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Optimization of TDZ Hormone on the Formation of Somatic Embryogenesis in Dendrobium Orchids (D.50TH Stage Beauty X D. Bobby Mesina)

Optimasi Hormon TDZ Terhadap Pembentukan Somatic Embryogenesis pada Anggrek Dendrobium (*D. 50TH Stage Beauty* X *D. Bobby Mesina*)

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ABSTRACT

The Dendrobium variety orchid is a plant that has rare potential and growth. This requires large amounts of seeds that can be propagated in vitro. This study aims to determine the effect of TDZ on the direct induction of somatic embryogenesis. The study was conducted using a completely randomized design (CRD) with one factor, namely media consisting of basal Murashige and Skoog (MS) media with growth regulator TDZ. Each treatment out of a total of seven treatments was repeated four times. The research was carried out from March 2022 to August 2022. The results showed that leaf explants (clonal) could only be induced in 2 treatments (1 mg/L and 1.5 mg/L) out of 28 experimental media treatment units. The earliest callus appeared in the 1 mg/L treatment with a growth time of 90 days after planting (DAP) and the earliest PLB appeared in the 1.5 mg/L treatment with a growing time of 45 days after planting (DAP), the percentage of explant growth that became PLB with 1.5 mg/L treatment resulted in a percentage of 20 % and the percentage of callus growth at 1 mg/L yields a percentage of 20%.

Keywords: TDZ, Dendrobium, Clonal, Somatic Embryogenesis

ABSTRAK

Anggrek varietas Dendrobium merupakan salah satu tanaman yang memiliki potensi dan pertumbuhannya yang langka. Untuk itu diperlukan bibit dalam jumlah besar yang dapat diperbanyak secara *in vitro*. Penelitian ini bertujuan untuk mengetahui pengaruh TDZ terhadap induksi somatik embriogenesis secara langsung. Penelitian dilakukan menggunakan rancangan acak lengkap (RAL) dengan satu faktor, yaitu hormon TDZ dengan konsentrasi 0,25 mg/L; 0,5 mg/L; 0,75 mg/L; 1 mg/L; 1,25 mg/L; 1,5 mg/L; dan 1,75 mg/L pada media MS. Setiap perlakuan dari total tujuh perlakuan diulang sebanyak empat kali. Hasil menunjukkan bahwa eksplan daun hanya dapat terinduksi pada 2 perlakuan (1mg/L dan 1,5 mg/L) dari 28 satuan percobaan media perlakuan. Kedinian muncul kalus pada perlakuan 1 mg/L dengan waktu tumbuh 90 HST dan kedinian muncul PLB pada perlakuan 1,5 mg/L dengan waktu tumbuh 45 HST, persentase pertumbuhan eksplan yang menjadi PLB dengan perlakuan 1,5 mg/L menghasilkan persentase sebesar 20% dan persentase pertumbuhan kalus pada 1 mg/L menghasilkan persentase sebesar 20%.

Kata Kunci: TDZ, Dendrobium, Clonal, Somatic Embryogenesis

INTRODUCTION

The dendrobium orchid is an orchid variety that has the third largest distribution, reaching a value of 1184 species in the world (Hidayati *et al.*, 2014). The problem faced by this orchid plant is the lack of production of orchid plants which is not comparable to the high demand for orchids, supported by data from 2016 Dendrobium orchid enthusiasts reached 17.61 million (BPS, 2018). Conventional orchid propagation takes a long time, so

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orchid propagation is done more quickly using seeds with tissue culture with the aim of not taking time and using vegetative parts such as leaves for uniform results (Andayani, 2007).

Orchid propagation using seeds produces a large number of plants, but the problem is that the seeds produced are not uniform (Sinha *et al.,* 2007). To produce uniform seeds, clonally propagated through somatic embryogenesis can be carried out. Formation of somatic embryogenesis can use sliced leaf explants or leaf shoots. The goal in establishing somatic embryogenesis is to maintain the quality of orchids that are identical to their parents and to meet high consumer demand for dendrobium orchids (Cardoso *et al.,* 2020), coupled with statements (Restanto *et al.,* 2021), plant propagation uses PLB explant sources. is one solution to produce orchids in large quantities.

Embryogenesis in explants can occur directly or indirectly or can occur through callus formation (Naz *et al.,* 2008). According to Chung *et al.* (2007), most of the somatic embryogenesis formed around leaf slices or close to leaf slices. Kriswanto *et al.* (2020), the media used for somatic embryogenesis multiplication is Murashige & Skoog (MS) solid media with the addition of TDZ hormone. The use of ZPT in the cytokinin group is useful for reducing the dominance of apical meristem growth and inducing adventitious and axillary shoots from the plant explants used. The best concentration for direct embryo induction was at 18.16mM TDZ Chung *et al.* (2005). The aim of using TDZ with various concentrations is to find out the best concentrations that produce direct somatic embryogenesis in leaf explants (Restanto *et al.,* 2018).

MATERIALS AND METHOD

Time and place

The research was carried out at the Plant Tissue Culture Laboratory, Faculty of Agriculture, University of Jember from March 2022 to August 2022. Histology preparations were made at the Microtechnic Laboratory, Department of Plants, Faculty of Biology, UGM.

Instrument and Materials

The tools used include LAF, autoclave, microscope, scalpel, magnetic stirrer, analytical balance, tweezers, culture bottles, and petri dishes. The materials used were MS media, agar-agar, sugar, distilled water, and young leaves of the Dendrobium sp orchid plant resulting from a cross (D. 50TH Stage beauty X D. Bobby Masina).

Experimental Design

The experimental design used a completely randomized design (CRD) using one factor. The treatment factor in the study was the TDZ growth regulator. There are 7 levels of concentration treatment consisting of 0.25 mg/L; 0.5mg/L; 0.75mg/L; 1mg/L; 1.25mg/L; 1.5mg/L; and 1.75 mg/L (Chung *et al.,* 2005). Each treatment was repeated 4 times, so there were 28 experimental units.

Experimental Procedure

Making media by mixing MS media stock solution, sucrose, and adding distilled water. After all the solutions are mixed, measure the degree of acidity using a pH meter until the solution shows the numbers 5.6-5.8. Then the solution was added with the TDZ hormone at the concentration according to the treatment, then added agar at a rate of 8 g/L and sterilized using an autoclave for ± 1 hour.

Somatic embryo induction was carried out using sliced young leaf explants from the Dendrobium sp. The explants were cut to a size of 1-1.5 cm, then planted in tubes using MS media with the addition of TDZ hormone. Each tube contains 5 pieces of explants. Leaf explants are planted by immersing the leaves in direct contact with the media.

Observation and Statistical Analysis

Variables observed included the early emergence of PLB and callus calculated from the time of growth from the beginning of planting until the day the response appeared, the percentage of growing explants that formed PLB and callus which was determined by calculating the number of PLB and callus divided by the total explants cultured multiplied by 100%, the development of embryogenesis somatic described using images and histological analysis performed on callus development. Observational data were then processed and analyzed

using ANOVA. Data in the form of images were analyzed descriptively

RESULT AND DISCUSSION

The results of the observations showed that only 2 treatments gave a response. The concentrations that succeeded in producing callus and PLB were 1 mg/L and 1.5 mg/L TDZ treatments. The concentration of 1 mg/L succeeded in growing callus with callus as early as 90 days after planting (DAP) as shown in Figure 1.

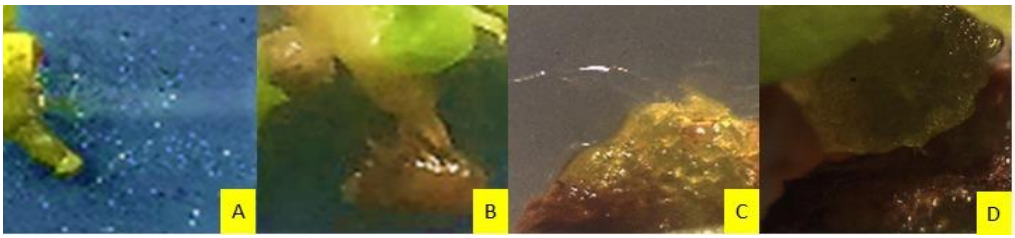

Figure 1. Callus formation at a concentration of 1 mg/L TDZ

The calculation results show that the t0 value of the combined blood clams without distinguishing between males and females is 0.1921 years or 2.3051 months. Age t0 is an initial condition parameter that determines the point in time when the shell has zero length. Based on the growth parameter values obtained, the Von Bertalanffy equation for blood clams from Rangsang Barat waters is obtained as follows Shown in Figure 1 (A) is a leaf explant used as planting material. Figure (B) at the age of 45 days after planting (DAP) explants have not shown a response. Figure (C) is the early onset of callus at 90 DAP. Figure (D) is a callus aged 135 DAP, The appearance of the callus is characterized by the growth of white granules and a crumb texture. Giving higher hormone concentrations given to explants can spur the formation of callus which will grow into somatic embryos. The process of somatic embryogenesis in orchids goes through 3 phases, namely the globular phase, the scutellar phase, and the coleoptilar phase as shown in Figure 2.

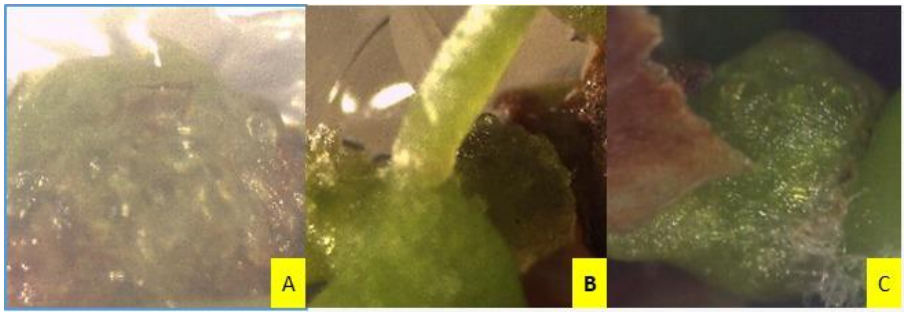

Figure 2. Development of somatic embryogenesis, A) globular phase, B) scutellar phase, C) collioptilar phase

Dewi et al., (2016), an embryo that begins to develop will undergo differentiation in the apical part of the embryo which consists of cells that are relatively small in size and actively divide. The cause of cell division is the presence of cytokinins in the induction media, where cytokinin hormones play a role in accelerating cell division. After passing through the scutellar phase, it then enters the coleoptilar phase which forms leaf primordia.

Treatment with a concentration of 1.5 mg/L was able to grow PLB directly with a growing time of 45 of days after planting (DAP). The development of PLB directly emerged from the area of the explants that were injured as a result of the cut marks at the start of planting and continued to divide until they showed green bulges. PLB proliferated and changed color to dark green.

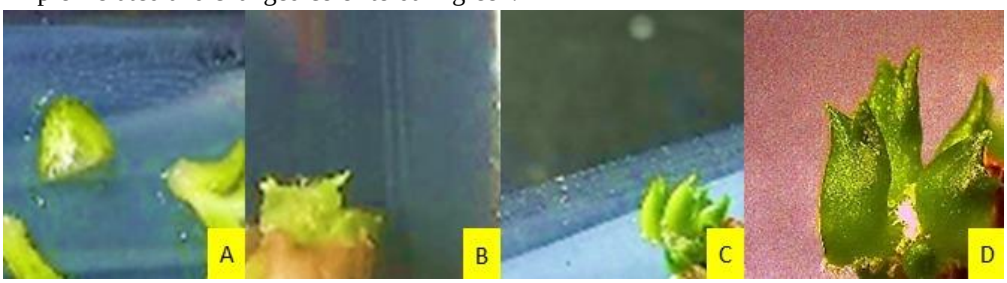

Figure 3. Formation of PLB at a concentration of 1.5 TDZ

Shown in Figure 3 (A) is a leaf explant planted. Figure 3 (B) is the initial PLB formation on the 45th day of DAP. The PLB that was formed continued to grow as shown in Figure 3 (C) at 90 DAP and Figure 3 (D) at 135 DAP. According to the statement of Hazubska-Przybył *et al.* (2020), swelling that occurs in explants is caused by cytokinin activity which causes cells to differentiate so that they can assist in PLB formation.

PLB formed can grow to form leaf and root organs. In orchids, there is a part of the somatic embryo that is actively dividing, namely the plumule pole. According to Restanto *et al.* (2018), roots in orchids are adventitious roots that can appear after differentiation of the shoots, although no root primordia were found in the embryos, somatic embryos still have cellular links with their supporting callus through structures such as suspensors so that the embryo can grow and develop into whole plants.

The addition of TDZ hormone to the media gave a different response to PLB and callus formation. Hormone concentration affects how fast or slow the explants respond to PLB formation. TDZ hormone concentrations of 1 mg/L and 1.5 mg/L had the same growth yield percentage of 20%, whereas, in the other treatments, there was no response or a percentage of 0%.

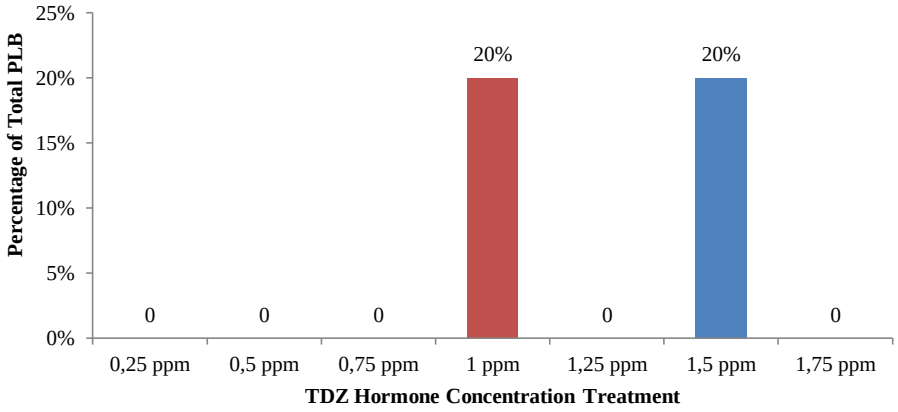

Figure 4. Percentage of the number of PLB

An indication of success in vitro plant propagation is the accuracy of using ZPT concentrations. The TDZ hormone affects the initial response of explants of *Dendrobium* sp. (Kurnianingsih *et al.*, 2020). Singh *et al.* (2003), the addition of the TDZ hormone at high concentrations causes accelerated development of explants in differentiation, that the success rate of PLB formation varies depending on the explants used and the composition of the media used.

Histological analysis was carried out on the best samples from several treatments that experienced a growth response. The callus used as a histological sample was a callus at a concentration of 1 mg/L which had entered the embryonic callus stage. Histological observations provide more information about embryogenic callus which is characterized by the presence of cell nuclei in the tissue observed using a microscope. The following histological observations from the induction results showed embryogenic callus and non-embryogenic callus based on the visual characteristics seen in (Figure 5).

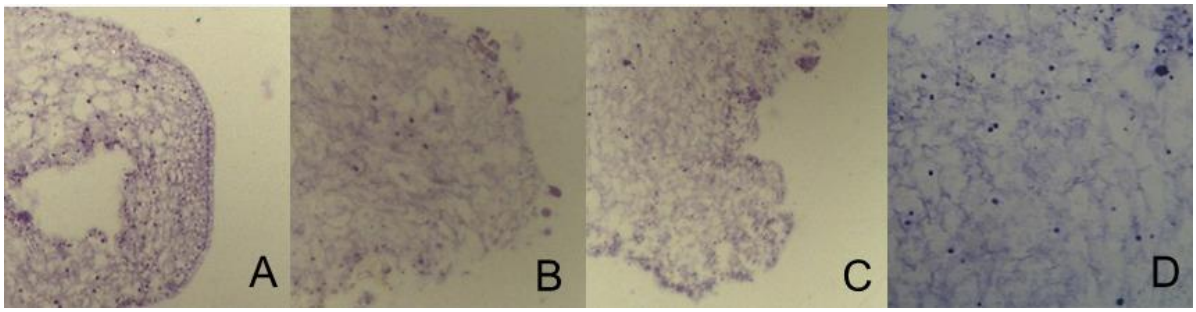

Figure 5. Somatic histology of orchid embryogenesis. A) globular phase, B) scutellar phase, C) collioptilar phase, D) non-embryogenic callus

As seen in Figure 5 (A) the globular phase is characterized by oval protrusions with surfaces that are meristematic or actively dividing. Figure (B) In the scutellar phase there is a bulge in the direction of growth. Figure (C) The formation of scutellar prospective leaf organs. Figure (D) is the histology of the non-embryogenic callus.

CONCLUSION

The treatment of TDZ hormone can produce callus growth and PLB at 1 and 1.5 mg/L treatment. A concentration of 1 mg/L TDZ was a treatment capable of producing a callus response with a growing time of 90 DAP while a concentration of 1.5 mg/L produced a callus with a growing time of 45 DAP. The percentage of callus and PLB that can be produced is 20%.

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