

International Conference on Food, Agriculture and Natural Resources, IC-FANRes 2015

The Application of Chitosan For Protocorm Like Bodies (PLB) Induction of Orchid (*Dendrobium sp*) In Vitro

Didik Pudji Restanto*, Boedi Santoso, Budi Kriswanto and Sigit Supardjono

Plant Tissue Culture Laboratory Faculty of Agriculture Jember University 68118 East Java Indonesia

Abstract

Indonesia has a tropical orchid species diversity is greatest in the world, but unfortunately, the conservation of orchids less attention from the government. At present the development of orchids in Indonesia is still at the level of conventional farming, while conservation is still lacking. If no one focuses on the conservation of orchids in Indonesia will become extinct due to an increase in illegal logging, forest fires and climate change will further accelerate the extinction of orchids in Indonesia. While it's still a few who are able to do the development and utilization of special orchids by tissue culture technology in particular. The best method to date in the preservation and propagation of orchids is by tissue culture, because through tissue culture are many things that can be done in comparison with conventional methods. The effect of fungal chitosan on the growth and development of seed in culture was investigated solid medium. The growth of seed explants into Protocorm Like Bodies (PLB) in solid medium was accelerated up to 4 times in the presence of fungal chitosan. The aim of the research was induction PLB with difference concentration of chitosan were 5, 10, 15, 20 and 25 ppm. In general, the best result at 15 ppm of chitosan with number of PLB, fresh weigh of PLB and number of plantlet. This concentration will be used for mass production by bioreactor system.

© 2016 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Peer-review under responsibility of the organizing committee of IC-FANRes 2015

Keywords: Dendrobium sp, Fungal Chitosan, Protocorm Like Bodies (PLB)

1. Introduction

Indonesia has tremendous biodiversity, one of them is the orchid. An estimated 5000 species have been grown in the tropical forests. This potential is extremely valuable to developers and orchid lovers in Indonesia, especially

* Corresponding author. Tel.: +62-274-589797
E-mail address: restanto.lmlit@unej.ac.id

the genetic potential to produce of orchid hybrids that have high commercial value. This potential will be meaningless if deforestation and over-exploitation of the forests occur.

In its development, the production of ornamental plants orchids that are experiencing significant growth. The countries that have advanced technology like in vitro biotechnology will dominate world exports of orchid plants. These countries are the Netherlands (33%), Japan (24%), Italy (11%), Thailand (10%) and other countries (14%). Reflecting on these countries, it is not wrong if the development of ornamental plants, Indonesia should develop a modern cultivation techniques (in-vitro) that can reduce the national import value of orchids.

Dendrobium is a type of ornamental orchids are very important and difficult to develop vegetatively. In generally, performed propagation in vitro by seed, but the problem do no produce a uniform crop and segregation occurs on flower color (Huan et al., 1978 in Fanfani and Rossi, 1989). The most possible is through the induction of Protocorm Like Bodies (PLB). According to Young et al. (2000), using 20 g PLB in biorektor for 8 weeks is able to produce 18,000 PLB identic with 18,000 uniform orchid seedling. The late references, it has been developed mass propagation of orchids through Somatic Embryogenesis (SE) induced from flower (Tokuhara *et al.*, 2003), stem, leaf and uxillary bud (Chen et al., 2008). Results of somatic embryogenesis has been reported to be the uniform, same with parent and good quality.

According to the aspect of the availability of genetic resources, human resources, soil and favorable climate, Indonesia has a huge potential for development of orchid plants. In addition to Indonesian people that plants are not only used for consumption purposes but also for purposes of aesthetic and religious or traditional ceremonies. From the existing problems the orchid researchers are trying to use biotechnological approaches through in-vitro techniques especially PLB formation

2. Methodology

2.1. Explants

The explants from *Dendrobium* orchid red flowers was crossed to white flowers which obtained from Orchid Association of Indonesia (P2AI) Jember branch (Arinta Jember). The sterilization used 96% alcohol and burned for 10 second.

2.2. Culture medium for orchids

The culture medium has been used a VW medium for orchids contained 20 g / l sugar, 150 ml / l coconut water, 0.525 g / l KNO₃, 0.25 g / l KH₂PO₄, 0.5 g / l (NH₄)₂SO₄, 0.007 g / l MnSO₄·4H₂O, 0.025 g / l MgSO₄·7H₂O, 0.028 g / l FeSO₄·7H₂O and 0.037 g / l na₂-EDTA. Growth regulators used auxin 10 mg / l, cytokines 5 mg / l and 2.4 D 2 mg / l.

2.3. Chitosan concentration

The used chitosan concentrations were 0 (as a control), 5, 10, 15, 20 and 25 ppm.

3. Results

3.1. PLB for explants

Tissue culture methods was developed to assist the micro propagation of orchid. In general, the orchid propagation through seeds, but these methods were not obtained equal result and there are segregation occurs at flower color. In this study, *Dendrobium* sp orchid as the explants resulted crossing of red and white flowers (Fig. 1).

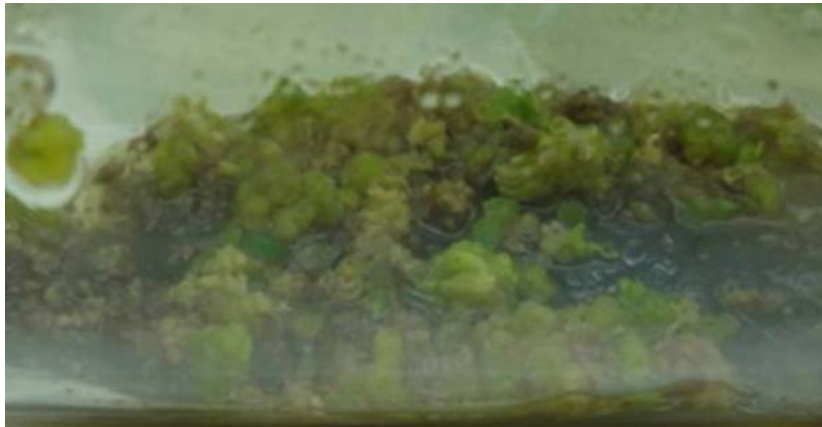


Figure1. PLB of *Dendrobium sp* (4 weeks) from crossed red flower with flower.

The PLB was subcultured on Vacint and Went (VW) liquid medium in 15% coconut water (CW). It was contained of cytokinin, auxin and gibberellin hormones as well as other compounds in order to stimulate the germination and growth of PLB *Dendrobium* orchid (Katuuk, 2000). The PLB have been subcultured in liquid medium for 3 weeks culture (Fig. 2)



Figure2. PLB after 3 weeks in liquid medium (VW).

In the illustration of Figure 2, shows the mass of PLB greater after incubating for 3 weeks in the solid medium. PLB formed in the same size are used for explants.

3.2. PLB formation

The PLB induction has been presence during two up three weeks in each treatment. Throughout treatment the chitosan concentration maintained on 15 mg/l was considered better results than other treatments. This finding proved that the growth rate of PLB were increased 2-times compared the control at 3, 6 and 9 weeks. Thus it followed by 10 and 20 ppm. Meanwhile the establishment of plantlets was occurred at 6 week after planting. Figure 3 illustrates the regeneration of PLB.

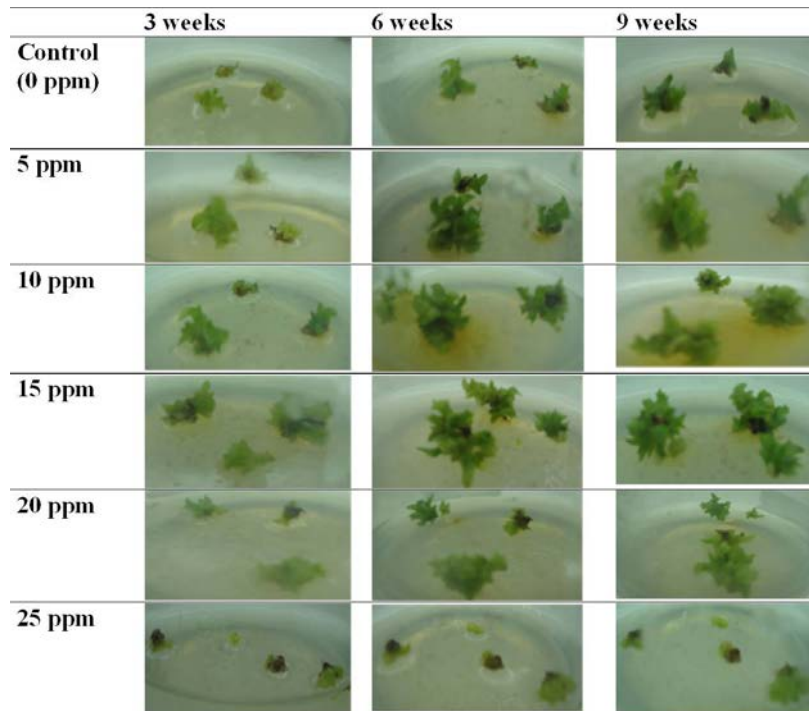


Figure 3 The effect of chitosan concentration (0, 5, 10, 15, 20 and 25 ppm) for inducing PLB and plantlet for 3, 6 and 9 weeks.

3.3. Fresh weight of PLB

The statistical analysis was revealed the fresh weight of PLB having significantly differences in chitosan treatment. Further, in the 15 ppm chitosan concentration has identified as the higher fresh weight of PLB at 3, 6 and 9 weeks were 0.53g; 0.75g, and 0.83g, respectively compared other treatments (Table 1).

Table 1. Fresh weight of PLB (g) of *Dendrobium* sp at 3, 6 dan 9 weeks

Chitosan (ppm)	3 weeks	6 weeks	9 weeks
0	0.16 a	0.20 a	0.27 a
5	0.33 b	0.39 a	0.42 ab
10	0.43 b	0.66 b	0.70 b
15	0.53 b	0.75 b	0.83 b
20	0.36 b	0.45 b	0.50 b
25	0.09 a	0.13 a	0.20 a

Note :the same word in the same row indicates non significant.

3.4. Number of PLB and Planlet

Based on the statistical analysis illustration, the number of PLB and plantlet indicate the chitosan was significant (Table 2). The chitosan application on 15 ppm was considered having better effect on the number of PLB and Plantlet compared to other treatments (0, 5, 10, 20 and 25 ppm).

Table 2. The number of PLB at chitosan concentration after 3, 6 dan 9 weeks

Chitosan (ppm)	3 weeks	6 weeks	9 weeks
0	2.66 a	4.00 a	6.00 a
5	2.33 a	4.66 a	8.00 ab
10	3.00 a	9.66 bc	10.33 b
15	3.33 a	11.00 bc	12.33 b
20	2.66 a	6.00 b	10.00 bc
25	1.00 b	1.33 d	3.00 c

Note : the same word in the same row indicates non significant.

The growth rate of PLB on solid media has gradually increased in each treatment observation throughout 0-25 ppm. It revealed the number of PLB has slightly developed in every week. Specifically, the treatment on 15 ppm indicated fastest growth rate of PLB and the number of plantlets compared with others. Furthermore, the growth rate and the number of plantlets PLB increased 2-times as seen as 6 weeks to 9 weeks observation with 12,33 number of PLB.

Thus Table 3 below indicated the concentration 15 ppm level, it is the highest number of plantlets. Though there are no differences between 10 and 20 ppm.

Table 3.The number of plantlet with chitosan concentration after 6, 7, 8 and 9 weeks.

Chitosan (ppm)	6 weeks	7 weeks	8 weeks	9 weeks
0	1.66 a	2.33 a	3.00 a	3.66 a
5	2.66 b	2.66 a	3.33 a	4.00 a
10	2.66 b	3.33 b	5.33 b	5.66 b
15	2.66 b	4.66 b	6.00 b	6.66 b
20	2.66 b	3.00 b	5.33 b	5.66 b
25	0.33 c	0.33 c	0.33 c	0.33 c

Note : the same word in the same row indicates non significant

Finally, in the 25 ppm observation illustrates the lowest number of plantlets which occurred at 6 week up to 9 weeks compared with other treatments.

4. Discussion

PLB was used as a source of explants on VW medium with the addition of 15% coconut water with different chitosan concentration (Fig. 3). According to Tuzlakogluet et al. (2004), the chitosan has a strong hydrophilic properties, because it can absorb water molecules surround. Therefore, a chitosan hydrogel functioned either as a barrier mass transfer (water vapor, O₂ and CO₂) or food additives carriers, such as antimicrobial agents and antioxidants.

In the PLB formation, the application of chitosan 25 ppm did not efficiently, due to the growth stagnancy. Hence, the PLB does not develop like fresh weight and number of PLB per weeks (Fig. 3). Chang and Chang (2000), stated that accumulated chitosan in a high concentration can inhibit cell division.

Chitosan is a derivative of chitin organic compounds, biomaterials in which currently widely used for various purposes, such as cleaning and purifying water, immobile bacterial cell enzymes, and food preservatives. According to Clandrkrachang (2002), the chitosan would increase the growth of orchids, flower production and increased resistance to fungi and viruses. Hadwiger et al. (2002), states that the mature orchid plants meristematic buds

initially formed of PLB when developed in VW solid media. Meristematic tissue growth depends on the size of the inoculums and the growth conditions. PLB was grown in solid medium and added with 15 ppm chitosan. Additionally, after three weeks, the shoot meristem *Dendrobium* sp may develop the increasing mass of plant cells 3 times after 6 weeks of development (Clandrkrachang, 2002).

A function of chitosan is to induce plants in order to enhance the biosynthesis of lignin and lignifications of plant cell walls so that it becomes more powerful and inhibit penetration of fungus pests (Reddy et al., 2005). Chitosan causes disorganization (messed) cells rapidly fungi, such as increased vacuole, cell wall thickening, distortion hyphae and cytoplasmic aggregation (Laflamme et al., 1999). Besides greatly affect the formation of PLB (Katuuk, 2000).

On the other hand, media is probably decided as a factor in propagation by tissue culture. The composition of the media used depends on the type of plant to be propagated, especially *Dendrobium* sp. Media that used solid media (VW) with the addition of different chitosan concentration, may enhance the wet weight and the number of PLB produced. In this method, the effect of chitosan as a treatment is measured in the range of 0-25 ppm. After six weeks of observation, treatment of 15 ppm chitosan appeared to be most optimal result in the growth and development of orchid as mentioned in the number of PLB and fresh weight of PLB (Table 1,2).

Chitosan can affect the growth of orchids, especially for plants in tissue culture proved by the increased fresh weight and the number of PLB *Dendrobium* sp. The increased fresh weight and the number of PLB influenced by chitosan in which may lead the signal to synthesize plant hormones such as gibberellins (Chandrkrachang, 2002). On the other hand, chitosan is a good material used as a growth stimulator solid media (Chandrkrachang, 2006)

Revealed on this study, chitosan is known in order to increase fresh weight of PLB on 15 ppm concentration, due to increased protoplasm, thus the increases of cells size and number. Further, protoplasm has growth due to the photosynthesis process (Noggle and Fritz, 1980). The food reserves are used in the plant metabolic processes that produce energy for plant growth. An increasing number of PLB and plantlet of *Dendrobium* sp, has unique characteristics not only to stimulate the plant growth but also induces the differentiation of plant tissues especially orchid *Dendrobium* sp (Chandrkrachang, 2006).

Furthermore, Ngeet et al. (2006), states that the effect of chitosan of *Dendrobium* sp can accelerate the growth and development of meristematic tissue of orchids. It proved that this study revealed in 15 ppm chitosan was identified as the highest number of PLB and plantlets than the 10 and 20 ppm after six weeks of observation. The ability of chitosan in the stimulation of orchid tissue It may improve and enable the cells to increase the ability of plants to further differentiation resulting in the number of plantlets (Park et al., 2002)

Finally, the study results showed that using of chitosan at 15 ppm had identified as the best result in detailed, it also show 12,33 number of PLB, 0,83 g fresh weight of PLB and 6.66 number of plantlets.

Acknowledgment

We would like to thank to DP2M-Ministry of Higher Education as funding source for 2014 and Orchid Association of Indonesia (P2AI) Jember branch (Arinta Jember).

References

- Chandrkrachang, S., 2002. The applications of chitin and chitosan in agriculture in Thailand. *Advances in Chitin Science* 5, 458-462.
- Chandrkrachang, S., 2006. Chitosan as a growth stimulator in orchid tissue culture, in agriculture in Thailand, *Plant Science* 170, 1185-1190.
- Chang C., Chang W.C., 2000. Effect of thidiazuron on bud development of *Cymbidium sinense* Willd in vitro. *Plant Growth Regulation* 30, 171-175.
- Chen, Y.L., 2008. Preparation and characterization of Water-soluble chitosan gel for skin Hydration. in Thesis submitted in fulfillment of the requirements for the degree of Master of Science.
- Hadwiger, S.J. Klosterman, J.J., Choi., 2002. The mode of action of chitosan and its oligomers in inducing plant promoters and developing disease resistance in plants, in: K. Suchiva, S. Chandrkrachang, P. Methacanon, M.G. Peter (Eds.), *Advances in Chitin Science* 5, 452-457
- Katuuk, J.R.P., 2000. Aplikasi Mikropropagasi Angrek Macan (*Grammatohyllum scriptum*) dengan Menggunakan Air Kelapa. *Jurnal Penelitian IKIP Manado*. 1a(iv), 290-298.
- Laflamme. S.G., Morel, H., 1999. Clona l multiplication of orchid, *The orchid; scientific studies*. Jhon Wiley and Sons. New York.

- Martin, K.P., Madassery, J., 2006. Rapid in vitro propagation of Dendrobium hybrids through direct shoot formation from foliar explants, and protocorm like bodies. *Sci Hort* 108, 95-99
- Nge, K.L., Nwe, N., Chandkrachang, S., Stevens, W.F., 2006. Chitosan as growth stimulator in orchid tissue culture. *Plant Science Journal* 170(6), 1185-1190.
- Noggle, G.R., Fritz, G.J., 1980. *Introduction Plant Physiology*. Prentice-Hall of India Private Ltd. New Delhi.
- Park, S.Y., Yeung, E.C., Chakrabarty, D., Paek, K.Y., 2002. An efficient direct induction of protocorm like bodies from leaf subepidermal cells of *Doritaenopsis* hybrid using thin-section culture. *Plant Cell Report* 21, 46-51
- Reddy, B., Malabadi, G.S., Mulgund, N., Kallappa. 2005. Micropropagation of *Dendrobium nobile* from shoot tip sections, *J. Plant Physiol.* 162, 473-478.
- Tokuhara, K., Mii, M., 2003. Highly-efficient Somatic Embryogenesis From Cell Suspension Cultures of *Phalaenopsis* Orchids By Adjusting Carbohydrate Sources. *In vitro cell Dev Biol Plant.* 39, 635-639.
- Zhang, D., Quantrick, P.C., 1997. Effect of Chitosan Coating on Enzymatic Browning and Decay During Postharvest Storage of Litchi (*Litchi chinensis*) fruit. *Postharvest Biol. Technol.* 12, 195-202.