Conference Paper

Somatic Embryo from Basal Leaf Segments of *Vanda tricolor* Lindl. var. pallida

Popy Hartatie Hardjo and Wina Dian Savitri

Faculty of Biotechnology, University of Surabaya, Kalirungkut Rd.,Surabaya 60292, Indonesia

Abstract

Somatic embryogenesis is one of techniques in plant micropropagation. The induction of somatic embryogenesis through callus phase was done on *Vanda tricolor* Lindl. var. pallida. This study aimed to find out the effect of naphtalene acetic acid (NAA) and benzyl amino purine (BAP) in inducing somatic embryogenesis via callus on the basal leaf segments of *Vanda tricolor* Lindl. var. pallida. The half-strength of Murashige and Skoog (· MS) medium with 1 % sucrose, incorporated with (0.02 mg · L^{-1} and 0.05 mg · L^{-1}) NAA and also 0.01 mg · L^{-1} BAP were used in this experiment. The best medium for embryogenic callus formation and proliferation was 0.05 mg · L^{-1} NAA in combination with 0.01 mg · L^{-1} BAP. The formation of somatic embryos occurred 30 d after the calluses were cultured on to ½ MS without the addition of plant growth regulator and subsequently formed shoots.

Keywords: basal leaf segments; somatic embryogenesis; *Vanda tricolor* Lindl. var. pallida.

Corresponding Author:
Popy Hartatie Hardjo
poppy_hardjo@staff.ubaya.ac.id

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1. Introduction

Vanda is one of orchid genera that consist of about 50 species. This genus is popular because its flower has an attractive color, big size and in particular species, it has a fragrant aroma. Until today, the reproduction of orchid is carried out by using semi-conventional method, where the orchid is crossed with itself or with the other plant but still is the same species. After that, the resulting seeds are usually cultured on the culture media and then grown until they reach the ideal condition for acclimatization. This procedure cause the offsprings are different from the parental. In addition, the time needed to produce the offsprings is too long.

One method for rapid propagations of orchid is the production of protocorm-like bodies (PLBs). The success of PLBs production is depend on the explants type, plant genotype, and plant growth regulator in the culture medium. Somatic embryos on orchid have been proven to have the same structure as protocorm, so that they are called protocorm-like bodies [1].

The process in which the somatic cells develop into embryos without performing gamete fusion is called somatic embryogenesis [2]. Somatic embryo not only can

be formed directly from cell and tissue or organ, but also through indirect pathway from callus. The stages in the indirect somatic embryogenesis are the induction of embryogenic callus, the embryo maturation, and the germination phase in order to produce a whole plant. Every step in the somatic embryogenesis needs the help of auxin and cytokinin in different combination ratio. Auxin has important role in somatic embryogenesis for induction and proliferation of embryogenic callus [3]. Rianawati et. al. [4] reported that callus induction from leaf segments of *Phalaenopsis* sp. developed on ½ MS medium incorporated with 10 mg · L^{-1} 2,4-Dichlorophenoxyacetic acid (2,4-D) and 0.1 mg · L^{-1} Thidiazuron (TDZ).

Study of somatic embryogenesis, especially on *Vanda tricolor* Lindl. var. pallida, has not been done before. Yet it has been done on another variety, e.g. Dwiyani et. al. [5] investigated callus induction on *Vanda tricolor* Lindl. var. suavis. The attempt to multiply *Vanda tricolor* Lindl. var. pallida via callus phase to form somatic embryo is needed to support the development of transgenic orchids and the conservation of rare orchids spesies. The objective of this experiment was to discover the effect of NAA and BAP in inducing somatic embryogenesis via indirect pathway on the basal leaf segments of *Vanda tricolor* Lindl. var. pallida.

2. Materials and methods

2.1. Materials

Explants source was the plantlet of *Vanda tricolor* Lindl. var. pallida collected from Handoyo Budi Orchid, Malang, East Java Province, Indonesia. The explants used were basal leaf segments. Murashige-Skoog (MS) (Phytotech) was used as the culture medium. NAA and BAP were the plant growth regulators applied as treatments.

2.2. Methods

This experiment was conducted with completely randomized design by using twenty explants per treatment. The treatments were (i) 0.02 mg · L^{-1} NAA; (ii) 0.05 mg · L^{-1} NAA; (iii) 0.02 mg · L^{-1} NAA + 0.01 mg · L^{-1} BAP; (iv) 0.05 mg · L^{-1} NAA + 0.01 mg · L^{-1} BAP; and (v) without NAA and BAP.

The basal leaf part was excised and cultured aseptically inside the culture bottle contained ½ MS medium with 1 % sucrose [6, 7], incorporated with the plant growth regulator(s) according to each treatment. The cultures were incubated in an incubator room at temperature of (24 ± 1) °C with 3 000 lux light intensity (1 lx = 1 lm · m^{-2}) . The subculture was performed in every 4 wk into the same treatment media. The resulting

Treatment	Starting date of callus initiation (average)[+)]	No. of explant formed callus (%)	No. of explant formed embryogenic callus (%)
Without plant growth regulator	-	-	-
0.02 mg · L^{-1} NAA	120 ± 5.7^a	5(1/20)	-
0.05 mg · L^{-1} NAA	75 ± 3.9^c	35(7/20)	29(2/7)
0.02 mg · L^{-1} NAA + 0.01 mg · L^{-1} BAP	90 ± 8.6^b	15(3/20)	67(2/3)
0.05 mg · L^{-1} NAA + 0.01 mg · L^{-1} BAP	60 ± 7.5^c	45(9/20)	77(7/9)
[+)]Mean ± SD values followed by different letter in the same column are significantly different based on DMRT test (p = 0.05)			

Table 1: The starting date of callus initiation, the percentage of explant formed callus, and the percentage of explant formed embryogenic callus on *Vanda tricolor* Lindl. var. pallida.

calluses were sub cultured into ½ MS with 1 % sucrose but without the addition of plant growth regulator in order to induce somatic embryo.

Observation was conducted in every week, including the starting date of callus initiation, the percentage of explant formed callus, the percentage of explant formed embryogenic callus, and the callus morphology. The data of 'the starting date of callus initiation' was analyzed statistically by ANOVA, followed by Duncan's Multiple Range Tests (DMRT) at α = 0.05.

3. Results and discussion

3.1. Initiation and proliferation of embryogenic callus

Initially, there is a swelling on the basal part of the leaf explant (Fig. 1A) that has occurred at 60 d after culture on the ½ MS medium contained plant growth regulator, while on the control (without the addition of plant growth regulator), the explants show necrosis sign and subsequently dies. The callus formation (Fig. 1B) occurs at 60 d on the explants treated by 0.05 mg · L^{-1} NAA + 0.01 mg ½ L^{-1} BAP and at 75 d on the explants treated by 0.05 mg · L^{-1} NAA. The both treatments do not differ significantly, but faster than the explants treated with 0.02 mg · L^{-1} NAA and the treatment with the addition of 0.02 mg · L^{-1} NAA + 0.01 mg · L^{-1} BAP (Table 1). According to Arditti [8], the meristematic area on the orchid leaf was limited on the epidermal cell from the basal part of the leaf. That is the reason, the swelling response on the explant continued with callus formation only occurs on the basal part of the leaf explants.

In the process of callus proliferation, the callus surface was starting to form a shiny green spheres at 90 d (Fig. 1C). In this experiment, 0.05 mg · L^{-1} NAA combined with 0.01 mg · L^{-1} BAP is capable to induce embryogenic callus, whereas 0.02 mg · L^{-1}

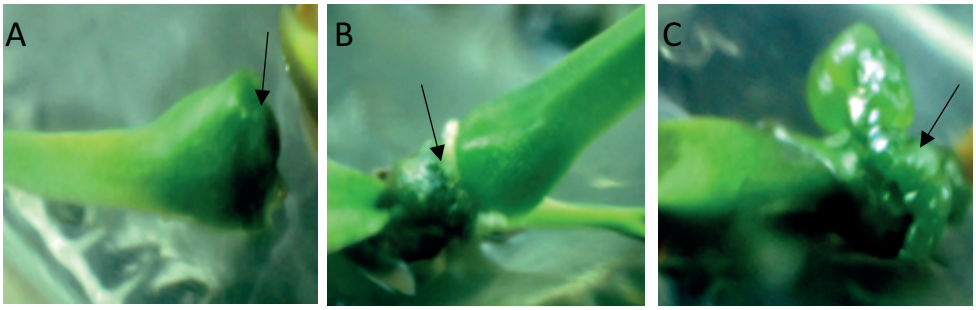

Figure 1: Early development of callus initiation on inner part of basal leaf segments A. The basal leaf explant is swelling; B. Callus initiation on basal leaf explant; C. Callus proliferation.

Treatment	Callus morphology
Without plant growth regulator	No callus is formed, the explants tissue is died
0.02 mg · L^{-1} NAA	Yellowish callus, no nodule is formed on the explants surface
0.05 mg · L^{-1} NAA	Yellowish callus, only a small part of the explants surface forms nodule
0.02 mg · L^{-1} NAA + 0.01 mg · L^{-1} BAP	Green callus, only a small part of the explants surface forms nodule, shiny
0.05 mg · L^{-1} NAA + 0.01 mg · L^{-1} BAP	Green callus, most of the explants surface forms nodule, shiny

Table 2: Callus morphology on basal leaf segments of *Vanda tricolor* Lindl. var. pallida at 120 d.

NAA alone is not effective to produce the embryogenic callus. Some researchers also reported that callus induction and proliferation of some orchid species, *e.g. Cymbidium* (Swartz.) [9], *Phalaenopsis* (Blume.) [10], *Dendrobium* (Swartz.) [11], and *Coelogyne cristata* (Lindl.) [12] occurred on the medium that contained both auxin and cytokinin, where the auxin concentration was higher than the cytokinin. The optimum concentration of NAA in this experiment is 0.05 mg · L^{-1}, supposing that it is lower than the previous study on somatic embryogenesis in *Vanda* [5, 13].

The highest percentage of callus formation (45 %) and of embryogenic callus (77 %) are taken place on the explants treated with 0.05 mg · L^{-1} NAA + 0.01 mg · L^{-1} BAP (Table 1). Visually, embryogenic callus is indicated by globular calluses on explant surface that form shiny green nodules (Table 2 and Fig. 2A). In particular species, somatic embryogenesis occurs because of the addition of cytokinin in combination with auxin in higher concentration, so that the proliferation of embryogenic cells happens. Cytokinin is an important constituent in cell proliferation

3.2. Somatic embryo formation

The nodular callus (Fig. 2A) that has sub cultured into ½ MS medium without the adding of plant growth regulator, develops into an embryo somatic-like structure or generally termed protocorm-like bodies (PLBs), just like described on Fig. 2B, 30 d after the initial

Figure 2: Callus development on basal leaf segments of *Vanda tricolor* Lindl. var. pallida to form somatic embryos and subsequently shoots A.Embryogenic calluses; B. Calluses are forming globular somatic embryos on ½ MS medium; C. Somatic embryos development to form shoots.

culture. This result is in line with the study conducted by Huan et al. [8] on Cymbidium, explaining that the PLBs formation from callus occurred on basal medium without plant growth regulator. In accordance with Mayer et al. [14], the adding of auxin could inhibit the PLBs formation on *Oncidium flexuosum* (Sims.), while the lower concentration of TDZ (1.5 μM) could increase the PLBs formation.

3.3. Plant regeneration

Somatic embryos produced from calluses of *Vanda tricolor* Lindl. var. pallida develop into shoots on ½ MS medium without plant growth regulator (Fig. 2C) 60 d after the initial culture. The same result has been reported by Chen and Chan [15] in *Phalaenopsis amabilis* [(L.) Blume]. On the contrary, Chen et al. [16] stated that PLBs from flower petiole explant of *Epidendrum radicans* (Lindl.) formed plantlets on MS medium contained 0.45 μM TDZ. In this study, the somatic embryos of *Vanda tricolor* (Lindl.) var. pallida are capable to form shoots on ½ MS medium without the help of plant growth regulator. This finding is assumed to happen because the embryogenic callus is derived from explants cultured on ½ MS medium incorporated with 0.05 mg · L^{-1} NAA and 0.01 mg · L^{-1} BAP, so that the effect of BAP is still exist on the embryogenic callus.

4. Conclusion

Embryogenic callus induction and proliferation from the basal leaf segments of *Vanda tricolor* Lindl. var. pallida occur on ½ MS medium enriched with 1 % sucrose that also contain 0.05 mg · L^{-1} NAA in combination with 0.01 mg · L^{-1} BAP. Embryogenic calluses then form somatic embryos or PLBs on the same culture medium composition but without plant growth regulator, followed by shoot formation.

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References

[1] Lee YI, Hsu ST, Yeung EC. Orchid protocorm-like bodies are somatic embryos. American Journal of Botany 2013;100(11):2121–2131.

[2] Jimenez VM. Involvement of plant hormones and plant growth regulators on in vitro somatic embryogenesis. Plant Growth Regulation 2005;47(2):91–110.

[3] Mei TA, Danial M, Mahmood M, Subramaniam S. Exquisite protocol of callus induction and protocorm-like bodies (PLBs) regeneration of *Dendrobium* Sonia-28. Australian Journal of Crop Science 2012;6(5):793–800.

[4] Rianawati S, Purwito A, Marwoto B, Kurniati R, Suryanah. Embriogenesis somatik dari eksplan daun anggrek *Phalaenopsis* sp. L. [Somatic embryogenesis from leaf eksplant of *Phalaenopsis* Orchids]. Jurnal Agronomi Indonesia 2009;37(3):240–248. [in Bahasa Indonesia]

[5] Dwiyani, RH. Yuswanti, Darmawati IAP. Induksi kalus pada tanaman anggrek *Vanda tricolor* Lindl. Varietas suavis, upaya penyediaan target transformasi melalui *Agrobacterium tumefaciens* [Callus induction on *Vanda tricolor* Lindl. orchid plant var. suavis as the attempt to provide transformation target via *Agrobacterium tumefaciens*] Bionatura 2013:15(2):114–116. [in Bahasa Indonesia].

[6] Teixeira da Silva JA. New basal media for protocorm-like body and callus induction of hybrid *Cymbidium*. Journal of Fruit and Ornamental Plant Research 2012:20(2):127–133.

[7] Jawan R, Joeplik GJ, Indan H, George MM, Gansau JA. Effect of basal media and carbon sources on callus culture maintenance of *Vanda dearei*. Borneo Science 2014:34:20–26.

[8] Arditti J. History of orchid propagation, plenary presentation. Asia-Pacific Journal of Molecular Biology and Biotechnology 2010:18(1) supplement:171–174.

[9] Huan LVT, Takamura T, Tanaka M. Callus formation and plant regeneration from callus through somatic embryo structures in *Cymbidium* orchid. Plant Science 2004:166:1443–1449.

[10] Tokuhara K, Masahiro MII. Induction of embryogenic callus and cell suspension culture from shoot tips excised from flower stalk buds of *Phalaenopsis* (Orchidaceae). In Vitro Cellular and Developmental Biology - Plant 2001:37(4):457–461.

[11] Juntada K, Taboonmee S, Meetum P. Somatic embryogenesis induction from protocorm-like bodies and leaf segment of *Dendrobium* Sonia 'Earsakul'. Silpakorn University Science and Technology Journal 2015:9(2):9–19.

[12] Naing AH, Chung JD, Lim KB. Plant regeneration through indirect somatic embryogenesis in *Coelogyne cristata* orchid. American Journal of Plant Sciences 2011:2(2):262–267.

[13] Chong ST, Ching QW, Xui LL, M. Maziah. A preliminary study of protocorm-like bodies (PLBs) induction using leaf explants of *Vanda* and *Dendrobium* orchids. Asia Pasific Journal of Molecular Biology and Biotechnology 2010:18(1):189–191.

[14] Mayer JLS, Stancato GC, Da-Gloria BA. Direct regeneration of protocorm-like bodies (PLBs) from leaf apices of *Oncidium flexuosum* Sims (Orchidaceae). Plant Cell, Tissue and Organ Culture 2010:103(3):411–416.

[15] Chen JT, Chan WC. Direct somatic embryogenesis and plant regeneration from leaf explants of *Phalaenopsis amabilis*. Biologia Plantarum 2006:50(2):169–173.

[16] Chen LR, Chen JT, Chang WT. Efficient production of protocom-like bodies and plant regeneration from flower stalk explants of the sympodial orchid *Epidendrum radicans*. In Vitro Cellular and Developmental Biology - Plant 2002:38(5):441–445.

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By: Hardjo, PH (Hardjo, Popy Hartatie)[1]; Savitri, WD (Savitri, Wina Dian)[1]

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Keywords

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Author Information

Reprint Address: Hardjo, PH (reprint author)

Univ Surabaya, Fac Biotechnol, Kalirungkut Rd, Surabaya 60292, Indonesia.

Addresses:

[1] Univ Surabaya, Fac Biotechnol, Kalirungkut Rd, Surabaya 60292, Indonesia

E-mail Addresses: poppy_hardjo@staff.ubaya.ac.id

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