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Mycorrhizal colonization of indigenous tropical tree species grown in peat swamp forests of Sumatera, Indonesia

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Abstract. The restoration of peat swamp forests in Sumatra island has become Indonesian government's priority to restore ecological functions and their utilization. Indigenous. However, little information is available on the status of arbuscular mycorrhizal (AM) fungi in Sumatera. The objective of this research was to know AM fungi colonization in indigenous of tropical peat swamp forests. Root samples of 28 tree species in 14 families grown in a peat swamp forest of Jambi, Riau, and South Sumatera. All soil and tree roots were grown in zeolite media and trapped in *Pueraria javanica* and *Shorgum bicolor* as host plants for four months in a green house. Roots were stained with 0.1% trypan blue and vesicles, arbuscles and internal hyphae of AM fungi observed under a compound microscope. The results have shown that 20 tree species (72%) were colonized by AM fungi, 4 tree species of the dipterocarps family (14%) were colonized by ectomycorrhizal fungi (ECM), and four other tree species (14%) did not find FMA or ECM colonization. It is suggested that utilization of mycorrhizas can increase early growth of some tree species grown in peat swamp forests and mycorrhizal application will be expected as a key technology to restore degraded peatlands.

1. Introduction

Peat-swamp forest in Indonesia disappeared for 61% of its area between 1990-2015, and only 6% remained untouched [1]. Disrupted carbon-storage peat-swamp forests resulted in higher level of green house gases emissions. Restoration of peat-swamp forest ecosystem is an urgent intersectoral priority project. Development of industrial plant forest for pulp and paper production is generally located in peat-swamp ecosystem which typically has acidic soil with very low pH and poor nutrient [2]. Mycorrhizal fungi played a fundamental role in exchanging carbon and nutrients in acidic peat soil where high amount of carbon and nutrients are stored in peat [3]. With this condition, local tree species, which could be widely developed in peat land are very limited. Among many species developed by forest for industrial purposes, *Acacia crassicarpa* as preferred for fiber and pulp production. The challenge for this species today is the lower productivity and increasing of planting cycle. In order to anticipate the reduction in raw material for pulp production, alternative species must be developed.

Arbuscular mycorrhizal (AM) fungi from *Glomeromycota* have been mutually associated with higher plant since ancient times. They form associations to most higher plant species (97%). A few plant families that were known not to form association with mycorrhizal fungi are *Brassicaceae*, *Chenopodiacea*e, and *Amaranthaceae* [4,5]. AM fungi was known to promote plant growth by enhancing nutrient (P, Cu, Zn, Mn, and Fe) absorption, modifying plant structure under drought condition, and improving soil aggregate structure by mycelia network [6, 7].

Surveys on local mycorrhizal fungi in Central Kalimantan revealed that fungal colonization was observed in various indigenous peat swamp trees [8]. Based on these preliminary surveys, AM fungal inoculations on peat swamp forest-originated trees were done. Obeservations revealed that AM fungi promote the growth of *Dyera polyphylla* and *Aquilaria filaria* in nursery after germination [9], and *Aloe vera* [10], *Ploiarium alternifolium* and *Calophyllum hosei* [11], *Alstonia pneumatophora* [12], *Shorea balangeran* after germination in nursery and also in the field, three years after planted [13]. *S. balangeran* and *D. polyphylla* were planted on degrade peat land [14], and *Lophopetalum javanicum* [15]. The aim of this study was to gain and develop AM fungal inoculants that promote the growth of selected tree species seedlings on rewetted peat-swamp land in mainland Sumatera, Indonesia.

2. Materials and Methods

2.1. Survey

Arbuscular mycorrhizal fungi exploration were done in Riau, South Sumatra, and Jambi. These provinces had the greatest peat-swamp forest area in Sumatra Island. Surveys were done for 8 days in each province on September to December 2016. Peat-swamp forest, including conservation and peat-mining-retired areas, were prioritized. Ten selected tree species, suggested by Asia Pulp & Paper (APP) experts, were prioritized as well to be collected their roots and rhizosphere soils. Survey team consisted of four researchers from Forest Research and Development Center, accompanied by APP research and development (R&D) team from respective locations.

2.2. Data analysis

In order to investigate AM fungal colonization, Roots were stained with 0.1% trypan blue and vesicles, arbuscles and internal hyphae of AM fungi observed under a compound microscope The roots were collected from rhizosphere soils of listed peat-swamp trees and/or seedlings (Table 1). All soil and tree roots were grown in zeolite media and trapped in *Pueraria javanica* and *Shorgum bicolor* as host plants for four months in a green house.

Table 1. List of indigenous trees which roots and rhizosphere soils were sampled for AM fungi isolation from peat-swamp forest in Riau, South Sumatra, and Jambi and their uses.

Order	Family	Species	Usage/application
Theales	Dipterocarpaceae	*Shorea balangeran*	Timber,pulp
		Shorea uliginosa	Timber
		Hopea mengarawan	Timber
		Parashorea aptera	Timber
	Tetrameristaceae	*Tetramerista glabra*	NTFP
Malpighiales	Hyperiaceae	*Cratoxylum arborescens*	Pulp
	Bonnetiaceae	*Ploiarium alternifolium*	NTFP
	Phyllantaceae	*Baccaurea macrocarpa*	Timber
	Euphorbiaceae	*Macaranga pruinosa*	Timber
		Endospermum diadenum	Pulp
Sapindales	Anacardiaceae	*Camnosperma auriculatum*	Pulp
		Spondias dulcis	Timber

NTFP : Non timber forest products

Table 1 (Continue)

Order	Family	Species	Usage/application
Gentianales	Apocynaceae	*Dyera polyphylla*	Timber,NTFP
		Alstonia pneumatophora	Timber, NTFP
		Alstonia scholaris	Timber, NTFP
	Gentianaceae	*Fragraea fragrans*	Pulp
	Rubiaceae	*Neolamarckia cadamba*	Timber, Pulp
Myrtales	Myrtaceae	*Tristaniopsis obovata*	NTFP, Pulp
		Eucalyptus pellita	Timber, Pulp
	Thymelaeaceae	*Gonystylus bancanus*	Timber NTFP
		Aquilaria malaccensis	NTFP
	Melastomaceae	*Melastoma malabatrichum*	
Rhizophorales	Rhizophoraceae	*Combretocarpus rotundatus*	Pulp
Fabales	Mimosaceae	*Acacia crassicarpa*	Pulp
	Fabaceae	*Diailum indum*	Timber
		Koompassia malaccensis	Timber, NTFP
Celastrales	Celastraceae	*Lophopetalum javanicum*	Timber, Pulp
Santalales	Olacaceae	*Ochanostachys amentacea*	Timber

NTFP : Non timber forest products

3. Results and Discussion

3.1. AM fungal symbiosis with indigenous tree species

AM fungal colonization was observed on collected wild seedlings from 9 orders, 12 family, and 28 species of peat-swamp trees. Observations revealed that 20 species (72%) were colonized by AM fungi. Furthermore, 4 dipterocarps (14%) were colonized by ectomycorrhizae, while 4 other species (14%) were not colonized by either AM fungi nor ectomycorrhizae (Table 2, 3, and 4).

Table 2. Percentage of AM fungal colonization in the roots of naturally-occurred local tree seedlings in peat-swamp forest ecosystem in Riau, Sumatra.

Species	Colonization (%)
S. balangeran	0±0*
C.arborescens	49±5
C.auriculatum	39±4
C.rotundatus	0±0
A.crassicarpa	29±11
E. pellita	0±0

* Dipterocarps are specifically ectomycorrhizal symbion.

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Based on microscope observation, AM fungal colonization was not visible on four dipterocarps; *Shorea balangeran*, *S. uliginosa*, *Hopea mengarawan*, and *Parashorea aptera*. However, ECM colonization was visible with varied colonization percentage. Previous study reported that dipterocarps were mutually symbiotic with ectomycorrhizal fungi and characterized as highly depended [16].

From all three provinces where surveys were conducted, neither full-grown trees or wild seedlings of mentibu (*Dactylocladus stenostachys*) were successfully found. AM colonization was found in roots of *Dyera pollyhylla* and unidentified spores of local AM fungi from peat-swamp forests in Sumatera (Figure 1). *Eucalyptus pellita* is not an indigenous Sumatran tree species and did not show root colonization from the collected roots from Riau or Jambi. However, AM fungal spores were successfully recovered from the rhizosphere soils of this species. Aside from AM fungi, *E. pellita* and *A. crassicarpa* were also observed to associate with ECM fungi from mainlad of China by APP R&D team, even produced fruit bodies of ECM which were identified as *Pisolithus arhizus*. This means both tree species were effectively associated with ectomycorrhizal fungi. Symbiosis with ECM were disrupted when fertilizer was applied.

Table 3. Percentage of AM fungal colonization in tree seedlings naturally found in peat swamp forest in South Sumatra (October 2016).

Species	Colonization (%)
G. bancanus	21 ± 10
M. cajuputi	12 ± 12
C. auriculatum	14 ± 14
D.polyphylla	42 ± 14
A. pneumatophora	0 ± 0
L. javanicum	9 ± 9
T. glabra	26 ± 13
A.malaccensis	12 ± 12
E. diadenum	0 ± 0
C. rotundatus	8 ± 7
S. balangeran	0 ± 0*
H. mengarawan	0 ± 0*
S. uliginosa	0 ± 0*
A. scholaris	5 ± 5
D. indum	0 ± 0
P. alternifolium	5 ± 5
T. obovata	13 ± 7

* Dipterocarps are specifically ectomycorrhizal symbion.

The low colonization percentage observed on each tree species might be due to growing conditions, host interaction, light condition, temperature, and nutrient competition between soil microbes. From 5 wild seedlings soil samples, not all collected seedlings were colonized by AM fungi, resulting in high standard error. On *A. crassicarpa* and *E. pellita*, naturally exist AM fungal colonization was not visible, probably due to intensive application of fertilizer inhibiting fungal association to plant roots. Application of mycorrhizal fungi can be done since at nursery to restore tropical peat swamp forest in Sumatera and Kalimantan. Some problems for restoration are to provide local seed tree species, because there is no information about fruiting seasons of tree species in peat swamp forests. There will be considered to select the best of single mycorrhizal species or consortium species, it means that mycorrhizal inoculant contain more than two mycorrhizal species or mixed species. Ectomycorrhizal inoculant can be applied to dipterocarps species origin from peat-swamp forests with spore suspension, tablets or with alginate beads [17].

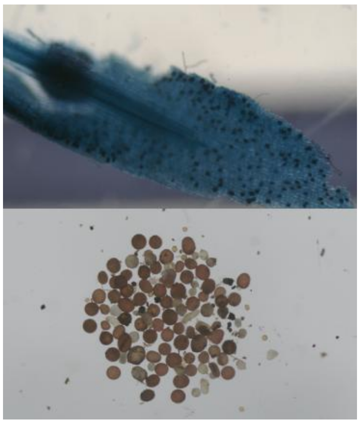

Figure 1. AM colonization in roots of *D. polyphylla* (above) and unidentified AM spores (below) from peat-swamp forests in Sumatera.

Table 4. Percentage of AM fungal colonization in tree seedlings naturally found in peat swamp forest in Jambi (October 2016).

Species	Colonization (%)
C. arborescens	32±8
M. pruinosa	9±8
E. diadenum	12±11
D. polyphylla	30±10
C. auriculatum	12±8
A. scholaris	7±7
A. crassicarpa	0±0
G. bancanus	29±12
M. malabatrichum	24±9
E. pellita	0±0
K. malaccensis	21±11
O. amentacea	52±9
N. cadamba	28±12
B. macrocarpa	29±15
S. dulcis	27±22
P. aptera	0±0*

* Dipterocarps are specifically ectomycorrhizal symbion.

4. Conclusion

Arbuscular mycorrhizal (AM) fungi has natural colonization with indigenous tree species from tropical peat swamp forest in Sumatera, Indonesia. The results of survey have shown that 20 tree species (72%) were colonized by AM fungi, 4 tree species of the dipterocarps family (14%) were colonized by ectomycorrhizal fungi (ECM), and four other tree species (14%) did not find AM fungi or ECM colonization. It is suggested that application of mycorrhizal fungi can increase the early growth of some tree species grown in peat swamp forests and mycorrhizal application will be expected as a key technology to restore degraded peatlands.

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