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In Vitro Regeneration of Dendrobium Through Somatic Embryogenesis from Leaf Explants

Rahmadyah Hamiranti[1*], Nanang Wahyu Prajaka[1], Yeni[1], Desi Maulida[1], Lisa Erfa[1]

[1]*Department of Food Plant Cultivation Lampung State Polytechnic, Jln Soekarno Hatta Rajabasa Bandar Lampung. Indonesia*

[*]*Corresponding Author: rahmahamiranti@polinela.ac.id*

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Abstract

The purpose of this study was to determine the best basic medium type and TDZ concentration for the development of somatic embryos from Dendrobium orchid leaf explants. Three replications were arranged factorial (2x3) in a completely randomized design for this study. First, there were two types of basic media, ½ MS and MS. The second factor was the concentration of cytokinin thidiazuron 1, 2 and 3 mg/l. Each experimental unit consisted of 5 culture bottles; each contained 5 explants. The research results showed that 1) The use of both types of basic media was able to induce callus formation on Dendrobium 'Gradita 31' orchid from leaf explants. 2) The use of 3 mg/l thidiazuron which combined with ½ MS or MS media was able to form primary callus faster than other treatments. 3) The higher percentage for embryo somatic and shoots formation were also found in 3 mg/l thidiazuron.

Keywords: Dendrobium, embryogenesis, thidiazuron

1 Introduction

Dendrobium orchids have high economic value by contribute for 34% of the orchid business, followed by Phalaenopsis, Vanda and other genera [1]. The high market, due to interest and selling price for Dendrobium seedlings is the reason for increasing productivity in the availability of true to type seedling with a large number. Propagation of Dendrobium orchids can be done with generatively (through seeds) or vegetatively. Orchid seeds cannot be grown directly on planting media in the field because orchid seeds do not have endosperm.

The process of fruit formation naturally rare occurs. Moreover, the formation of Dendrobium orchid fruit takes quite a long time, around 3-4 months until the seeds are ready to be sown as in vitro culture [2]. Vegetative propagation through seedlings cannot be used as a mass propagation method because the planting material produced is very limited (2-4 seedlings per year). Meanwhile, keiki (seedling at the tip of the bulb) will only appear when mature Dendrobium orchids undergo stress condition [3].

Numerous studies have been conducted on orchid somatic embryogenesis in vitro. Hapsoro and Yusnita [4] stated that somatic embryogenesis is defined as the process of creating an embryo (a plant structure that already has root and shoot formation) from nonzygotic parts of the plant body (leaves, roots, stems, hypocotyl, etc.). The composition of the basic media is an important factor in the somatic embryogenesis of orchid plants. MS media is most widely used in the regeneration process through somatic embryogenesis in several types of orchids such as *Dendrobium*, *Spathoglottis*, *Cattleya*, *Rhynchostylis* and *Grammatophyllum* [5]–[11]. Several research results [12]–[15] showed that the use of half strength of MS media was able to supply sufficient nutrients to encourage the development of somatic embryos in *Dendrobium* and *Phalaenopsis*.

The ability of plant regeneration to respond on nutrients and growth regulators is species-specific, meaning that plants from different genotypes will respond differently in their plant regeneration patterns even though they have been given the same treatment[16] . In vitro plant regeneration is regulated by the balance and interaction of plant growth regulators (PGR) contained in the explant (endogenous) and with the PGR absorbed from the growth medium (exogenous). In certain proportions, the use of PGR can stimulate embryo formation [17]. Therefore, it is necessary to carry out a research regarding regeneration procedures through somatic embryogenesis which appropriate to the genotype of Dendrobium orchid plants. This research aimed to obtain the best type of basic media and concentration of TDZ for the formation of somatic embryos from Dendrobium orchid leaf explants.

2 Material and Methods

This research was carried out at the Tissue Culture Laboratory, Department of Food Plant Cultivation, Lampung State Polytechnic, Rajabasa, Bandar Lampung, from April to September 2023. The plant material was seedlings of Dendrobium 'Gradita 31' that obtained from Balai Penelitian Tanaman Hias. The explant was leaf segments with a size of ± 1 cm^2. Explant was planted as adaxial faces on medium (the top surface of the leaf touched the medium). Planting was carried out in LAFC under aseptic conditions. Explants were cultured in a culture room at a temperature of 25ºC ± 2ºC with a subculture interval of 4 weeks on the same medium.

The media were MS [18]and half strength of MS (½ MS). ½ MS was modified from MS into half concentration of macro elements. These two basic media were enriched with thiamine-HCL (0.1 mg/l), nicotinic acid (0.5 mg/l), pyridoxine-HCL (0.5 mg/l) and myo inositol (100 mg/l). Other ingredients added were sucrose 30 g/l, ascorbic acid 200 mg/l, citric acid 150 mg/l and plant growth regulator (as needed). Then adjust the pH to 5.8. The addition of 1 N NaOH was carried out if the initial pH was less than 5.8, conversely if the initial pH was more than 5.8 then 1 N HCL was given. After adjusting the pH to 5.8 then the media was cooked by adding 7 g/l of agar powder. After its boiled then the media was poured into culture bottles at a rate of 20 ml per bottle. The media-filled bottles were sealed with transparent plastic, fastened with rubber bands, and autoclaved for 15 minutes at 121ºC with a pressure of 1,2 kg/cm^2.

The sterilizing tools for culture bottle sterilized were detergent, 0.5% sodium hypochlorite solution, and 70% alcohol. A variety of instruments are used, such as pipettes, pH meter, analytical scales, culture bottles, aluminum foil, plastic wrap, rubber bands, label paper, cameras, hand sprayers, magnetic stirrers, beakers, tweezers, scalpels, and laminar air flow cabinets (LAFC).

A completely randomized design with three replications arranged factorial (2x3) was used in this study. The first factor was the type of basic media, ½ MS, and full MS. The second factor was the TDZ concentration 1, 2 and 3 mg/l combined with 2,4-D 0.5 mg/l.

Each experimental unit consisted of 5 culture bottles, each contained 5 explants. The observation data for each observation variable was analyzed for diversity using the F test, and if there are significant differences between treatments, then the middle value will be separated using the tukey test at a significance level of 0.05. Observation variables included callus performing periode, percentage of explants forming callus and somatic embryo, and percentage of shoot formation.

3 Results and Discussion

Planting were used the part of the leaf with leaf vein which the tip, base and sides of the leaf were not used. After that, the explants were cultured in a culture room under dark room conditions. The first primary callus appeared on day 77 after planting in the 3 mg/l TDZ treatment on both ½ MS and MS media. Furthermore, primary callus appeared on day 85 in the TDZ 2mg/l treatment and day 87 in the TDZ 1mg/l treatment. (Table 1).

The primary callus was subcultured into the same media for forming somatic embryo. At week 16, somatic embryo formation appeared. It started from the embryogenic callus which forms a green globular structure. In this phase, globular embryos were transferred to a culture room with bright room conditions. Two weeks after being transferred, the globular embryo then elongates (coleoptile) and began to form shoots after 1 week. Physiological and morphological changes in shoot formation through somatic embryos from leaf explants can be seen in Fig 1.

According to [17] the indirect embryogenesis phase started from the induction phase where the explants run into dedifferentiation of cells that form callus and become competent, in response to the addition of TDZ. Afterwards, the competent cells respond to become embryogenic callus which then transformed to somatic embryo phase with the globular stage then the coleoptile stage and finally the regeneration phase to become a shoot. The results of research [19] state that the embryogenesis process in *Phalaenopsis amabilis* plants started from the formation of pro-embryos which appear close to the cut side of leaf. The pro-embryo then enlarges and forms a globular embryo. Mature embryo does not simultaneously undergo

development into coleoptiles, procambium and then shoots emerge. But there are some cells that are left behind in their development so they are still in the globular stage. Hence, in one clump of somatic embryo, we can see all the various stages from globular, coleoptile and then developing into plantlets.

The data from Table 1 showed that the TDZ concentration had a different effect, but the two basic media had the same effect on the time of callus performed, somatic embryo formation and shoots formation. Giving TDZ 3mg/l gave the highest percentage of somatic embryo formation and shoots formation. This was in line with several studies [12], [20], [21] that the use of TDZ 3 mg/l was able to provide the highest response in each phase of orchid embryogenesis. The performance of shoots can be seen on Fig 2.

The ability of TDZ to induce the formation of somatic embryo is thought to be because TDZ could attach to one side of the Cytokinin Binding Protein (CBP) that found in the cell membrane so that it can stimulate endogenous cytokinin production. Thus, increasing cytokinin levels in cells will stimulate cell division and stimulate tissue morphogenesis [22]. The use of ½ MS basic media was able to provide the same effect as MS media. This means that in the process of forming somatic embfigryo for Dendrobium 'Gradita 31' orchid, ½ MS media was able to supply sufficient nutrients during the embryogenesis process. ½ MS media can be used as a cheaper alternative in the mass multiplication process through somatic embryogenesis, because the concentration of chemical ingredients was only half of the composition from MS media. This is also supported by several studies [12]–[15] that the use of ½ MS media is able to induce the formation of somatic embryos in *Dendrobium* and *Phalaenopsis*. Low nitrogen concentrations in the media were also reported able to induce higher embryo growth in coffee plants [23], *Santalum album* [24] and sorghum [25].

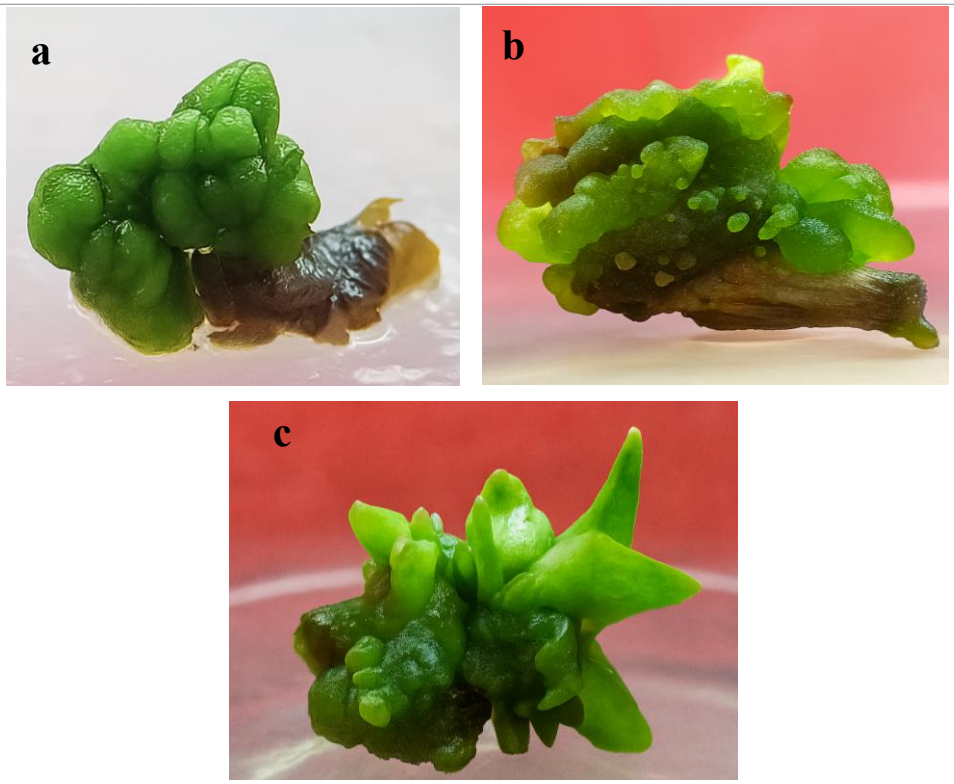

Figure 1. Somatic embryogenesis development of Dendrobium 'Gradita 31' (a) globular phase, (b) elongated embryo (coleoptile), (c) shoots formation.

Many studies have reported that auxin and cytokinin play an important role in the process of plant embryogenesis. On this research, we used 0.5mg/l 2,4-D as the auxin. The use of low concentrations of auxin combined with high levels of cytokinin has also been widely reported in orchid plants such as *Dendrobium malones* [11], *P. amabilis* [19], *Vanda tessellates* [26] and *Phalaenopsis* 'Sogo Vivien' [27]. On the other hand, another research for embryogenesis of *Phalaenopsis* 'Hong kong' single used of 3mg/l TDZ for whole stages was able to support embryogenesis process [12]. Other report [28] single auxin (3mg/l 2,4-D) was able to induce somatic embryo of *Vanda sumatrana*. The differences in the response of each orchid species to the type and concentration, cytokinin-auxin alone or in combination are very clearly visible, even though they are still in the same plant family. This is because plants have different genetic characteristics and their ability to respond the signals from the same growth regulator [16], [29], [30].

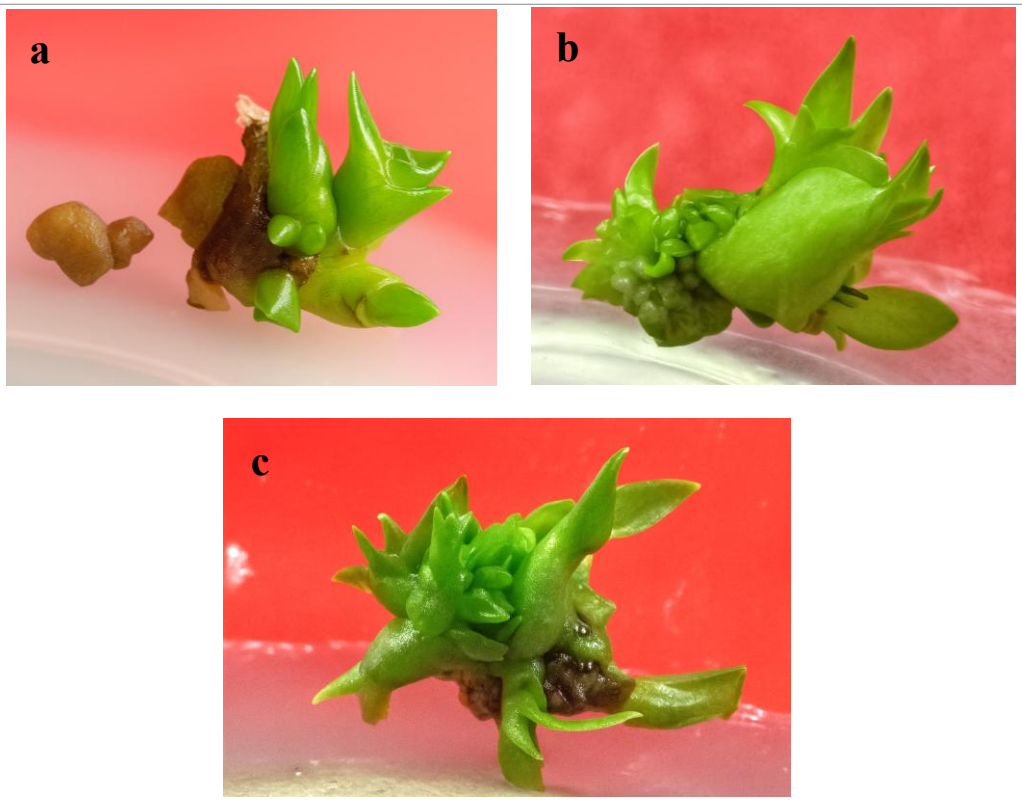

Figure 2. Shoots performance on different concentration of TDZ (a) 1mg/l, (b) 2mg/l, (c) 3mg/l.

Table 1. Effect of basic media and different concentration of TDZ on somatic embryogenesis Dendrobium 'Gradita 31'

Treatments	Callus performing period (days)	Primary callus (%)	Embryo somatic (%)	Shoots forming (%)
½ MS+ 1mg/l TDZ	86.00b	76.67a	58.33b	71.67b
½ MS+ 2mg/l TDZ	85.33b	83.33a	73.33ab	85.00ab
½ MS+ 3mg/l TDZ	77.00a	91.67a	91.67a	91.67a
MS+ 1mg/l TDZ	87.00b	75.00a	53.33b	70.00b
MS+ 2mg/l TDZ	85.67b	81.67a	66.67ab	83.33ab
MS+ 3mg/l TDZ	77.67a	91.67a	93.33a	90.00a

Figure 3. Performance seedling of Dendrobium 'Gradita 31' (a) globular phase microscopic (40x), (b) seedling (24 week), (c) seedling for acclimatization (30 week), (d) seedling 5 month after acclimatization

4 Conclusions

Based on the research on the effect of basic media and different concentration of TDZ on somatic embryogenesis Dendrobium ‘Gradita 31’, the conclusions were drawn:

1. The use of both types of basic media was able to induce callus formation on Dendrobium 'Gradita 31' orchid from leaf explants.
2. The use of 3 mg/l thidiazuron which combined with ½ MS or MS media was able to form primary callus faster than other treatments.
3. The higher percentage for embryo somatic and shoots formation were also found in 3 mg/l thidiazuron.

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