference Concentration of Polyamines on PLBS Proliferation

Full half-plbs characteristic were studied. For half-plbs, a sterile surgical blade was used to cut them longitudinally. Full and half-plbs were cultured on MS medium added different concentrations (0, 0.2, 0.4, 0.6, 0.8 and 1 mM) of sperimidine, spermine and putrescine and added 3% sucrose.

With 0.1 N HCl (hydrochloric acid)or KOH (potassium hydroxide), The culture medium's pH was increased to 5.7. Before adding 0.75%, autoclave at 1.05 $kgcm^{-2}$ for 15 minutes at 121°C. The culture was kept at 252°C for 14 hours under fluorescent lamps with a 12.5 $mol/m^2/s$ photoperiod. Plbs induction (%), no. of plbs (plbs/explant), no. of shoots (shoots/explant), shoot induction (%), height (cm.), and no. of leaves (leaves/plant) were all measured after one month of culture.

C. Effect of Culture Media on Shoot Induction

Plbs (250 mg of fresh weight) derived from plbs proliferation were grown on different strength MS media [MS and 12 MS] supplemented with 0.8% agar or 0.2% phytagel and 3% sucrose. The culture condition was the same condition as the previous experiment. After cultured for 1 month, fresh weight (mg.) and no. of shoot (shoots/explant) were recorded.

D. Effect of Culture Media on Root Induction

Shoot explants (2-3 cm shoot length) generated from plbs proliferation were cultivated on MS media with and without 0.2 percent activated charcoal (AC), 3 percent sucrose, and 0.75 percent agar [MS and 12 MS]. The cultures were incubated at 25±2°C for 14 hours and then exposed to light. After a month of culture, the height (cm), root induction (%), number of roots (roots/plant), and root length were measured (cm.).

E. Effect of Various Types of Growing Media on Plant Growth and Development of D. pulchellum

Complete plantlets (3-4 cm. height) were successfully acclimatised to the greenhouse environmental. Plantlets potted in different potting growing media (plastic pot, spathe, charcoal smash and spathe: charcoal (1:1)). After 1 month of culture, the frequency of survival rate (%).

F. Data Analysis

A completely randomise design (CRD) was used to statistically analyze the data, and the Duncan's multiple rang test was used to distinguish the means between the treatments (DMRT).

III. RESULT AND DISCUSSION

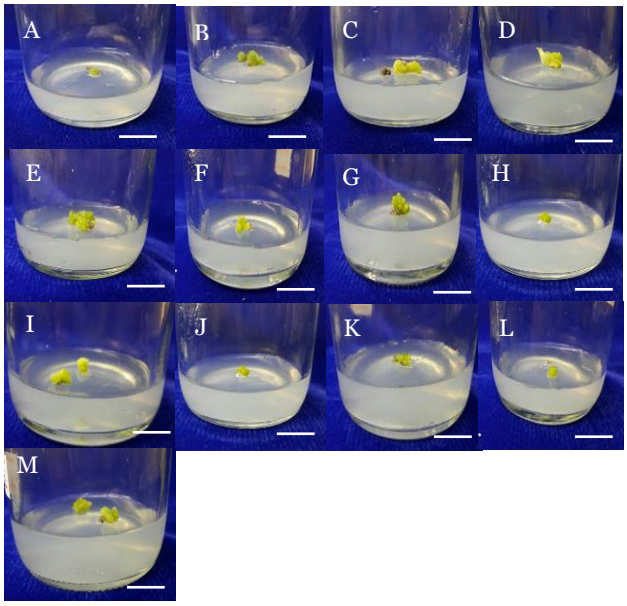
A. Effects of Type and Difference Concentration of Polyamines on PLBS Proliferation

The effects of varying polyamine concentrations on various factors were investigated.cFull-plbs and half-plbs cultured on MS medium containing different concentrations (0, 0.2, 0.4, 0.6, 0.8 and 1 mM) of sperimidine, spermine and putrescine, 3% sucrose. MS medium mixed with 1 mM spermidine was used to develop half-plbs, according to the results, gave the best plbs induction at 80% and no. of protocorm-like bodies at 4.11 plbs/explant (Table 1) The character of the protocorm is round. Green clumps together in large numbers and found that small shoots occur (Figure 1). Full-plbs were grown in MS media containing 1 mM spermidine gave the highest of shoot induction (80%), no. of shoot (1.67shoots/explant), height (1.31cm.) and no. of leaves (3.63 leaves/plant) (Table 2). The characteristics of the shoots and leaves were found, as shown in Figure 2. Polyamines in varied concentrations were applied to full-plbs and half-plbs. Concentration of spermidine is effective in increasing plbs induction, no. of plbs, shoot induction, no. of shoot, height and no. of leaves compared to other polyamines. Kumar and Palni (2013) discovered that high concentrations of spermidine and spermine in Gladiolus hybrid culture media without plant growth regulator led to cormlet development. Furthermore, Ovono *et al.* (2010) found that the presence of exogenous putrescine improved the growth of endogenous auxin and Dioscorea cayanensis–Dioscorea rotundata complex contains putrescine. Therefore, it results in an increase in the number of protocorms light bodies when plants absorb and obtain the right amount. Resulting in development and growth there is an increase in the number and also affects the induction of plants to grow. For example, spring wheat polyamine and sugar production could be stimulated and stressed by PEG treatment. (Grzesiak *et al.,* 2013). Spermine is a tetramine. Spermidine is a triamine while putrescine is a diamine. The hydrophobic interaction is caused by the methylene group. As a result, the plbs may not absorb exogenous putrescine as well as other polyamines. Putrescine may accumulate in cultured media, generating stress as a result of its toxicity at certain concentrations. (Takao & Miyakawa, 2006).

Table 1. Effect of types and difference concentrations of polyamines on proliferation of plbs obtaining from half-plbs explant for 3 months of culture

Type of polyamines	Concentrations (mM)	Protocorm-like bodies (induction)	No. of protocorm-like bodies (plbs/explant)	No. of shoot (shoot/explant)
0	0	65	2.76	0
spermidine	0.20	46.66	2.58cd[1/]	0
	0.40	61.66	3.19d	0
	0.80	68.33	3.07bc	0
	1	80	4.11a	1
spermine	0.20	43.33	3.39b	0
	0.40	53.33	3.03bc	0
	0.80	50	3.37b	0
	1	60	3.41b	0
putrescine	0.20	53.33	3.09bc	0
	0.40	61.66	3.06bc	0
	0.80	63.33	3.09bc	0
	1	68.33	3.32b	0
F-test		ns	*	ns
C.V. (%)		56.63	3.15	1.00

[1/]Different letters indicate significant difference at $p \leq 0.05$ (DMRT)
ns : non significant difference
* : significant difference at $p \leq 0.05$

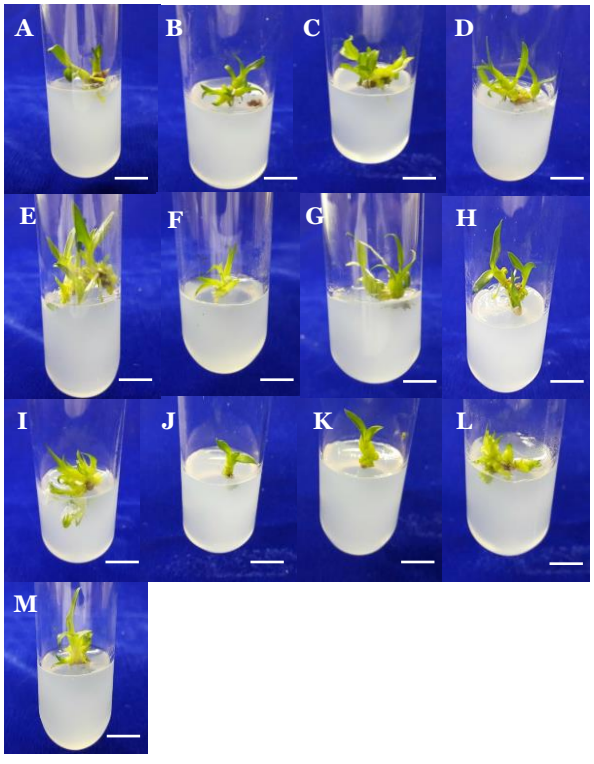


A MS-free
B 0.20 mM spermidine
C 0.40 mM spermidine
D 0.80 mM spermidine
E 1.00 mM spermidine
F 0.20 mM spermines
G 0.40 mM spermines
H 0.80 mM spermines
I 1.00 mM spermines
J 0.20 mM putrescine
K 0.40 mM putrescine
L 0.80 mM putrescine
M 1.00 mM putrescine

Figure 1. Plbs proliferation of *Dendrobium pulchellum* produced from half-plbs explants was grown for three months on MS medium with various types and concentrations of polyamines. (bar = 0.5 cm.)

Table 2. Effect of types and difference concentrations of polyamines on plbs proliferation obtaining from full-plbs explant for 3 months of culture

Type of polyamines	Concentrations (mM)	Shoot induction (%)	No. of shoot (shoot/ explant)	Height (cm.)	No. of leaves (leaves/ plant)
Control	0	61.66	1.20abc	1.07abc	3.60
Spermidine	0.20	61.66	1.20abc	1.19ab	3.56
	0.40	63.33	0.86c	1.07abc	2.80
	0.80	76.66	1.33abc	1.08abc	3.60
	1	80.00	1.67a	1.31a	3.63
spermine	0.20	53.33	1.10abc	0.81bc	2.46
	0.40	50.00	1.16abc	0.99bc	3.53
	0.80	53.33	1.33abc	1.02abc	3.10
	1	63.33	1.50ab	1.00abc	3.36
putrescine	0.20	43.33	1.03abc	0.74c	3.16
	0.40	50.00	1.43bc	0.68c	3.36
	0.80	60.00	1.30abc	0.88ab	3.26
	1	61.33	1.36abc	0.95abc	3.53
F-test		ns	*	*	*
C.V%.		58.63	1.20	1.12	3.51



A MS-free
B 0.20 mM spermidine
C 0.40 mM spermidine
D 0.80 mM spermidine
E 1.00 mM spermidine
F 0.20 mM spermines
G 0.40 mM spermines
H 0.80 mM spermines
I 1.00 mM spermines
J 0.20 mM putrescine
K 0.40 mM putrescine
L 0.80 mM putrescine
M 1.00 mM putrescine

Figure 2. Plbs proliferation of *Dendrobium pulchellum* derived from full-plbs explant were cultured on MS medium added type and difference concentration of polyamine for 3 months of culture (bar = 0.5 cm)

B. Effect of Culture Media on Shoot Induction

When different culture media were investigated of *D. pulchellum*, plbs were placed on MS medium added with 0.2% phytagel gave the highest fresh weight (351.76 mg.) and no. of shoot (22 .76 shoots/explant), significant difference with the other treatments (Table 3). Figure 3 depicts the features of shoot development after 3 months of culture. On the *D. sonai* 5 orchid, a similar effect was obtained by halving the MS media concentration, which resulted in a considerable rise in plbs production within 4 weeks (Zhang *et al.,* 1993). MS medium with a high ionic concentration of nutritional salts at full strength was reported by Zhang *et al.* (1993). Plants' physical or biochemical reactions to water stress are altered when agar is added to culture media (Ibrahim *et al.,*

2005). Hyperhydricity is a condition that occurs when a plant has an abundance of water and nutrients.(Senaratna, 1992). In addition, the gelling agent in semisolid media give matrix of spreading phenolic compound which is toxic and released by stressed plant tissue (Cervelli & Senaratna, 1995; Faria *et al.,* 2002).

Table 3. Effect of culture media on plbs proliferation of *D. pulchellum* after 1 month of culture

Culture media	Fresh weight(mg.)	No. of shoot (shoots/explant)
MS +0.8 % agar	266.07b	15.56c
½ MS +0.8 % agar	264.33b	17.53bc
MS +0.2 % phytagel	351.76a	22.76a
½ MS +0.2 % phytagel	278.40ab	27.50b
F-test	*	*
c.v%.	2.88	8.1

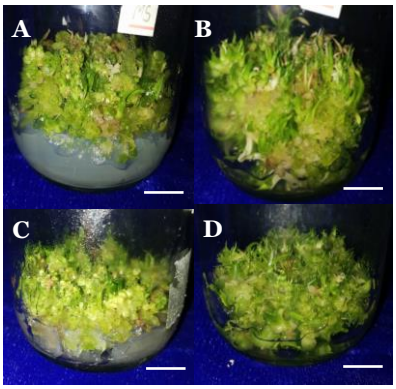


Figure 3. Characteristics of plbs proliferation and shoot induction of *D. pulchellum* were cultured on different culture media (bar = 0.5 cm.)

A. MS+0.8% agar
B. ½ MS+0.8% agar
C. MS +0.2 % phytagel
D. ½ MS +0.2 % phytagel

C. Effect of Culture Media on Root Induction

Shoot explant (2-3 cm. shoot length) were thorough cultured on MS and ½ MS added with and without 0.2% AC and 3% sucrose. The result found that MS medium supplemented with 0.2% AC gave the highest root induction (100%), no. of roots (7.13 roots/plant) and root lenght (5.07 cm.), significant difference with the other treatments (Table 4, Figure 4). *D. lindleyi* were cultivated on nutrition media stimulation supplemented with 0.2% AC provided the greatest results, similar to Satinder *et al.* (1997) [25]. *In vitro*, Cattleya walkeriana grew best with 2 g/l AC, according to Faria *et al.* (2002). AC-supplemented culture media appeared to trigger root induction more effectively than culture medium without AC. The inhibitory phenolic and carboxylic chemicals produced by cultured tissues may be able to be absorbed by AC (Ravishankar and George, 1997). Several authors have reported that AC was added to nutrient media because of its effective benefits and phenolic complex absorption (Caldas *et al.,* 1990); root stimulator (Kim and Lee, 1992); (Choi & Chung, 1989), rhizome growth (Fridborg & Erikkson, 1978); development improvement (Waes, 1987); and toxic substance absorption in media (Satinder *et al.,* 1997). Waes (1987) used in order to improve the development of cells *in vitro*, AC triggered plantlet 18 species of native European orchids are being developed. *in vitro* with 0.02% -0.03 % AC.

Table 4. Effect of culture media on root induction of *D. pulchellum* after 1 month of culture

Culture media	Height (cm.)	Root induction (%)	No. of roots (roots/plant)	Root lenght (cm.)
MS-free	4.70b[1/]	100	4.40b	4.71
MS+0.2 %activated charcoal	5.07a	100	7.13a	5.17
½ MS-free	4.89b	90	4.50a	4.95
½ MS+0.2% activated charcoal	4.30c	95	4.53a	4.58
F-test	*	ns	*	ns
C.V%.	4.56	98.95	5.61	4.61

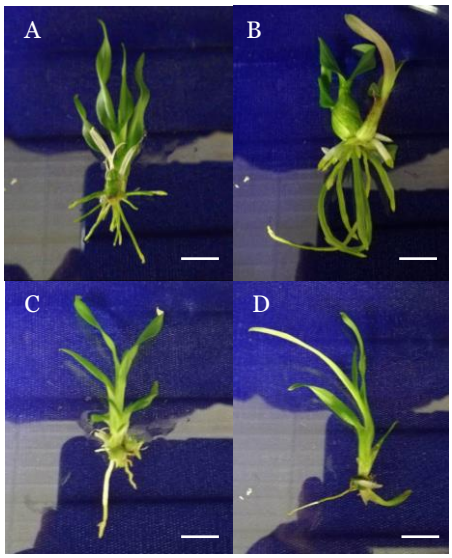


Figure 4. Characteristics of root formation of *D. pulchellum* were cultured on different culture media (bar = 0.5 cm.)

A. MS -free

B. MS+0.2 %activated charcoal

C. ½ MS-free

D. ½ MS +0.2 %activated charcoal

D. Effect of Various Types of Growing Media on Plant Growth and Development of D. pulchellum

Complete plantlets (3-4 cm. height) were successfully acclimatised to the greenhouse environmental. Plantlets potted in survival rate of 100% after 1 month of transfer. Moreover, after transferred complete plantlets to acclimatise in spathe, charcoal smash and spathe : charcoal (1:1) gave the highest survival rate at 100% while plastic pot gave survival rate at 90 for 1 month (Figure 5 and 6). This result found that complete plantlets were potted in with growing media (spathe, charcoal smash and spathe : charcoal (1:1)) gave survival rate better than plastic pot (without growing media). Therefore, the properties of the growing medium affect the plant growth. For spathe, it can show bluish points or areas (Winston, 2017). Charcoal smash is added to growing media mix to filter impurities. Charcoal can be an ideal growing medium for epiphytic and other plants that require airflow around their roots or that soak up moisture and micronutrients from the ambient air to sustain themselves.

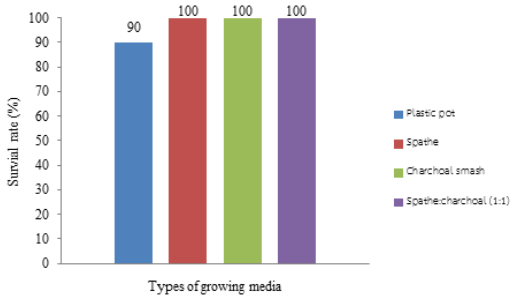


Figure 5. Percentage of survival rate of *D. pulchellum* in different growing media for 1 month

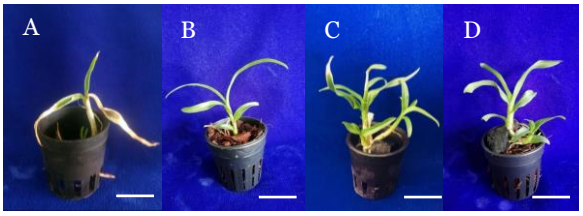


Figure 6. Complete plantlets of *D. pulchellum* to acclimatise in different growing media for 1 month

A. Plastic pot B. Spathe

C. Charcoal smash D. Spathe : charcoal (1:1)

IV. CONCLUSION

Half-plbs cultured on MS medium with 1 mM spermidine produced the highest percentage of protocorm-like bodies (80%) and number of protocorm-like bodies (4.11 plbs/explant). Shoot induction (80%), number of shoots (1.67shoots/explant), height (1.31cm), and number of leaves (3.63 leaves/plant) were all highest in full-plbs 1 mM spermidine was added to MS medium during culture. Plbs were grown in MS medium containing 0.2% phytagel gave the highest fresh weight (351.76 mg.) and no. of shoot (22.76 shoots/explant). For root formation, 0.2% activated charcoal was added to MS medium gave the highest root induction (100%), no. of roots (7.13 roots/plant) and root length (5.07 cm.), significant difference with the other treatments. Complete plantlets to acclimatise in spathe, charcoal smash and spathe : charcoal (1:1) gave the highest survival rate at 100% while plastic pot gave survival rate at 90 for 1 month.

V. ACKNOWLEDGEMENT

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