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Original Research Article

The Influence of Thidiazuron on Direct Somatic Embryo Formation from Various Types of Explant in *Phalaenopsis amabilis* (L.) Blume Orchid

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A B S T R A C T

Phalaenopsis amabilis is an important national flower of Indonesia as a parent for orchid breeding, so that needs a good strategy to produce high number of plants. The objective of this research is to analyze the use of thidiazuron (TDZ) for producing high number of plantlets, through directly induction of somatic embryos (SEs) from various explants. The method was used 20 each of protocorms, leaves, stems and roots as explants. The explants were dissected transversely, then put on various culture media: New Phalaenopsis (NP) and NP + (1, 2, 3) mgL^{-1} TDZ. Cultures were maintained at 25°C with continous white light. The formation of SEs was observed every week for 8 weeks. The results showed that SEs formation increased inline with the addition of TDZ concentration to the NP medium, for both velocity and amount of SEs formation. In NP0, SEs were formed at (26.07 ± 0.73) days after inoculation of protocorm, whereas on NP + (1, 2, and 3 mgL^{-1}) TDZ, SEs were formed at (17.85 ± 0.67) days, (15 ± 0.64) days, and (11 ± 0.64) days, respectively. All types of explants formed SEs on NP + TDZ (1−3 mgL^{-1}), whereas only 14 of 20 protocorms produced SEs (70%), and 8 of 20 stems formed SEs (40%) in NP0. In roots, SEs was formed on NP + 2 mgL^{-1} TDZ and NP + 3 mgL^{-1} TDZ. For stems, the highest amount of SEs (28.25 ± 1.07) was reached on NP + 3 mgL^{-1} TDZ, followed by protocorm (23.30 ± 1.13) SEs and roots (8.25 ± 0.68) SEs. In contrast, in NP0, the amount of SEs was very low (1.25 ± 0.46) from stem and (1.50 ± 0.65) from protocorms, there was no evidence of SEs formation in the leaves and roots.

1. Introduction

Somatic embryogenesis is defined as a process in which a bipolar structure, resembling a zygotic embryo, develops from a non-zygotic cell without vascular connection with the original tissue (Arnold et al. 2002). In that process, somatic cell differentiate into a plant without the involvement of fertilization or gamete fusion (Smertenko and Bozhkov, 2014). Somatic embryogenesis has emerged as a powerfull tool for studying plant development because somatic embryos (SEs) resemble zygotic embryos and undergo almost the same developmental stages (Elhiti et al. 2013). Therefore, somatic embryogenesis has been considered to be a suitable system for plant mass propagation and for regeneration of transgenic plants (Bhattacharyya et al. 2016).

In recent years, somatic embryogenesis protocols have been successfully developed in several orchids, including *Dendrobium* (Bhattacharyya et al. 2016; Kaewubon and Meesawat, 2016), *Cymbidium* (da Silva and Winarto, 2016), *Oncidium* (Mayer et al. 2010), *Cattleya* (Cueva-Agila et al. 2016), *Vanda* (Hardjo et al. 2016) and *Phalaenopsis* (Winarto et al. 2016). However, the selection of suitable types and sources of explant are critical factors for obtaining a successful culture in somatic embryogenesis system (Feng and Chen, 2014).

Currently, some techniques of propagation have also been developed for a number of orchid species through somatic embryogenesis from various types of explant including leaves (Jainol and Gansau, 2017), roots (Meilasari and Iriawati, 2016), shoot tips (Van et al. 2012), stem nodal (Hong et al. 2010), seed-derived protocorms (Mahendran and Bai, 2016), and protocorm-like bodies (PLBs) (Li and Xu, 2009). Furthermore, the proccess of somatic embryogenesis could be induced either directly from epidermal and sub-epidermal cells of explants (Moradi et al. 2017) or indirectly via intervening callus (Niknejad et al. 2011). However, plant regeneration from callus is often associated with genetic and cytological variation making the strategy less desirable for large-scale clonal multiplication (Anjaneyulu and Giri, 2011). Direct

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somatic embryogenesis is beneficial with its reduced time for plant propagation as well as with minimized culture-induced genetic changes (Jayanthi et al. 2011).

Generally, somatic embryogenesis is considered to occur in response to modifications in the level of available growth regulators, especially auxins and cytokinins in tissue culture media (Moradi et al. 2017). Thidiazuron (TDZ) is a potent regulator for *in vitro* propagation system in a wide variety of plants (Guo et al. 2011). For some years, TDZ has been generally used to culture orchid tissue, which could induce organogenesis and high-frequency of direct somatic embryogenesis, either alone or in combination with other growth regulators (Mahendran and Bai, 2016; Wu et al. 2012). Moreover, many studies reported that the use of TDZ alone could induced direct SE in *Phalaenopsis* orchid (Feng and Chen, 2014). TDZ displayed primaryly cytokinin-like activity and can be used as a subtitute for both auxin and cytokinin (Kou et al. 2016). Besides, Bhataccharyya et al. (2016) reported that TDZ showed better efficacy over other purine-type cytokinins (BA or Kinetin) even at low concentrations.

Phalaenopsis amabilis is a native Indonesian orchid and one of the most important parental species of *Phalaenopsis* hybrids (Semiarti et al. 2007). Semiarti et al. (2013) reported that the number of propagated plants of this orchid using seeds was still limited because of highly dependent on the existance and the quality of siliques that resulted from pollination. Moreover, the continuity of mass propagation of this orchid to produce large number of uniform seedlings in a relatively short time to meet the market demands is also still limited. Therefore, to solve that problem, this study was taken to evaluate the influence of TDZ on direct SEs formation from various types of organs of *P. amabilis* as explants.

2. Materials and Methods

2.1. Plant materials and culture conditions

A silique of *P. amabilis* (Java ecotype) plant (Figures 1A and 1B) following 120 days of pollination was collected from Titi Orchids Nursery, Harjobinangun-Pakem, Sleman, Yogyakarta. The silique was wiped with 70% alcohol then passed over a fire and waited until the fire went out. This work is done for three times. After sterilized, the seeds were taken from the silique and sown on a solid New Phalaenopsis (NP) medium (Islam et al. 1998; Semiarti et al. 2010). Thereafter, the cultures were incubated in 100 mL flask at temperature of 25 ± 1°C with 1000 lux of continuous light. Four-week-old protocorms (developing orchid embryos) (Figure 1C) and 6-month-old orchid seedlings (Figure 1D) were used as the source of explants.

Roots and leaves of the 6-month-old seedlings and 4-week-old protocorms that were cut transversely, and stems were used as explants. The explants were put on NP solid medium supplemented with TDZ (0, 1, 2, 3 mgL^{-1}), and cultures were maintained at temperature of 25 ± 1 °C with 1000 lux intensity of continuous light. Subcultures were conducted every two weeks. Detailed observation on morphology of SEs formation was conducted every day and photographs were taken once a week for eight weeks using dissecting microscope (Eschenbach, Germany).

2.2. Histological analysis on the differentiation of SEs

Histological analysis on the differentiation of SEs was observed for each developmental stage of SEs in the surface of explants by anatomic preparation using paraffin method according to Ruzin (1999). The anatomic samples in the glass slides were observed using light microscope (Olympus, Japan).

2.3. Data analysis

A total samples of 320 explants grouped randomly into 16 treatment groups. Each group consisted of 20 explants cultured in 4 Petri dishes (diameter 100 mm × height 15 mm), each Petri dish contained 5 explants. All treatment means were compared by following Duncan's Multiple Range Test. Significant differences between means were presented at the level of $P \leq 0.05$.

3. Results

3.1. Induction of SEs

Based on percentage data of SEs formation after 8 weeks of culture, it was known that SEs could be induced in all treatments, except on leaf and root explants that cultured on hormone-free medium and root explants cultured on NP medium supplemented with 1 mgL^{-1} TDZ (Table). Within four weeks some explants turned to yellow and became necrotic. However, only 70% of protocorms and 40% of stem explants were successfully formed SEs in TDZ-free medium.

In the presence of TDZ in culture medium, 100% of SEs were successfully formed from protocorm and stem explants. The initial embryos emerged as protruding nodular masses from the surface of

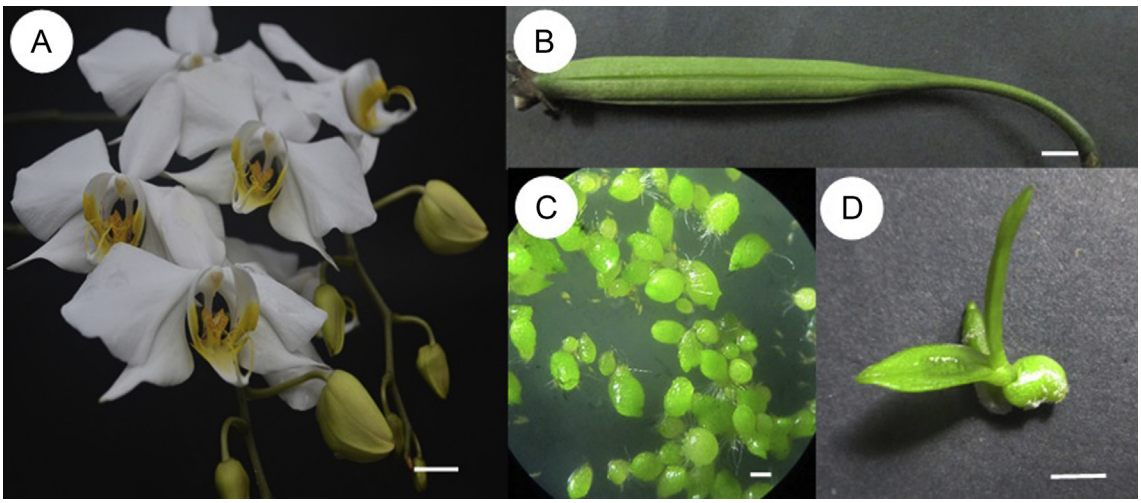

Figure 1. Phenotype of *P. amabilis*. (A) A bunch of flowers for the material of self-pollination to get silique. (B) 4-month-old silique. (C) 4-week-old protocorms. (D) 6-month-old seedling (bars a, b, d = 1 cm; bar c = 1 mm).

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