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Morphological Characteristics of Ectomycorrhizas on Merbau [*Intsia bijuga* (Colebr.) O. Kuntze]

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Merbau [*Intsia bijuga* (Colebr.) O. Kuntze] is one of valuable timber tree in south-east Asia which has been known having ectomycorrhizae (EcM) though being ignored. Identification of the ectomycorrizae is prime important for being basis of further studies. This study investigated the EcM fungi associated with Merbau by using both sporocarp morphology and EcM morphotypes. Morphological characters of sporocarps and basidiocarps of the fungi and EcM morphotypes obtained from seedlings and trees from natural and plantation stands of merbau, as well as from nurseries were compared to the description of those resulted from baiting method. Only one species of ectomycorrhizal fungus was found associated with merbau [*Intsia bijuga* (Colebr.) Kuntze] which has not been described yet. The fungus formed mycorrhizae with monopodial pinnate branching. The fungus was identified belonging to the genus of *Scleroderma*. The fungus was more common to occur beneath merbau seedlings than trees. The sporocarps may be yielded under greenhouse condition and could be cultured in MMN agar media, thus it facilitates to the production of inoculums used for further studies.

Key words: Merbau, ectomycorrhizae, *Scleroderma*, morphological characters, morphotype

INTRODUCTION

Merbau [*Intsia bijuga* (Colebr.) O. Kuntze] belongs to family of Fabaceae, subfamily of Caesalpinoideae which is geographically distributed from Madagascar towards Malaysia, Indonesia, Australia, and Polinesia. Main ecological regions of this species in Indonesia are Sumatera, Borneo, Celebes, Java, Moluccas, East Nusa Tenggara, and Papua. However, this species is naturally dominant in all tropical lowland forest of Papua and Papua Barat Province of Indonesia. The species considers as one of valuable timber tree species in South-East Asia and is the main targeted species for commercial production of sawn timber (Dress 1938; PROSEA 1994).

Merbau, as subfamily of Caesalpinoideae, is characteristically not a nodulated tree, and has been well known exclusively forming ectomycorrhizas (EcM). No evidence is being reported yet that this tree species has an association with endomycorrhizas (vesicular-arbuscular mycorrhizas) fungi (Smith & Read 2008). Mycorrhiza association takes importance roles in mechanisms of survival for plants in areas with poor soil conditions by enhancing the plant ability to take up nutrients especially phosphorus which are frequently present as limited factors for plant growth (Pedersen & Silvia 1996; Smith & Read 2008). Until now, the role of fungi involved in the symbiosis of EcM with merbau is unknown.

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Although the ectomycorhizal association has long been recognized (Wattling *et al*. 2002; Smith & Read 2008), the comprehensive study of the ectomycorrhizae on merbau is having less scientific attention. On the other hand, identification of the ectomycorrhizal fungi is prime important for further studies. EcM fungi may be identified based on morphology of sporocarp (Brundett *et al*. 1996), morphotype of the EcM (Agerer 2006) and later molecular biology (Smith & Read 2008). However, although molecular biology is considered as more accurate method for identification yet, the classical method based on morphology and morphotype are still regarded as more applicable and practical methods to be used in the field. Recently, only Tedersoo *et al*. (2007) identified 15 species of EcM fungi associated with natural stands of merbau of the Seycheles by using DNA sequencing of mycorrhizal root tips, but their study was lack of morphological descriptions.

The objective of the present study was to investigate the EcM fungi associated with Merbau by using both sporocarp morphology and EcM morphotypes. The results obtained in our work increases the knowledge of morphology characters of the fungi and EcM which provides helpful basic information for further studies.

MATERIALS AND METHODS

Site of Study. The Study site is placed at Manokwari district West Papua Province, geographically lies between 133°58'60"- 134°04'03"E, and between 0°46'05"- 0°50'38"S. It consists of beach forests and low land forests which are stretched from the beaches to hills with the elevations of 110 m. In this area merbau stands are commonly found

in groups and associated with other vegetations such as *Pometia pinnata* J.R. Forster & J.G. Forster, *Calophyllum inophyllum* L. and *Palaquium amboinense* Burck. Soils are sandy soils in the beach forests and calcareous soils in lowland forest. There are also plantation and non-permanent nurseries of merbau in this area. The stands in plantations were 50 years old with planting distance of 4 x 5 m. Meanwhile, the nursery is placed at Bogor District West Java Province, geographically located in between 106°43'30"E and 06°33'15"S. Soil in this site is originated from alluvial deposits.

Field Survey and Baiting Method. Natural stands, plantations and nurseries of merbau in Manokwari District, West Papua Province and a nurseries in Bogor District, West Java were visited three times during the months of December 2006 to March 2007 for collecting sporocarps, merbau ectomycorrhizal root tips, merbau wildlings/ seedlings and soils. Each sporocarps found were photographed to describe its size and colour, and further dried in an oven at 50 °C for 24 h and stored at room temperature. Fresh sporocarps were also brought to the laboratory for isolation. Root samples were collected using a trowel and digging around the trees to a maximum depth of 20 cm, following the root system. The root samples were taken out with the soil to minimize damages to ectomycorrhizal tips, placed in plastic bags, subsequently transported to the laboratory and stored in refrigerator at the temperature of 4 °C until its examination.

Baiting method was performed to get sporocarps of EcM fungi associated with merbau especially the fungi which colonized during seedling stage, as well as EcMs formed by the association. Merbau wildlings/seedlings with the height of 20-50 cm (approximately 4-8 moths old) which were collected from the field were further planted in pots filled with soil media taken from natural stands of merbau. The baiting plants were maintained in greenhouse. The sporocarps and EcMs produced through this method were described and compared to the characters of those found in the field.

Morphology and Anatomy Characterization. The main features were described based on sporocarps and basidiospore morphologies using naked eyes and a compound microscope, oftenly requiring a 100x oil immersion objectives. Colours, measurements and shapes of all microscopic characteristics were obtained in 3% KOH, 70% Et.OH and Melzer's reagent. Spores were also examined under scanning electronic microscope (SEM). All macroscopic and microscopic characteristics were used for determination and identification by comparing them to the references (Giovanni 1985; Rifai 1987; Largent & Baroni 1988; Sims *et al*. 1995; Brundrett *et al*. 1996; Keizer 1998; Chen 2006; Watling 2006; Sanon *et al*. 2009).

Ectomycorrhizal root tip fragments were fixed in FAA (5% Formalin, 5% acetic acid, and 90% alcohol) for 24 hours. Cross sections of ectomycorrhizal were observed under a compound microscope for histology studies of morphotypes (following Agerer 2006).

Characterization of *Scleroderma* sp. Fresh sporocarps of *Scleroderma* sp. from the field were clean and washed in sterilized water containing 40% alcohol for 10 sec, followed by sterilization in 0.2% $HgCl_2$ for 10 sec. Slices of the sporocarp were planted in Modified Melin-Norkrans (MMN) agar media. Composition of MMN agar media followed the receipt of Kjøller and Bruns (2002) with little modification, i.e. 8.0 g glucose, 2.5 g malt extract, 1 g yeast extract, 50 mg $CaCl_2 \cdot 2H_2O$, 25 mg NaCl, 150 mg $MgSO_4 \cdot 7H_2O$, 250 mg $(NH4)_2HPO_4$, 500 mg KH_2PO_4, $FeCl_3$ (1%, v/v) 2 ml, 1 mg thiamine-HCl, and agar 15 g, for 1000 ml. The growth of fungi in MMN agar media was recorded, including the diameter of the growing fungus colony for 2 months and, the description of the colour and the textures of mycellia.

RESULTS

Identification based on the morphological characters of sporocarps and basidiospores of the specimens collected from beneath the natural stands, plantations and nurseries of merbau, it was found only one species i.e. *Scleroderma* sp. forming ectomycorrizae with merbau. Strongly confirmation that only one species of fungus formed EcM with merbau was obtained by carefully observing the connection of the root systems of the plant with the mycelia system of the fungi, and the consistency of occurrences of the fungi in the fields. Furthermore, histological analysis of all EcM root samples showed similar characteristics of morphotype. The fungus sporocarps most commonly occurred on the root system of merbau seedlings than that on mature trees.

Through baiting method, the sporocarps were successfully produced on 8-12 months after planting the wildlings in the greenhouse. Furthermore, based on the characteristics of the sporocarps and the morphotype of the mycorrhizae, the fungus and the mycorrhizae found in the field were identical.

Scleroderma sp. is a member of Basidiomycota, fam. Sclerodermataceae. The species has basidioma: epigenous, globose, later irregular when mature, 0.66 ± 0.17 cm in diam., with smooth surface, creamy white to brownish white. Peridium: rigid, simple, 0.2-1.0 mm thick, yellowish white. Sliced fresh of basidioma produced yellow exudates in Et.OH. Gleba: yellowish to light brown when young and dark brown when mature, without columella but having external basal pad. Clamp connection: present. Badiospores: pale brown in Melzer's reagent and KOH, globose with reticulate ornamentation, 8 ± 10 ì m in diam. (Figure 1).

Ectomycorrhizae formed very thick mantel (12 ± 25 ì m), rough surface, creamy white in colour and monopodial pinnate or simple branching in the part of the young ectomycorrhizae, but becoming irregular pinnate in the base parts of the older ectomycorrhizae, sometimes ectomycorrhizae roots were interwoven very densely. The mantel structure was consisted of one layer of pseudoparenchymatous tissues. Moreover, hartig net was well developed within epidermal layer (Figure 2).

Scleroderma sp. was sucessfully isolated and cultured *in vitro* in Modified Melin-Norkrans (MMN) agar media using slices of sporocarp. Isolation using ectomycorrhizal root tips and mycellia were failed due to contamination.