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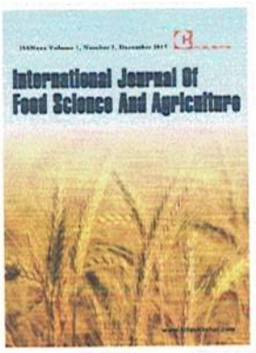

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Effect of Benzyl Adenin on the Growth of Black Orchid (*Coelogyne pandurata* lindl.) Planlets in *in vitro* Culture

Lucky Prayoga*, Rochmatino, Kamsinah

Faculty of Biology, Universitas Jenderal Soedirman, Jl. Dr. Soeprano No. 63, Karangwangkal Purwokertro, Central Java, Indonesia.

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***Corresponding author:** Lucky Prayoga, Faculty of Biology, Universitas Jenderal Soedirman, Jl. Dr. Soeprano No. 63, Karangwangkal Purwokertro, Central Java, Indonesia.
Email: lucky.prayogo@unsoed.ac.id

Abstract

Conservation of black orchids (*Coelogyne pandurata* Lindl.) is a high priority because the natural population is decreasing significantly. A typical method for orchid conservation is farming. The aims of this research were to examine the effects of adding benzyladenine (BA) to medium on the total number of buds, shoot initiation, and the number and length of roots. Our research method was based on a completely randomized design, in which various concentrations of BA (0, 0.5, 1, 1.5 and 2 ppm) were added to Vacin and Went medium. The BA concentration significantly affected the growth of black orchid plantlets. The most prominent effect was seen at 0.5 and 1 ppm/L BA; however, increasing the concentration over 1 ppm/L inhibited bud growth and the number and length of roots. Our data suggest that optimization of the BA concentration added to medium leads to accelerated growth of black orchid plantlets. *In vitro* propagation with the addition of BA to Vacin and Went media can be applied for black orchid conservation.

Keywords

Benzyladenine, Black Orchid, Concentration, Conservation, Plantlets

1. Introduction

The black orchid (*Coelogyne pandurata* Lindl.) is a natural orchid species endemic to Kalimantan that is protected by the government of Indonesia because its population is decreasing significantly [1]. Typically, declines in orchid populations are driven by biotic and abiotic factors related to growth, development and reproduction [2]. Factors causing the rapid decline in the black orchid population include excessive harvesting for commercial trade, illegal logging, domestication, land conversion and deforestation. Global warming has also negatively affected the natural orchid population [2]. Furthermore, black orchids exhibit low resistance to generative propagation in nature; this is because orchid seeds do not have an endosperm and thus require mycorrhizal assistance to supply compounds needed for germination [3]. Therefore, effective and efficient technologies for mass orchid propagation must be developed to meet commercial demands for successful orchid conservation [4]. Therefore, it is necessary to make efforts to conserve rare orchids and increase the production of black orchid seeds with plant propagation, both generative and vegetative propagation by in vitro culture [5]

Currently available cultivation techniques for *in vitro* production of orchid seedlings or plantlets include asymbiotic germination and micropropagation techniques, which can be employed for the large-scale production of clonal plantlets [6]. Efforts to rescue and propagate black orchids have employed *in vitro* culture techniques, which enable rapid propagation of explants, preservation of germplasm and multiplication of plants that are difficult to reproduce conventionally [7]. Adenine sulfate is often used as a supplement during *in vitro* plant propagation, because adenine is a cytokinin ana-

logue involved in many aspects of plant development, such as stimulation of somatic embryogenesis, caulogenesis and adventitious and axillary shoot formation [8] and [9]. Furthermore, unlike other cytokinins, adenine does not exhibit inhibitory effects on root formation [10]. Adenine may promote similar effects during *ex vitro* propagation; however, this possibility has yet to be explored experimentally.

The addition of benzyladenine (BA) to *in vitro* culture medium potentially enhances the germination ability of seeds. Increasing the concentration of BA increases the number of roots in *Nigelia sativa* and *Allium cepa* plants; however, at high concentrations, BA inhibited root growth [11]. Moreover, BA supplementation increased the number of shoots and roots in rice and the number of flowers and leaf thickness in calla lily [12]. However, research on the effects of BA on the *in vitro* culture of black orchids is limited. This study aimed to investigate the influence of the BA concentration on the number and length of roots, number of shoots, and the speed of black orchid plantlet appearance.

2. Methods

2.1. Experimental Design

This research was conducted at the Laboratory of Plant Physiology, Jenderal Soedirman University over a 5-month period from 02/07/2018 to 06/21/2018. The experimental methods were based on a completely randomized design. The treatments comprised application of five concentrations of BA (0, 0.5, 1, 1.5 and 2 ppm/L), repeated 15 times to obtain 60 experimental samples. To subculture the black orchid plantlets, plantlets were removed from their culture bottles using flame-sterilized tweezers and placed on a petri dish. Healthy plantlets of similar sizes were then selected using tweezers and added to Vacin and Went treatment medium. Each bottle contained two plantlets. The mouths of the culture bottles were heated and closed with aluminum foil, tied with a rubber band and then covered with a wrapper. Culture bottles containing plantlets were stored on a culture rack and kept clean to avoid contamination. Observations and measurements were taken over a 10-week period.

2.2 Data Analysis

Data were analyzed using analysis of variance and the least significant distance test (confidence level of 5%).

3. Results and Discussion

The addition of different concentrations of BA to black orchid plantlets significantly influenced the shoot appearance time, number of shoots and number and length of roots ($F = 3.36$; $P < 0.05$) (Table 1).

Table 1. Effects of benzyladenine on plantlet growth (mean ± standard deviation; n = 32)

Treatment BA (ppm/L)	Number of shoots	Shoot appearance (days after planting)	Length of roots	Number of roots
0	1.8 ± 0.9	38.1 ± 11.5	4.28 ± 1.08	1.9 ± 0.90
0.5	2.2 ± 0.9	52.12 ± 11.6	10.7 ± 4.3	3.5 ± 1.3
1	2.0 ± 0.7	63.5 ± 10.48	12.16 ± 2.8	3.2 ± 1.5
1.5	4.08 ± 1.32	44.6 ± 14.10	6.9 ± 2.8	1.8 ± 0.93
2	1.9 ± 0.9	45.3 ± 9.7	4.42 ± 0.67	2.1 ± 0.99

The number of shoots in plantlets increased after supplementation with 0.5, 1, and 1.5 ppm/L BA, whereas shoot growth appeared to be inhibited at 2 ppm/L BA. The increase in shoot number was due to the cytokinin activity of BA, which stimulates shoot growth. The greatest number of shoots (4.08 ± 1.32) was observed after 1.5 ppm/L BA treatment. These results corresponded with those of previous studies that found an increase in the shoot number following BA addition (12) and (13) and a decrease in shoot number after supplementation with high BA concentrations (14) The 2 ppm/L BA concentration also inhibited bud growth in orchid plantlets.

Shoot initiation was delayed at BA concentrations of 0.5 and 1 ppm/L; however, this effect was attenuated at 1.5 and 2 ppm/L BA concentrations; a likely reason for the delayed shoot initiation was inhibition of proliferation by BA. Shoot ends in certain doses, whereas at high doses the resistance is reduced. Our results are similar to those of Mat-Yasin et al. [14], who investigated the propagation medium of *Spathoglottis plicata*. Hartati et al. [15] found that the addition of NAA and coconut water to medium for *in vitro* black orchid propagation has increasing number of roots. However, at physiological concentrations, cytokinins activate the shoot apical meristem and shoot growth but suppress the root apical meristem and root growth [16] Cytokinins promote cell division, stimulate the release of lateral buds from apical dominance, delay leaf senescence, and enhance chloroplast development. Furthermore, conflicting studies indicate that cytokinins can both promote and inhibit root initiation and development, although studies on this topic are limited. In sum, the effects of cytokinins on *in vitro* plant development appear to differ from those observed *in vivo*.

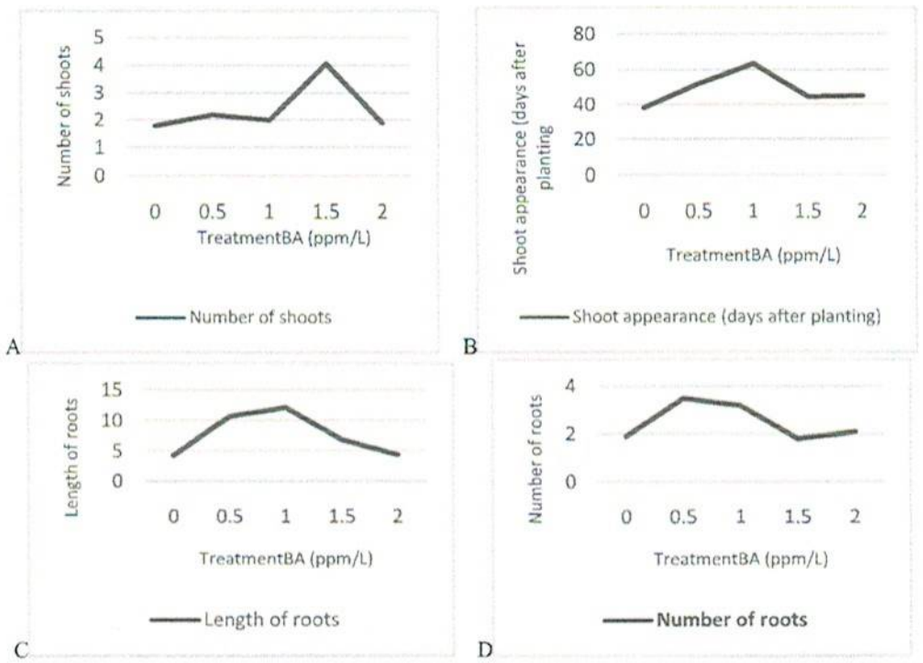

Figure 1. A. trend of number of shoots; B. trend of shoots appearance; C. trend of length of roots; and D. trend of numbers of roots.

The longest roots were found after treatment with 0.5 and 1 ppm/L BA, and root length decreased at higher BA concentrations. On the other hand, no significant difference in root number was observed among the BA concentrations. Cytokinins are a class of plant hormones that stimulate axillary bud formation and inhibit rhizogenesis [17]. Moreover, cytokinins influence several growth and de velopmental processes in plants, and the nature of their actions depends on the cytokinin concentration. Another major role of cytokinins is stimulation of cell division. Many cytokinin effects manifested at the organism level, such as the formation of vascular tissues in roots, are determined just by cytokinin capability of stimulation of definite precursor cell proliferation (Figure 1).

Plant growth along the longitudinal axis is determined by the shoot and root apical meristems, which are influenced by cytokinins and auxins. Cytokinins promote cell division and expansion, especially in the leaves and cotyledons. The BA concentration also influences callus development; Ellaleem et al. [18] reported that the combination of BA and tridiazeron affects the total number of tomato calluses. Sardoi [19] found that 400 ppm/L BA increased the offset of *Aloe barbadensis*. A high concentration (1,000 ppm/l) of BA was required for optimal regeneration of melons [20, 21].

4. Conclusion

In conclusion, the addition of BA to the culture medium of black orchid plantlets affected the number of shoots, bud initiation time, and length and total number of roots. These effects were dependent on the BA concentration applied. Our results may contribute to the conservation of black orchids via enhanced *in vitro* culture.

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