Effect of AMF Inoculation on the Growth of Combretocarpus rotundatus (Miq) on a Peat Soil from Central Kalimantan

(For Restoration Ex-Mega Rice Project Central Kalimantan)

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Abstract: *For the restoration of degraded peat swamp forest, silvicultural techniques using Arbuscular Mycorrhizal Fungi (AMF) are considered necessary. AM fungi symbiotic relationships play important role for the growth and survival of trees. The objective of this study is to observe the AM fungal status of a typical peat swamp forest in Block C at Kelampangan in Central Kalimantan which has been cleared and disturbed. Currently the site is occupied by various pioneer plants. Soil samples from the rhizosphere of five pioneer vegetation (Melastoma sp, Combretocarpus sp, Acacia sp, Cratoxylon sp, and Nephrolepsis sp) were collected and spores were isolated from the soil using a wet sieving and decanting method. Greatest spore number was observed in the rhizosphere of Melastoma sp, followed by Acacia sp, and Combretocarpus sp. Three genera of AM fungi were found, namely Glomus, Gigaspora, and Acaulospora. A field experiment was conducted to examine the effect of the arbuscular vesicular mycorrhizal fungus Glomus sp 1, Glomus sp 2 and Gigaspora sp and seedling media on the growth of Combretocarpus rotundatus (Miq). In general the AM fungi used in this study showed beneficial effect on plant growth parameters. The colonization by Glomus sp 1 produced the highest plant height, stem diameter and leaf number per plant. On the peat soil medium, Combretocarpus rotundatus (Miq) showed a high degree of dependence on mycorrhizal association, increasing with the age of the plants.*

Key words: *Peat-swamp forest, ex-MRP, AMF*

Tropical peat lands make up approximately 12% (30-45Mha) of the total global peat lands (Immirizi and Maltby, 1992), of which Indonesia supports the greatest area, 20 Mha (Radjagukguk, 2001). In the past decade large areas of the Indonesian peat lands have experienced serious damage as a result of human activities such as logging and drainage. Pearland site development is often associated with the construction of drainage canals in order to make the land usable for agriculture or, more often, for oil palm and pulp wood plantations. Canals and ditches are not only built to control and lower the water level but also to facilitate access to the peat swamp forest and for transportation of timber logs (Jaenicke and Siegert, 2008). After forest clearance, peat becomes more *susceptible to fires and flooding, resulting in a downward cycle of retro-succession, with floral diversity becoming dominated by shrubs, ferns and sedge (Page et al., 2008). When undisturbed, peatlands play an important role in the global carbon (C) cycle, storing about 450 Gt C, which the amount is about 30% of the global soil carbon pool (Gorham, 1991), and peat lands act as carbon sinks (Page et al., 2002), support extremely high levels of floral and faunal biodiversity (Cheyne et al., 2007), maintain stable hydrological and nutrient cycling systems (Wösten et al., 2006) and provide means of livelihood for local communities (Smith, 2002).*

The Indonesian Government has a national program to accelerate rehabilitation of degraded peat swamp forest. Peat soil has an extremely low pH and revegetation of the peat soil is difficult because of the high concentration of toxic elements, such as aluminium, and poor nutrient availability. However, it is not easy to rehabilitate this ecosystem immediately, because it is necessary to select and produce high quality tree seedling species for rehabilitation. In order to implement successful rehabilitation programs, transferable methods need to be developed. This paper considers the use of mycorrhizae inoculation for seedling transplants as one of these potential methods.

Mycorrhizae are fungi that form symbiotic relationships with plant roots. The two most common forms of mycorrhizae are ectomycorrizae and arbuscular mycorrhizae fungi (AMF). In exchange for sugars produced by the plant, the mycorrhizae provide additional phosphates, nitrates, and other micro and macronutritents, can increase water supply through times of drought, and reduce susceptibility to soil-borne pathogens (Dell, 2002). Mycorrhizae play a crucial role in ecosystem processes, including plant and soil community composition and ecosystem resource capture (Rillig, 2004).

Studies in the tropics, of the relations between mycorrhizae, their plant hosts and their ecosystems have been under studied in relation to the temperate zone, though in recent years this has begun to be remedied (for example, Kiers et al., 2000; Lovelock and Ewel, 2005; Tawaraya et al., 2003). These studies have shown mycorrhizae are just as important in tropical as in temperate biomes. Tawaraya et al., (2003) established that in TPSF 17 of 23 Dipterocarp species hosted both AMF and ectomycorrhizae. Equally, once the correct mycorrhizal species was established for a given host species, the symbiosis was shown to increase plant biomass, height, leaf production and nutrient assimilation, under nursery conditions (Turjaman et al., 2005; Turjaman et al., 2006).

Arbuscular Mycorrhizal Fungi (AMF) are symbiotic associations, formed between plants and soil fungi that has essential role in plant growth, plant *protection, and soil quality that benefit both partners. The phytobiont correspond to approximately 80% of plant species and the fungi are classified in the phylum Glomeromycota, including nine genera; Glomus, Paraglomus, Acaulospora, Entrophospora, Gigaspora, Scutellospora, and Archaeospora (Dalpe, 2004).*

The symbiotic relationship between AMF and the roots of higher plants contributes significantly to plant nutrition and growth (Auge, 2001; Amaya-Carpio et al., 2009). These positive responses in productivity to AMF colonization have mainly been attributed to the enhanced uptake by AMF of relatively immobile soil ions such as phosphorus, potassium, calcium, magnesium, sulphur, iron, zinc, copper, and manganese (Marschner et al., 1994; Liu et al., 2007), but also involve the enhanced uptake and transport of far more mobile nitrogen ions, particularly under drought conditions (Liu et al., 2007). Enhanced plant growth might also result from soil-borne pathogen (e.g. nematodes, pathogenic fungi, bacteria) protection (Cardoso and Kuyper, 2006; St-Arnaud and Vujanovic, 2007), improved soil structural development and aggregate stabilization (Miller and Jastrow, 2000; Picone, 2003; Rillig, 2004; Wright, 2005).

AMF symbiosis, a natural association between the roots of higher plants and arbuscular mycorrhizal, are important in trees, because AMF are believed to improve host plants growth, water relations and acquisition of nutrients especially P from soil (Bucher, 2007). Mycorrhizas affect the maintenance of vegetation in various ecosystems, and may play an important role in tropical peat swamp forest. AMF may be formed even in trees, which grow in the peat swamp forest.

Combretocarpus rotundatus Miq is classified into fast-growing tree species and has tolerance to strong light, drought and high soil temperature condition in their regenerate and survivorship (Saito et al., 2002). This species is also an important timber tree in tropical peat swamp forest in Indonesia. C. rotundatus is distributed over scattered locations of Kalimantan and Sumatra islands, and attains height of up to 30 m and diameter of up to 60 cm. This species is common and often gregarious in tropical peat swamp forest.

Peat land of Central Kalimantan is distributed in the lowland area, about 5 – 35 m above sea level, and stretches approximately 200 km inland (Fig. 1). It holds nearly 27% of the total peat land on Kalimantan Island (Shimada, 2001). Some large rivers, such as the Kapuas, Barito, and Katingan, flow from the mountains of northern side of the peat lands through the peat lands to the Java Sea. Peat thickness varies among peat land types, from approximately 0.5 to 9.7 m (Shimada et al., 2001). Tropical peat swamp forest occupies most of the Central Kalimantan area. Part of the forest has been either cleared for cultivation and logging or destroyed by peat land fires. The study site is located in the Kelampangan forest. A *large part of this forest has been degraded both by cultivation and human settlement and by the enormous forest and peat fires in 1997.*

METHODS

Isolates of VAM fungi were collected from rhizosphere of pioneer plants in Ex Block C Mega Rice Project (MRP) in Central Kalimantan (Fig. 1). Five different pioneer plants, namely Combretocarpus sp, Acacia sp, Melastoma sp, Cratoxylon sp, and Nephrolepsis sp were compared. Isolation and identification of AM fungal spore were conducted in the Laboratory of Microbiology, Faculty of Agriculture, Gadjah Mada University in Yogyakarta. The identification followed Manual for Identification of VAM fungi and examination was conducted under a dissecting microscope (Brundrett et al., 1996).

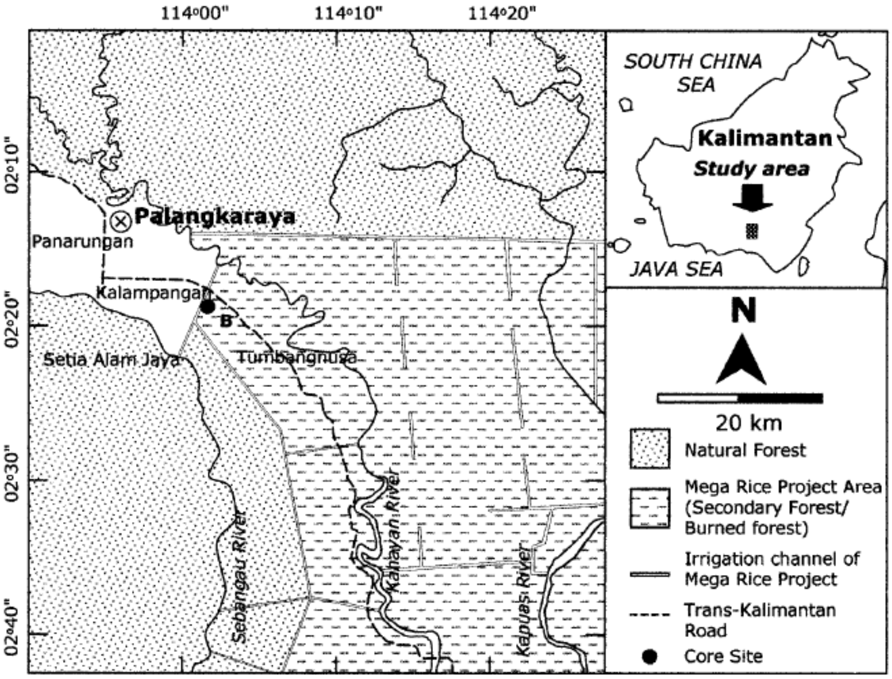

Figure 1. Map of the study site (after Yulianto et al., 2004).

Seeds of C. rotundatus were the surface sterilized in 70% alcohol for 5 min, subsequently rinsed with sterilized water and germinated on sterilized peat soils. Four week old uniform seedlings were transplanted into plastic pots (30 x 30 cm) containing 2.0 kg autoclaved (0.11 MPa, 121^{0}C, 2 h) peat soil with a pH of 3.3, and 5.91 mg kg^{-1} available phosphorus. The experimental pots were placed in a plastic greenhouse under natural light conditions from September, 2007 to January, 2008, which had no controlling temperature equipment. The average day/night temperature was $29/21^{0}$C and the relative humidity was 70-90%.

The AMF used in this study were Glomus sp TD15, Glomus sp D32, and Gigaspora sp obtained from Block C ex-PLG Central Kalimantan. The inoculation with AMF was added as soil from pure pot cultures containing spores and *mycorrhizal root fragments, 30 g/pot of inoculum was placed directly below the seedlings. Non-AMF treatments (control seedlings) received the same weight of autoclaved growth media.*

The experimental treatments were made up of three AMF inoculations (Glomus sp TD15, Glomus sp D32, Gigaspora sp, and non-AMF) under greenhouse nursery condition and were arranged in a completely randomized design. Each treatment had six replicates and, hence, there were a total of 24 pots with one seedling per pot.

Five months after planting, plant height, stem diameter and leaf number per plant were recorded and the AMF and non-AMF seedlings were harvested. All of the seedlings were separated into roots and shoots, dried in hot-air oven at 75^0C *for 2 d, and dry weights of shoots and roots were recorded. Part of fresh roots was carefully washed, cut into 1 cm long root segments and fixed by FAA at least 24 h. The root samples were cleared with 10% KOH solution, stained with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970), and examined microscopically for root colonization. The AMF infected percentage was calculated by the following formula: AMF colonization (%) = 100 x root length/root length observed (Smith and Read, 2008).*

The experimental data were subjected to analysis of variance (ANOVA) with Statistical Analysis System (SAS) 8.1 software, and Fisher's Protected Least Significant Difference (LSD) (ρ=0.05) was used to compare treatment means.

RESULTS

The results showed that AM fungi was abundant in rhizosphere of pioneer plants and identification to the spore revealed that there were three genera of AM fungi, namely Glomus, Gigaspora, and Acaulospora (Table 1 and Fig. 2, 3).

In the nursery conditions, Glomus sp TD15, Glomus sp D32, and Gigaspora sp, formed AMF in Combretocarpus rotundatus Miq seedlings (Table 2). AMF colonization was higher than 60% in all inoculated seedlings. There was significant difference between three AMF species and control seedlings. AMF colonization was highest in seedlings colonized by Glomus sp TD15 than with the other AM fungi species. AM fungi colonization of C. rotundatus using spores of Glomus sp TD15, Glomus sp D32, and Gigaspora sp increased plant height, stem diameter (Fig. 4, and 5).

Table 1. Type and Spore Number Of VAM Fungi in The Rhizosphere of Pioneer Plants.

No.	***Plant***	***Spore number per 100 g soil***	***AMF species***
1.	*Combretocarpus sp*	*68*	*Glomus sp;Acaulospora sp; Gigaspora sp*
2.	*Acacia sp*	*108*	*Glomus sp; Gigaspora sp*

3.	*Melastoma sp*	*263*	*Glomus sp;Acaulospora sp; Gigaspora sp*
4.	*Cratoxylon sp*	*8*	*Glomus sp;Acaulospora sp*
5.	*Nephrolepsis sp*	*7*	*Glomus sp*

Table 2. Plant Growth and AMF Colonization of C. Rotundatus Inoculated With Three VAM Types 5 Months After Transplanting Under Nursery Conditions.

Treatment	***Plant growth***				***AM Colonization (%)***
	Shoot height (cm)	***Stem diameter (mm)***	***Leaf number per (plant)***	***Dry weight (g/plant)***	
Control	*10.26 c**	*2.16 c*	*10 c*	*4.45 c*	*9 c*
Glomus sp TD15	*41.96 a*	*5.16 a*	*36 a*	*39.51 a*	*74 a*
Glomus sp D32	*26.30 b*	*4.33 a b*	*28 a b*	*35.23 b*	*68 b*
Gigaspora sp	*16.43 b c*	*4.16 a b*	*27 a b*	*11.34 c*	*63 b*

Values followed the same letter are not significantly different ($\rho<0.05$)

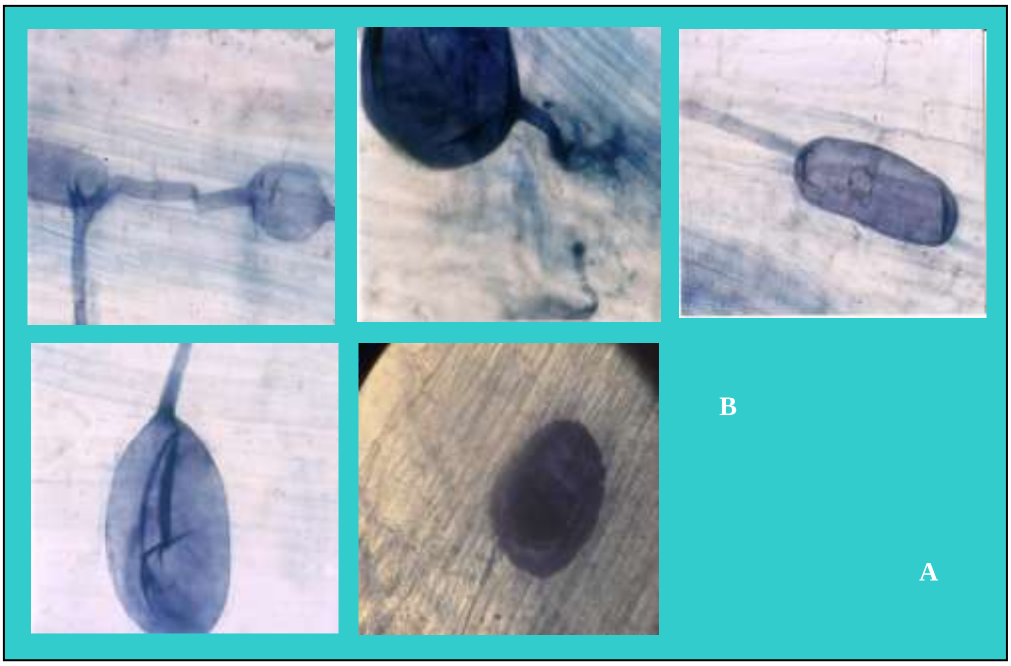

Figure 2. Vesicular (V), intraradical hyphae (A), and extraradical hyphae in

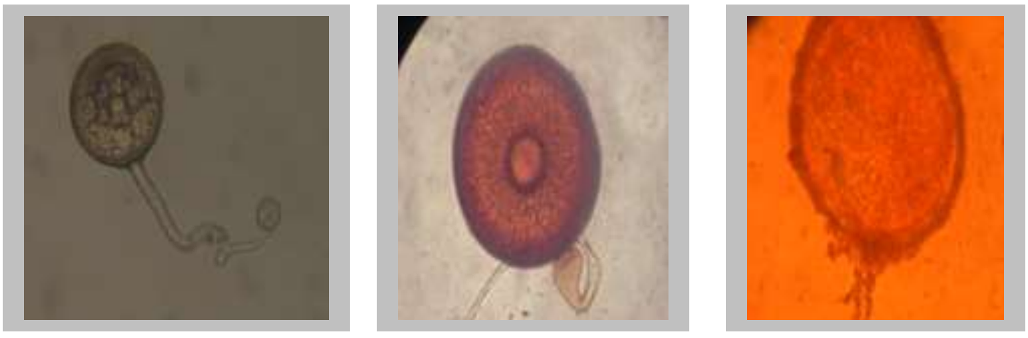

Figure 3. Spores of Glomus sp (A), Color: a continuum from white to yellow-brown, Shape: globose, Spore wall: three layers. Gigaspora sp (B), Color: yellow-brown, Shape: Globose to subglobuse, Spore wall: consisting of three layers. and Acaulospora sp (C), Color: orange-brown, Shape: globuse, Spore wall: Three layers.

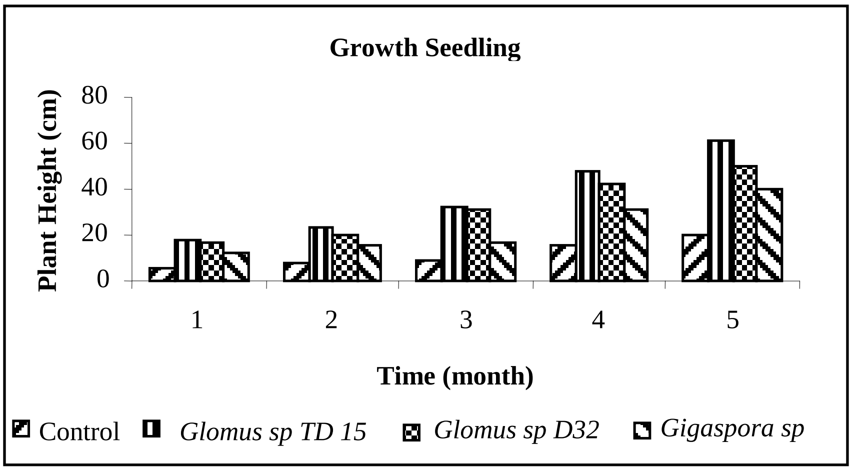

Figure 4. Height increase with time of Combretocarpus rotundatus plants inoculated with three AMF types.

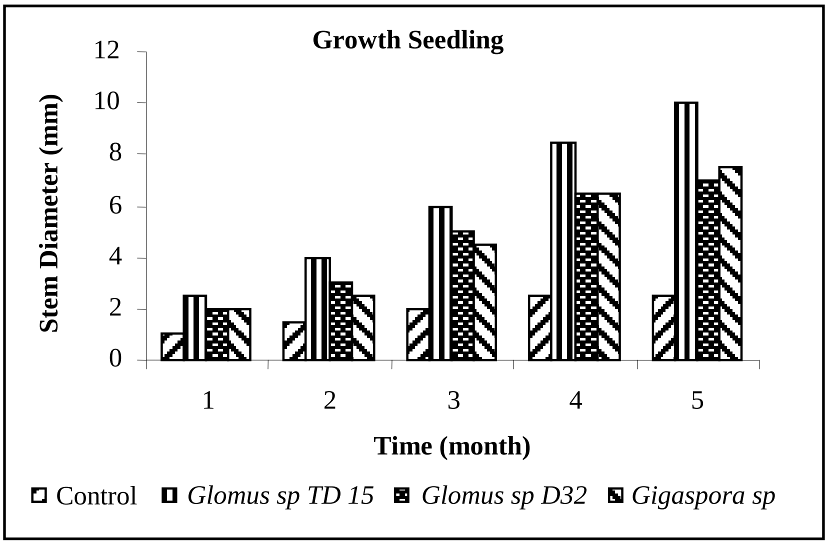

Figure 5. Stem diameter increase with time of Combretocarpus rotundatus plants inoculated with three AMF types.

DISCUSSION

The greatest spore number was obtained in the rhizosphere of Melastoma sp (263 spores), followed by rhizosphere of Acacia (108 spores), and rhizosphere of Combretocarpus sp (68 spores). AM fungi are ubiquitously associated with the large majority of plant families in different ecosystems across the world ranging from the tropics to subtropics (Gai et al., 2006). Opik et al., (2006) suggested that the location effect on AM fungal communities might be significant through a meta-analysis of 26 publications that reported AM community compositions in different locations of the world. They also pointed out, however, that there were too few studies on fungal communities of the same plant species at different site.

Even though the AM fungi of trees in peat swamp forest has been reported (Tawaraya et al., 2003), the role of AM fungi in species originating from peat *swamp forest needs to be clarified. This result demonstrate for the first time that AM fungi have positive effects on Combretocarpus rotundatus Miq after five months under nursery conditions. Colonization by three AMF species was identified as the most appropriate fungi for improving early growth of Combretocarpus rotundatus Miq after five months under greenhouse nursery conditions. Therefore, Glomus sp TD 15 is to be AMF candidate for production of C. rotundatus under nursery conditions. Similar result has also been reported by Morte et al., (2008) inoculation with Glomus mosseae significantly increased the growth of Phoenix canariensis. The positive effect of Glomus sp colonization on Canary palm has also been observed under different growth condition (Morte and Honrubia, 2002) and in other palms like Bactris gasipaes. Smith and Read, 2008) concluded that, the arbuscular mycorrhizal fungi supply nutrients to their host plant, especially phosphorus. The fungi absorb phosphate from the soil through their extraradical hyphae and incorporate it into the cytosolic pool, while the excess of phosphorus (P) is transferred to the vacuoles and translocated to the intraradical hyphae (Saito, 2000). The forms of P involved in long distance transport along the hyphae are probably inorganic orthophosphate (Pi), poly-phosphate and organic phosphate such as Phosphate-ester (Ezawa et al., 2002). Ecological categories of AMF may be more useful for summarizing fungal performance in the nursery conditions (Brundrett et al., 2005).*

The AM fungi expand their filaments in soil and plant roots. This filamentous network promotes bi-directional nutrient movement where soil nutrients and water move to the plant and plant photosynthates flow to the fungal network. The structures produced by fungi are intraradical and extraradical spores, intraradical hyphae, and extraradical hyphae. Intraradical AM fungal mycelium form a network around and inside cortical cells of plant roots, extraradical AMF mycelium can spread throughout the soil surrounding the root system and increase the ability to explore soil areas, accessing water and nutrients for plant roots. Benefits to plants are improved water and nutrient uptake, enhanced P transport, and drought and disease resistance (Dalpe, 2004).

Survival rates of seedlings are important for the first establishment of plant growth of Combretocarpus rotundatus. The ability of AM fungi to increase crop growth and yield, attributed to better nutrition in phosphorus-poor and phosphorus-fixing soils, have been reported for hundreds of plants species (Auge and Moore, 2005). Mycorrhizal symbiosis can also improve host responses to other environmental limitations, such as drought (Brundrett et al., 1996; Alguacil et al., 2006; Aliasgharzad et al., 2006; Aroca et al., 2007), salinity (Auge, 2004; Gupta and Rautaray, 2005; Cho et al., 2006).

CONCLUSION AND SUGGESTION

Conclusion

In conclusion, colonization by Glomus sp TD15 consistently increased the growth of five months old Combretocarpus rotundatus seedlings. Glomus sp TD15 could, therefore, be used to inoculate Combretocarpus rotundatus Miq in nurseries because this fungi was well adapted to the environmental conditions and had a superior effect on plant growth as compared with the other AMF types.

This study may also contribute to the future application of AM fungi to restoration of peat swamp . Our finding that "generalists" can colonize host plants over a low of pH values is novel, and it is expected that these fungi may have a great potential as "universal fungi" for environmental restoration if they can be isolated successfully.

Suggestion

Indigenous AM fungi are suggested to be potentially good inoculants for native peat swamp tree species under greenhouse nursery conditions and would be beneficial for the restoration of degraded peat swamp forest.

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