



Characteristics of Fungi Contaminant in Tissue Culture of *Bromheadia finlaysoniana* (Lind.) Miq

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ABSTRACT

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Generative propagation of orchid plants has problems because orchid seeds do not have endosperm that need to be multiplied using tissue culture. Contamination is a limiting factor in the multiplication of plants in tissue culture. This study describes the types of contaminants found in *Bromheadia finlaysoniana* callus. This research was carried out in two stages. The first stage is carried out culture of various explants of *Bromheadia finlaysoniana* using murashige and Skoog media with enrichment of hormone Benzylaminopurine 1 mg/l; (Naphthalene acetic acid) 0.5 mg/l; 100 mg mynositol; pyridoxine-HCl 0.5 mg/l; thiamine-HCl 0.1 mg/l; nicotinic-acid 0.5 mg/l; glycine 2 mg/l. The second stage is observed and the percentage of contaminant fungi. The observed contaminants character includes the colour, the direction of growth, and the hyphae colony's shape. Determination of contaminants type compared to the morphology of references. The result is the highest contamination in flower stalk explants, 81%; most colonies on leaf explants are 28. Contaminant fungi grow predominantly of fungi with white and grey colour and a rough surface shape. The macroscopic character of contaminant fungi in tissue cultures mostly comes from the class of Deuteromycetes and Zygomycetes.

INTRODUCTION

Orchids are famous for their beauty, various shape, size, flower colours, and distinctive scents which create a relaxing effect. The long durability of orchids results in their high economic value. *Bromheadia finlaysoniana* , one natural orchid species, is only found in a few conservation and protected forests such as Sintajo Raya conservation forest and Rimbang

Baling sanctuary (Puspitanangtyas (2009). Generative propagation of orchids often has several physiological constraints, such as the ability of orchid seeds to germinate, the need for a very long time to germinate and, the need for a symbiosis of orchids with mycorrhizal fungi. Orchid seeds have very little or almost no endosperm, so the availability of food reserves at the beginning of seed germination causes germination to occur (Bey *et al* 2006).

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Orchid propagation is more often done vegetatively, namely by tissue culture techniques. Tissue culture is a technique for isolating plant parts, whether in the form of organs, tissues, cells, or protoplasm, and then culturing these plant parts on artificial media with sterile and constrained environmental conditions for those orchids that need to be propagated using tissue culture techniques. Tissue culture is a technique for multiplying and culturing plant parts quickly on artificial media under sterile and controlled environmental conditions (Santoso *et al* 2019). Orchid propagation through tissue culture has been studied extensively to conserve and propagate orchid seeds (Setiaji and Annisa 2020).

However, explants resulting from tissue culture are often contaminated by several microbes. Tissue culture was constrained Orchid plant extracts propagated by high levels of contaminants. Based on Heriansyah & Indrawarnis (2019), leaf and stem explants had a high percentage of roots and seeds. This contamination may emerge from explants (both internal and external), media contamination, or less sterile tools and working area. Microbes groups that may contaminate the explants are fungi, bacteria, viruses (carried away by propagation material) and nematodes that can inhibit the growth of explants into callus or whole plants in the media *in vitro*. Fungi dominate contamination in tissue culture compared to other microbes (Wati *et al.* 2020).

Fungi as the major contaminants in tissue culture should be identified. Determination of morphological characteristics of the fungi is the first step in identification. This study aims to describe contaminants of fungi species in the tissue culture of *Bromheadia finlaysoniana*.

MATERIALS AND METHODS

This research was conducted in the Network Culture Laboratory of Riau Islamic University. The series of activities carried out in the research include:

Sterilization and media manufacture

The tools used in tissue culture activities and to detect fungi are sterilized first by

autoclaving at a pressure of 2 atm and a temperature of 121°C for 15 minutes (Gunawan *et al.*, 2004). MS medium with hormone enrichment 6-BAP (Benzylaminopurine) 1 mg/l; NAA (Naphthalene acetic acid) 0.5 mg/l; 100 mg myoinositol; pyridoxine-HCl 0.5 mg/l; thiamine-HCl 0.1 mg/l; nicotinic acid 0.5 mg/l; glycine 2 mg/l; 30 g/l sucrose and 10 g/l agar; with a pH adjustment of 5.7 using NaOH and/or HCl. Media sterilization using autoclave at 1210C; 1.2 kg/cm² for 30 minutes.

Planting Explanation

Orchid plant explants before sterilization. The explants were sterilized through graded sterilization using 70% hypochlorite, 95% alcohol and, distilled water. All parts of the explant were waiting under running water, then immersed in 70% hypochlorite solution while shaking for 15 minutes, and then soaked in 95% alcohol in the same way and then rinsed with distilled water. The explants were cut to 0.5 mm x 1 mm, then inserted into sterile media, and then incubated in a culture chamber to induce callus. Culture growth was observed daily for contamination. Contamination was observed visually every day until the seventh day in culture bottles. The fungal morphology observed were colony colour, colony growth direction and, colony surface shape.

RESULTS AND DISCUSION

Contaminating fungi grow in all isolated cultures. Percentage of contaminant fungi that grow on tissue culture *Bromheadia finlaysoniana* in some explant varied from 18%-81% (Table 1).

The highest percentage of contaminant fungi in *Bromheadia finlaysoniana* tissue culture came from the flower (81%), and the lowest one came from roots (18%). Colonies of fungi of varying amounts overgrew Fungal-contaminated cultures. Total colonies of fungi contaminants that grow the most are found in the leaf explant culture of 28 colonies, although the percentage of contaminants is only 75%. Entire colonies of fungi contaminants that grow are calculated from the differences in the morphological character of

Table 1. Percentage and total fungi contaminating *Bromheadia finlaysoniana* (Lind.) Miq tissue culture

Explant source	Contamination percentage (%)	Total colonies of fungi
Roots	18	4
Stems	68	18
Flower stalks	81	25
Leaves	75	28

the fungi that grow on each explant bottle.

The differences in percentage and number of fungi contaminant colonies in each explant are thought to be due to differences in the structure of each explant. Leaf explants have a wider structure so they can stick to each other during the sterilization process. According to Haris *et al.* (2009), contamination is a limiting factor for the success of tissue culture that can come from (1) plant material factors, external (epiphytic microbes) and internal (endophytic microbes), (2) small organisms that enter the media, (3) culture bottles and equipment that is less sterile, (4) work environment and cultural space, (5) human error, and temperature and humidity factors, (6) wet months, contamination will be high. According to Juarna (2016), leaf explants are more susceptible to the appearance of contamination compared to other plant organs. Widiastoety (2001) states that contamination of planted explants can occur due to externally and internally infection.

External contamination will appear two to three days after planting. Internal contamination will occur after four days after planting. In this study, the average fungi grow three days after planting. Macroscopic characters from observations of contaminant fungi in orchid tissue culture in several explants obtained various amounts. The differences between these fungi are characterized by colony color, growth direction and, the shape of the colony surface (Table 2).

Macroscopic observations that include colony color, colony growth direction, and colony surface shape were the first steps in identifying microbes. Differences in each colony color, the direction of growth, and surface shape are inferred colonies that grow are different types of fungi. From macroscopic observations of contaminant fungi, there were two types of colonies on the root explant with color colonies olive and whitish colony growth symmetric and colony texture velvety smooth (Figure 1). Two types of colonies on stem explants with same colony color grey and colony growth symmetric and colony texture powdery rough and velvety smooth (Figure 2). Three types of colonies on the explant of flower stalks with colony color, grey, dark grey, and beige, colony growth same symmetric and colony texture rough smooth. The leave explant five types different from the color types of colonies the direction of growth was dominant symmetrical and diverse colony texture (Figure 3).

Table 2. Macroscopic character of fungi contaminant on some explants of *Bromheadia finlaysoniana*

Explant	Macroscopic Character		
	Colony color	colony growth	Colony texture
Roots	Olive	Symmetric	Velvety Smooth
	Whitish	Symmetric	Velvety Smooth
Stems	Grey	Asymmetric	Powdery rough
	Grey	Asymmetric	Velvety Smooth
Flower stalks	Grey	Symmetric	Velvety Rough
	Dark grey	Symmetric	Velvety Rough
	Beige	Symmetric	Velvety Rough
Leaves	Olive	Symmetric	Velvety Rough
	Whitish	Symmetric	Velvety Rough
	Grey	Symmetric	Velvety Smooth
	Whitish	Asymmetric	Powdery rough
	Whitish	Symmetric	Velvety Smooth

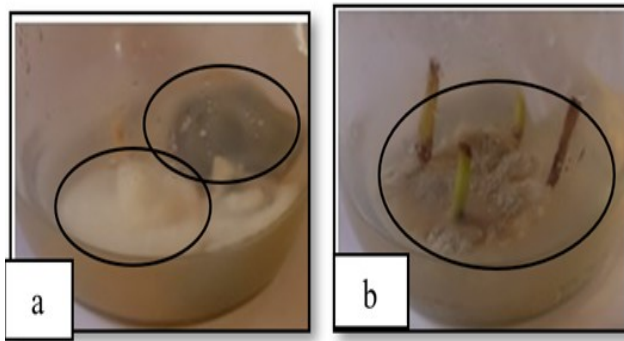


Figure 1. fungi contaminant a. fungi contaminant on roots explants, b. fungi contaminant on stems

In each treatment, the growing contaminant fungi were dominated by white-grey fungus with a rough surface, in line with research conducted by Oratmangun *et al.* (2017), who observed fungal contamination in callus *Catharanthus roseus* (L.), G. Don, that the growing contamination fungus is dominated by white and grey colony colors with a rough surface shape. The macroscopic character of fungal contaminants in tissue culture comes mostly from the class of Deuteromycetes and zygomycetes. The class of Deuteromycetes has macroscopic features of white, grey to blackish colony color with symmetrical growth direction and rough surface, while classed zygomycetes have a grey, green colony color with a spread growth direction and a rough surface (Waluyo, 2004).

Contaminants in culture are influenced by several factors, including the cleanliness of the tools, materials, and explants used. Differences in contaminant fungi that grow in several cultures are forgotten because of the different types of explants used. According to Juarna (2016), there is a different character of fungi contaminants mushrooms that grow on the culture of leaf explants and leaf stalk explants. The similar character of contaminants in each culture is alleged because of the treatment and the same growing media. According to Waluyo (2004), microorganisms need nutrients that act as energy sources and cell building materials for survival. The necessary foodstuffs are water, energy, carbon, mineral, and nitrogen. The need for nutritional substances varies for each microorganism. Oratmangun *et al.* (2017) have similar characters of contaminant mushrooms in the use of medium media MS (Murashige and Skoog).

CONCLUSIONS

The highest fungal contamination in *Bromheadia finlaysoniana* explants was found in leaves. White and grey colonies colours dominated the morphological characteristics of each fungi contaminant with symmetrical growth directions and coarse



Figure 2. fungi contaminant on stem explants



Figure 3. fungi contaminant on stem leaves

colony textures. The macroscopic characters have shown that the fungi were determined as Deuteromycetes and Zygomycetes class.

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