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IN VITRO PROPAGATION AND ACCLIMATIZATION OF BLACK ORCHID (COELOGYNE PANDURATE LINDL.)

Authors: I.A. Astarini, V. Claudia, N.K.A.P. Adi, S.K. Sudirga, N.P.A. Astiti

Keywords: embryo rescue, Knudson C, W3 media, organic media, coconut husk

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Abstract:
Black orchid is an epiphytic orchid found mainly in Borneo Island, Indonesia. This orchid currently is facing a large conservation problem and threatened to extinction due to human exploitation. It is therefore an urgent requirement to develop efficient propagation technique to conserve this species. This research aims to determine growth response and the ability of black orchid seeds to grow in different medium and to establish the best media for acclimatization. The experiment was divided into 2 stages, seed growth in vitro, and acclimatization. Three different media were employed during seed growth in vitro, i.e. Western 3 media (W3), Knudson C media (KdC) and organic media (O). Each treatment consists of eight replicates. Acclimatization stage used 4 types of media; moss, fern stem, charcoal and combination of charcoal and coconut husk. Each consists of 8 replicates. Results shows that seeds started to grow at 3 weeks after sowing in W3 media, and 5 weeks after sowing in other medium. At 12 WAP, seed growth reached phase 5 (third leaf formed) on W3 medium, whilst on KdC and organic media, reached phase 4 (second leaf formed embryo). Within 3 months, the highest shoot produced was on W3 media (581 shoots), whilst on KdC and organic media produced 255 and 191 shoots respectively. Almost all plantlets (98%) survived during acclimatization period. Plantlets grow best on moss, compared to other media.

- Full Text (PDF format, 4698271 bytes)

http://www.actahort.org/books/1078/1078_21.htm
In Vitro Propagation and Acclimatization of Black Orchid (Coelogyne pandurata Lindl)

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Keywords: Embryo rescue, Knudson C, W3 media, organic media, coconut husk

Abstract

Black orchid is an epiphytic orchid found mainly in Borneo Island, Indonesia. This orchid currently is facing a large conservation problem and threatened to extinction due to human exploitation. It is therefore an urgent requirement to develop efficient propagation technique to conserve this species. This research aims to determine growth response and the ability of black orchid seeds to grow in different medium and to establish the best media for acclimatization. The experiment was divided into 2 stages, seed growth in vitro, and acclimatization. Three different media was employed during seed growth in vitro, i.e. Western 3 media (W3), Knudson C media (KdC) and organic media (O). Each treatment consists of eight replicates. Acclimatization stage used 4 types of media; moss, fern stem, charcoal and combination of charcoal and coconut husk. Each consists of 8 replicates. Results shows that seeds started to grow at 3 weeks after sowing in W3 media, and 5 weeks after sowing in other medium. At 12 WAP, seed growth reached phase 5 (third leaf formed) on W3 medium, whilst on KdC and organic media, reached phase 4 (second leaf formed embryo). Within 3 months, the highest shoot produced was on W3 media (581 shoots), whilst on KdC and organic media produced 255 and 191 shoots respectively. Almost all (98%) plantlets survived during acclimatization period. Plantlets grow best on moss, compared to other media.

INTRODUCTION

Indonesia has high diversity of orchids. It is estimated 1.400 species of native orchids found at Borneo, 1.126 species at Sumatera, 769 species in Java, 500 species in Sulawesi, 369 species in Molucca and 200 species in Nusa Tenggara (Semiarti et al., 2007). Popular orchids among consumers include Dendrobium (34%), Oncidium Golden Shower (26%), Cattleya (20%), Vanda (17%) and an increasing popularity orchid species, black orchid (Coelogyne pandurata Lindl.) (3%) (Martin et al., 2006).

Black orchid belong to sympodial ephypites from the Orchidaceae family. It has shrub type, where each plant connected by rhizome and usually has many pseudobulbs (Kartohardiprodjo and Prabowo, 2009). Flower of black orchid come in a 15-20 cm raceme, consisting approximately 14 flowers. Petal has light green color, and the labelum has a violin shape and black in color. The flower has sweet scent, with flowering period of March – June (Sastrapradja et al., 1976). This species currently has a serious conservation problem and threatened to extinction due to human exploitation, such as illegal harvest from the habitat,
illegal logging and land clearing. Furthermore, it has a very short period of flowering, so it is difficult for natural hybridization to occur (Untari and Pusptaningtyas, 2006).

It is therefore an urgent requirement to develop efficient propagation technique to conserve this species. A number of media formulations have been employed on in vitro culture of orchids, for e.g. Vacin and Went (VW), Murashige and Skoog (MS), White, Gamborg (B5), Gautheret, Schenk and Hilderbrandt (SH), Nitch & Nitch, Lloyd & McCown (WPM), Knudson C (KdC), Western 3 (W3), alternative media (Hyponex/Gandasil D, Vitabloom D), and organic media using natural ingredients such as coconut water, banana, potato and sweet potato (Wattimena, 1988). The objective of this research was to determine growth response and the ability of black orchid seeds to grow in different medium and to establish the best media for acclimatization.

MATERIAL AND METHODS

The experiment was divided into 2 stages, seed growth in vitro, and acclimatization.

1. Seed Growth in vitro. Capsule of black orchid were collected from CV. Gede Ayu Bali Orchid, Kerobokan, Badung, Bali. Four months old black orchid capsules (9-10 cm length) were sowed in 3 different media, W3 + 60 g/L banana puree + 7.5 g/L agar + 20 g/L sucrose + 1 ppm BAP, Knudson C (KdC) + 20 g/L sucrose +1 ppm BAP, and organic media (O) which consist of banana puree 60 g/L + potato extract 150 ml/L + 20 g/L sugar + 7.5 g/L agar + 0.5 g/L active charcoal + 1 ppm BAP. Capsules were washed using detergen, followed by soaking in 2 g/100 mL fungicide for 30 minute. Capsule were then washed 2 times using sterile water, brought inside the laminar and surface sterilized using alcohol for 10 second, followed by flaming 3 times, 2 second each. Sterile capsule were then placed in sterile petri dish, cut into half and culture on media. Each bottle/media consists of 5 scoop orchid seeds which were distributed evenly to all media surface (Figure 1). There are 8 replicate to each treatment combination. Variable observed include start of growing time (measure when 25% of the seed produce green embryos), growing phase (1-5) (Semiarti et al., 2010) and number of shoot per week up to 12 week.

2. Acclimatization. Media were steamed for 30 minute before used. When it cooled down, all media were immersed in 2 g/L fungicide for 5 minute, followed by dipping in Green Hyponex 2 g/L overnight. Media were then distributed to small pots and leaved for 3 days. Plantlets were removed from culture bottles, cleaned from agar and debris under running tap water, followed by immersing in 2 g/L fungicide for 5 minute to inhibit fungal growth and ready for transferred to pots. There are 4 media trialed; moss, fern stem, charcoal and combination of coconut husk and charcoal, each consists of 8 replicates. Transferred plantlets were kept inside tissue culture lab for 4 weeks before transferred to shade house with 80% paranet shade. Percent survival was observed every week for 8 week.

RESULTS AND DISCUSSION

Orchid seed started to grow 3 week after planting (WAP). At 12 WAP, seed growth reached phase 5 (third leaf formed) on W3 media, whilst on KdC and organic media, reached phase 4 (second leaf formed embryo) (Fig 2a). Within 3 months, the highest shoot produced was on W3 media (581 shoots, Fig. 3a), whilst on KdC and organic media produced 255 and 191 shoot respectively (Fig 2b, 3b, 3c). W3 media contained 2 types of potassium, i.e. potassium dihydrogen phosphate and potassium chloride which possibly induce faster growth compared to other media. Knudson C media was employed best to enhance growth of Dendrobium sp.
Addition of organic compound play an important role since it contained vitamins, amino acid nucleic acid, phosphorous and growth hormone such as auxin and gibberelin that induce seedling growth and development (Widiastoety and Bahar, 1995).

Almost all plantlets (98%) survived during acclimatization period in all treatment combinations. Plantlets grow best on media containing moss, compared to fern stem and combination media. Charcoal alone was not a good media as it cannot hold and provide moist for the plantlet, causing wilting of plantlet. Acclimatization is an important stage in plant tissue culture, to obtain a healthy plant grows in the field. Plants need good roots to absorb nutrients and able to pursue healthy growth. Therefore in the acclimatization process, appropriate media is needed to support roots growth and provide nutrients for the plantlet. The benefit of using fern stem is it is slowly decomposed and has good nutrient for orchid growth. Moss has a soft texture, retain water and nutrients for orchid needs. Charcoal is slowly decompose but has very little nutrient, while coconut husk is easy to decay but has good nutrient.

**CONCLUSIONS**

In vitro propagation of black orchid was successfully achieved in all media. The best media was W3 in which faster growth and more shoots were produced within 12 weeks. Easily prepared organic media give good alternative to reduce cost of media. Acclimatization was successful in all type of media. Moss is the best media, but combination of coconut husk and charcoal will give a more affordable option for orchid grower.

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**Literature Cited**


Figures

Fig. 1. Surface sterilization of black orchid capsule and seed culture in vitro.

Fig. 2. A. Growth phase of orchid seeds. B. Number of shoot produce on each media.

Fig. 3. Black orchid seedling at 12 WAP. A. W3 media, B. KdC media, C. Organic media