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Inhibition of Dendrobium bicaudatum Reinw. ex Lindl *growth using Paclobutrazol for in vitro conservation*

Penghambatan Pertumbuhan *Dendrobium bicaudatum* Reinw. ex Lindl., dengan Pactobutrazol untuk Konservasi secara In Vitro

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ABSTRACT

D. bicaudatum orchid has become threatened with extinction. Environmental damage due to human activities and overcollection of orchids has triggered the extinction of orchids in their natural habitat. In situ and ex situ conservation measures need to be taken to reduce the threat of extinction for orchids. This research aims to investigate the effect of paclobutrazol (PBZ) on culture mediums as a plant growth inhibitor, one of which is in vitro conservation measures, and was conducted experimentally using a completely randomized design. The independent variables were the concentrations of PBZ of 0, 1, 3, and 5 mg L^{-1}, respectively, which were added to the growing medium in the form of solid MS medium. The medium-term conservation of D. bicaudatum orchids with the addition of paclobutrazol exhibited a very significant result. Inhibition of vegetative growth such as the number of leaves and root length was exhibited by 1-5 mg L^{-1} PBZ, while the stem diameter, as one of the dependent variables, increased more in all treatment groups than in the control group. Reduced number of roots occurred in the groups of treatments of 1 mg L^{-1} PBZ and 3 mg L^{-1} PBZ.

Keywords: extinction, in Vitro culture, reduction,

ABSTRAK

Keberadaan anggrek *D. bicaudatum* mulai mengkhawatirkan. Kerusakan alam akibat aktivitas manusia dan pengambilan anggrek di alam secara berlebih memicu kepunahan anggrek pada habitat asal. Tindakan konservasi tanaman baik secara *in situ* maupun *ex situ* perlu dilakukan untuk mengurangi ancaman kepunahan anggrek tersebut. Penelitin bertujuan untuk menyelidiki efek dari paclobutrazol pada media kultur sebagai penghambat pertumbuhan tanaman, salah satu upaya konservasi secara in vitro. Penelitian dilakukan secara eksperimental menggunakan rancangan acak lengkap. Variabel bebas penelitian adalah konsentrasi PBZ berturut-turut 0, 1, 3, dan 5 mg L^{-1} yang ditambahkan pada medium tanam berupa MS padat. Hasil penelitian menunjukkan konservasi jangka menengah anggrek *D. bicaudatum* dengan penambahan pactobutrazol menunjukkan pengaruh yang sangat nyata. Penghambatan pertumbuhan vegetatif seperti jumlah daun dan panjang akar terdapat pada PBZ 1-5 mg L^{-1}, sedangkan pada variabel diameter batang terjadi pembesaran pada semua perlakuan dibandingkan kontrol. Reduksi jumlah akar terjadi pada perlakuan PBZ 1 dan 3 mg $L^{-1.}$

Kata kunci: kepunahan, kultur *in Vitro*, reduksi

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INTRODUCTION

Dendrobium bicaudatum Reinw. ex Lindl. is one of the epiphytic orchids originating from Sulawesi. The characteristic feature of *D. bicaudatum* orchid is that they have horn-like flowers with slightly twisted petals (Utami *et al*., 2014). *D. bicaudatum*, section Spatulata species has the potential as a source for crossbreeding because it can produce new characters from its uniqueness. In their natural habitat, *D. bicaudatum* orchid has become threatened with extinction. Environmental damage due to human activities and overcollection of the orchids trigger their extinction in their natural habitats. Plant conservations, both in situ conservation (in their natural habitats; Chen *et al*., 2009; Havens *et al*., 2014) and ex situ conservation (outside their natural habitats; Havens *et al*., 2006; Li and Pritchard, 2009; Mounce *et al*., 2017) need to be done to reduce the threat of extinction for these orchids. Conservation of plant genetic resources is an important factor in food security and biodiversity.

In addition to applying low temperature and luminosity (Carvalho *et al*., 2016), ex situ plant conservation by in vitro culture uses inhibitor that needed to slow down growth and increase the interval between subculture cycles. In this regard, the use of paclobutrazol has been widely reported (Ardigusa & Suksma, 2015; Indrayanti *et al*., 2019; Gimenes *et al*., 2018; Habibah and Sumadi, 2013; Vaz *et al*., 2012; Wen *et al*., 2013. According to Fletcher and Hofstra (1990), paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1H-1, 2, 4-trizol-1-yl)-pentan- 3-ol] is a member of the triazole family, which has growth-regulating properties. Growth regulator has been reported to play an important role in stress response and adaptation (Vineeth *et al*. 2016; Sharma and Dubey 2005). According to Kishore *et al*. (2015), Soumya *et al*. (2017), and Bisht *et al*. (2018), paclobutrazol (PBZ) is a compound that has an anti-giberellic acid (GA) effect by inhibiting the oxidation of ent-kaurene into ent-kaurenoic acid in the biosynthesis of gibberellic acid and improving photosynthetic capacity and water balance in leaves. Paclobutrazol is often used as a morphoregulator (Bañón *et al*., 2005).

According to the results of research conducted by Gimenes *et al*. (2018), the use of PBZ at various concentrations was able to reduce root growth in *Zygopetalum crinitum* in vitro. The same result was also seen in Grammatophyllum orchids (Habibah and Sumadi, 2013). In strawberry plants, PBZ was able to significantly reduce water consumption (Parvin *et al*., 2015). Paclobutrazol has also been reported to be very useful to make dwarf varieties of crops, including temulawak (Syahid, 2007), apples (Kopenek and Karoglu, 2011), and sunflowers (Ribeiro *et al*., 2011). This research aimed to develop a medium-term storage procedure for *D. bicaudatum* orchid in vitro by investigating the effect of paclobutrazol as a plant growth inhibitor on culture medium.

RESEARCH METHOD

This research was conducted from May to November 2021. *In vitro* conservation was conducted at the Tissue Culture Laboratory, Faculty of Agriculture, Udayana University, Jalan Pulau Moyo, Pedungan, Denpasar Selatan. The explants used were protocorm aged 66 days after sowing.

The research was conducted experimentally using a completely randomized design. The independent variables were PBZ concentrations of 0, 1, 3, and 5 mg L^{-1}, respectively, which were added to the growing medium of solid MS medium. The dependent variable was protocorm growth, which was indicated by number of leaves, number of roots, root length, and stem diameter. Meanwhile, the control variable was the growing medium, namely, solid MS medium, and the temperature of the growing room and incubation room which ranged from 23–25 °C. Each treatment combination was performed with five repetitions. The experimental unit consisted of culture bottles with MS medium, each of which was planted with ten protocorms. The medium used was solid Murashige and Skoog (MS) medium (1962). Solid MS medium was obtained by adding 8 g of agar as a solidifying agent for each liter of medium. The pH of the medium was adjusted to reach 5.8 with the addition of HCl and NaOH. After that, the medium was poured into culture bottles and sterilized using an autoclave. After cooling, they were stored in the culture room for 4-5 days to ensure that there was no contamination and they were ready to be planted. The protocorm used as the explant was 124 HST, with a globular shape and was planted with 1.5 g of the media.

The bottles containing the protocorms were placed on the culture rack randomly according to the experimental design in a closed incubation room at 23-25 °C. The growth of the protocorms on the mediums in the treatment groups was observed up to four months after planting. The quantitative data obtained were analyzed using ANOVA according to the design used. If the treatment has a significant effect, the least significant difference (LSD) test at a 5% significance level will be conducted as a follow-up test.

RESULT AND DISCUSSION

Observations were conducted after four months of protocorms being planted on the mediums with the application of paclobutrazol. The significance of the treatments on the observed variables is presented in Table 1. The growth of the protocorms of the *D. bicaudatum* orchid *in vitro* using PBZ was significantly and very significantly affected, indicated by the number of leaves, number of roots, root length, and stem diameter. These results are different from the research conducted by Deswiniyanti and Lestari (2018), where paclobutrazol has not shown a real effect on the growth and development of orchid seeds *Vanda tricolor.*

Table 1 and Figure 1 show that *D. bicaudatum* orchid plantlet leaf formation was inhibited when the plantlets were administered with 1 and 3 mgL^{-1} PBZ, 18% significantly different from plantlets treated with 5 mg L^{-1} PBZ and plantlets in the control group (without PBZ). The same trend also occurred in the variable of number of roots. The highest reduction in the number of roots was in the treatment group with 1 mg L^{-1} PBZ (33.3%), while the lowest was in the treatment group with 5 mgL^{-1} PBZ, which was not significantly different from that in the control group. Further, the greatest inhibition of the variable of length of *D. bicaudatum* root occurred in the treatment group with 3 mg L^{-1} PBZ and the smallest inhibition occurred in the treatment group with 1 mg L^{-1} PBZ. These results were significantly different from those of the control group. The results above showed that the administration of 1-5 mg L^{-1} PBZ had an effect on plantlet growth. The activity of PBZ in inhibiting the formation of gibberellins resulted in reduced cell elongation in *D. bicaudatum* plantlets. The effect increases with the increase in the concentration of inhibitor. While in Prasayu et al. (2021) research, PBZ concentration of 9 mg L^{-1} is the optimal concentration in inhibiting *Grammatophyllum speciosum* synthetic seed growth. This means that each plant species shows different responses to high or low concentration of PBZ.

These results corroborate the research of Habibah & Sumadi (2013) and Syahid (2007) which stated that PBZ with a concentration of about 5.0 mg L^{-1} can be used as a growth inhibitor, including growth inhibitor for plants of the Prunus genus, where the greater the concentration of paclobutrazol does not cause greater inhibition (Krizan *et al*., 2007). The results of this research also corroborate Habibah & Sumadi (2013) and Zheng *et al*. (2012) who suggested that paclobutrazol is a retardant that inhibits the biosynthesis of gibberellins which play a role in the process of plant cell and tissue elongation. Paclobutrazol also reduces tissue metabolic activity and can inhibit vegetative growth processes. Several other studies have shown the inhibitory activity of PBZ on plant growth at various levels, depending on the species (Bello-Bello *et al*., 2015; Padilla *et al*., 2015; Roussos *et al*., 2016; Gimenes *et al.,* 2018).

In this research, the inhibition of the vegetative growth process of *D. bicaudatum* plantlets was indicated by the inhibition of increasing the number of leaves, number of roots, and root length on the mediums in the treatment groups. This is in line with the *in vitro* culture of pepper, which experienced a reduction in the number of leaves with paclobutrazol treatment (Yelnititis & Bermawie 2001). Meanwhile, the addition of PBZ to mediums with various concentrations caused an enlargement of the stem diameter, which was significantly different from that of the control group. Figure 1 showed that the largest diameter, 0.34 cm, was exhibited by plantlet treated with 1 mg L^{-1} PBZ, and the smallest diameter, which was 0.10 cm, was in the control group. The reduction in growth that occurred caused the distance between the leaves to be shorter so that the diameter of the stem became larger. According to Fletcher *et al.* (2000), reduced plantlet height was a consequence of induced gibberellin inhibition, exemplified by reduced internode elongation. Paclobutrazol (PBZ) has also been reported to suppress elongation growth in plants by interfering with cell division and enlargement processes with its anti-gibberellin activity.

The morphological differences of plantlets grown on mediums with PBZ and plantlets in the control group are presented in Figure 2. The leaves of *D. bicaudatum* orchid grown on medium with PBZ looked thicker, shorter, and dark green in color. Paclobutrazol has been reported to inhibit the normal catabolism of abscisic acid (Marshall *et al*., 2000). In the catabolism process, PBZ causes an increase in the concentration of abscisic acid in leaves. Abscisic acid causes stomata to close and reduces water transpiration from the leaves.

Table 1. The growth of protocorms of *D. bicaudatum* up to 120 days after planting on mediums with the addition of various concentrations of paclobutrazol

PBZ Concentration (mg L^{-1})	Number of Leaves	Number of Roots	Root Length (cm)	Stem Diameter (cm)
0	4.71 a	7.71 a	1.60 a	0.10 b
1	3.86 b	5.14 b	0.69 b	0.34 a
3	3.86 b	5.43 b	0.61 b	0.30 a
5	4.43 a	8.57 a	0.64 b	0.27 a
LSD 5%	0.67	1.38	0.25	0.08
SD	0.43	1.69	0.48	0.11

Note: Means followed by the same letters are not significantly different according to the LSD test at 5%

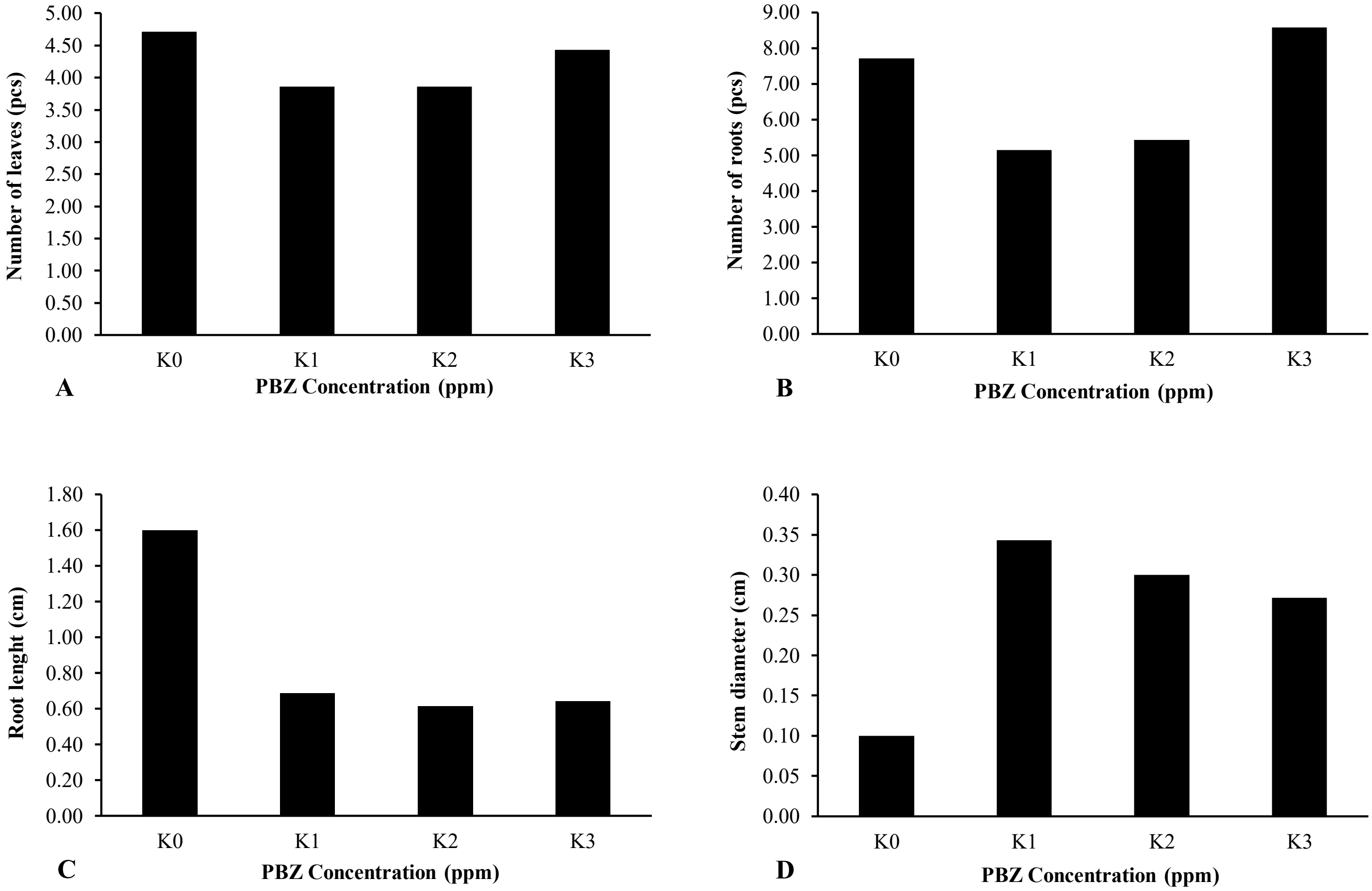

Figure 1. The Effect of PBZ on several variables of protocorm growth at 120 days after planting. K0 (medium without PBZ); K1 (MS+ 1 mg L^{-1} of PBZ); K2 (MS+ 3 mg L^{-1} of PBZ); K3 (MS+ 5 mg L^{-1} of PBZ). A. variable number of leaves; B. variable number of roots; C. variable lenght of root; D. variable diameter of steam.

Figure 2. Growth of *D. bicaudatum* orchid plantlets at 120 days after planting (A) D. bicaudatum on MS+PBZ medium. (B). *D. bicaudatum* on medium without PBZ that was used for the control group, bar =1.43 cm.

CONCLUSION

The medium-term conservation of *D. bicaudatum* orchids with the application of paclobutrazol showed a very significant result. Inhibition of vegetative growth in the form of growth in the number of leaves and root length was exhibited by the groups of treatments of 1-5 mg L^{-1} PBZ, while the stem diameter increased more in all treatment groups than in the control group. Reduction in the number of roots occurred in the treatments of 1 mg L^{-1} PBZ and 3 mg L^{-1} PBZ. These results indicate that paclobutrazol with concentration between 1-5 mg L^{-1} can be used for short-term conservation of *D. bicaudatum* plantlets but it is recommended to use PBZ concentration of 1 mg L^{-1} because it is more efficient.

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