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Host specificity of ectomycorrhizal fungi to common dipterocarps in Bangladesh

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Abstract: Host specificity of ectomycorrhizal fungi to common dipterocarps in Bangladesh were studied in the research field of the Department of Agroforestry, Bangladesh Agricultural University, Mymensingh during July 2008 to May 2010. The common dipterocarps of Bangladesh are *Dipterocarpus turbinatus* (Garjan), *Hopea odorata* (Telsur), *Shorea robusta* (Sal) etc. are ectomycorrhizal. Etomycorriza (ECM), the major mycorrhizal types form symbiotic association between fungi and feeder roots of higher plants. Seedlings of sal, garjan and telsur were established in the Agroforestry research field during July-August, 2008. Sporocarps of *Laccaria, Inocybe, Suillus, Russula* and *Scleroderma* were collected from sal forest of Bangladesh and its spores were germinated in the agarose media. Germinated spores (with agarose media) of *Laccaria, Russula, Inocybe, Suillus* and *Scleroderma* were inoculated to the previously established seedlings of sal, garjan and telsur. After inoculation root samples of all dipterocarps were examined under dissecting microscope and ECM fungi colonized root tips were characterized based on their specific color, texture and emanating hyphae. It was observed that all the selected ECM fungi were successfully established in case of Sal roots. *Laccaria sp*, *Russula sp* and *Inocybe sp.* were found to be colonized in both Garjan and Telsur roots; while *Russula sp.* and *Scleroderma sp.* did not colonize in Garjan and Telsur seedlings roots indicating that these ECM fungal species are not suitable in association with these dipterocarps. Vegetative growth of all dipterocarps seedlings that colonized successfully ECM fungi results significantly higher growth than ECM fungi non-inoculated and un-colonized seedlings of all tree species (Sal, Garjan and Telsur).
Key words: Dipterocarps, host specificity, ectomycorrhizal fungi.

Introduction

Dipterocarpaceae is the most important tree family ecologically and economically in South-East Asia. In Bangladesh, dipterocarps are scattered in the tropical wet evergreen, tropical semi-evergreen and tropical moist deciduous forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Sylhet, Mymensingh and Gazipur districts. The common dipterocarps of Bangladesh are *Dipterocarpus turbinatus* (Garjan), *Hopea odorata* (Telsur), *Shorea robusta* (Sal) etc. The *Shorea robusta* is the predominant species of tropical moist deciduous forests of Bangladesh commonly known as Sal forest. These dipterocarps are basically timber tree and produce good quality timber used for making furniture, poles, pillars, paneling, beams, planking, piling, and other important construction purposes.

All members of the dipterocarps are ectomycorrhizal. Ectomycorrhiza (ECM), one of major mycorrhizal types, is the symbiotic association between fungi and feeder roots of higher plants. Host plants that are colonized by ECM fungi include many tree species belonging to Pinaceae, Betulaceae, Fagaceae, Salicaceae, Myrtaceae, and Dipterocarpaceae, including major components in most temperate and many tropical forests (Smith and Read, 1997). Since almost all fine roots of these host species were colonized by ECM fungi, ECM tips and mycelia are ubiquitous in forest soil.

In ECM symbioses, fungal partners provide significant amount of nutrients to host plants, e.g. up to 90% phosphorous and nitrogen in host plants are supposed to be derived from ECM fungal partner. Without the nutrient support from fungal partners, it is difficult for host plants to grow and survive under natural soil conditions. In return, host plants provide fungal partners with photosynthetically fixed carbohydrates. This carbohydrate supply enables ECM fungi to have advantages over other microorganisms to scavenge soil nutrient pools. With such carbohydrate supply from host plants, ECM fungi can spread mycelia, produce sporocarps, and accomplish their life cycles. Therefore, both partners in ECM symbiosis obligately depend on each other to grow and survive in natural environments. Because of the physiological importance of ECM symbioses and their dominance in forest ecosystems, ECM fungi play important roles in various ecological processes in developed forests (Hogberg and Hogberg, 2002). Indeed, ECM root tips and ECM mycelia account for more than 50% soil respiration and a substantial part in carbon and nutrient cycling in forest ecosystems (Vogt *et al.* 1982).

The forests of Bangladesh are nutrient poor in nature because of severe denudation where ectomycorrhizal association seems to be indispensable for growth and survival of dipterocarps seedlings. ECM communities are tremendously diverse and host specific (Molina *et al.* 1992). In Bangladesh, more than 20 ECM species of *Laccaria*, *Russula*, *Amanita*, *Scleroderma*, *Suillus*, *Lactarius* genus colonize with different dipterocarps (Islam *et al.* 2007), but their specificity to different dipterocarps species so far are not identified. So, it is necessary to identify the ECM species which are specific to different dipterocarps.

Materials and Methods

Host specificity of ectomycorrhizal fungi to common dipterocarps in Bangladesh were studied at the research field, Department of Agroforestry, Bangladesh Agricultural University, Mymensingh during July 2008 to May 2010. The experimental site is geographically located at about 24º75´ North latitude and 90º50´ East longitudes (Khan, 1997). Seedlings of three common dipterocarps of Bangladesh viz. *Dipterocarpus turbinatus* (Garjan), *Hopea odorata* (Telsur) and *Shorea robusta* (Sal)) were collected from different forest nurseries and established in the experimental field. Sporocarps of ECM fungi viz. *Laccaria, Inocybe, Suillus, Russula* and *Scleroderma* were collected from different sal forest (Madhupur and Bhawal Range) of Bangladesh. Spores of these ECM fungi collected from sporocarps were germinated in the agarose media. Germinated spores (with agarose media) of all ECM fungi were inoculated to the pre-established seedlings of sal, garjan and telsur following the Randomized Complete Block Design (RCBD) with three replications. All dipterocarps seedlings were also grown without inoculation as control. After successful inoculation, sporocarps development along with these dipterocarps saplings was observed during May (2009 & 2010). After sporocarps observation root samples of all dipterocarps were collected during the period of 10-15 August of 2009. These collected root samples were carefully washed and examined under the dissecting microscope. ECM fungi root tips were characterized based on their specific color, texture and emanating hyphae. Growth of all dipterocarps saplings (inoculated and non-inoculated) were also observed by recording data of plant height and stem girth. All recorded data were analysed by using computer package programme MSTAT-C (Russell, 1986) to find out the statistical significance and the mean differences were evaluated by least significance difference (Gomez and Gomez, 1984).

Results

Ectomycorrhizal (ECM) fungal establishment

Sporocarp development: Establishment of ECM fungi in association with dipterocarps saplings was observed by observing sporocarps development. Sporocarps of *Laccaria*, *Inocybe* and *Suillus* were found in the basal area of all dipterocarps saplings i. e. *Shorea robusta*, *Dipterocarpus turbinatus* and *Hopea odorata* saplings (Table 1). Sporocarps of *Russula* and *Scleroderma* were found only in the basal area *Shorea robusta*. Sporocarps of these two ECM fungi were not found at the basal area of *Dipterocarpus turbinatus* and *Hopea odorata* saplings (Table 1).

Table 1. ECM fungal establishment in association with roots of dipterocarps sapling

Dipterocarps	ECM fungal root tips and sporocarps production				
	Laccaria	*Russula*	*Inocybe*	*Suillus*	*Scleroderma*
Shorea robusta	√	√	√	√	√
Dipterocarpus turbinatus	√	×	√	√	×
Hopea odorata	√	×	√	√	×

√ = established i. e. found in association sapling roots/produced sporocarps
× = not established i. e. not found in association with saplings roots/no sporocarps were produced

ECM fungal root tip characterization: Root tips of all ECM fungi were examined under a dissecting microscope. All examined root tips were characterized based on their specific color, texture and emanating hyphae. It was found that results from ECM root tips observation were very much similar with sporocarps observation where all ECM fungi were successfully established in association with *Shorea robusta*, saplings root. *Laccaria*, *Inocybe*, and *Suillus* were found in association with both *Dipterocarpus turbinatus* and *Hopea odorata* saplings root. ECM fungi *Russula* and *Scleroderma* were not colonizing in *Dipterocarpus turbinatus* and *Hopea odorata* saplings root (Table 1).

Growth of dipterocarps saplings: Growth of all dipterocarps saplings were observed by recording the data of height and girth after one and two years of transplantation (Table 1, Figs. 1- 3). Height and girth of all ECM fungi inoculated *Shorea robusta* saplings were statistically similar which were longer and vigorous and significantly shorter and weak saplings were observed in the non-inoculated saplings (Table 2, Fig. 1).

Height and girth of *Dipterocarpus turbinatus* saplings in all ECM fungi colonize (*Laccaria*, *Inocybe*, and *Suillus* inoculated) saplings were statistically similar which were longer and vigorous than ECM fungi un-colonized (*Russula* and *Scleroderma* inoculated) and non-inoculated saplings, ECM fungi un-colonized and non-inoculated saplings were statistically similar which were shorter and weak (Table 2, Fig. 2). Growth (height and girth) of *Hopea odorata* were exactly same with the growth of *Dipterocarpus turbinatus* (Table 2 and Fig. 3).

Table 2. Growth characters of dipterocarps saplings after two years in inoculated and non-inoculated condition

Dipterocarps		Inoculated saplings					Non-inoculated saplings
		Laccaria	*Inocybe*	*Suillus*	*Russula*	*Scleroderma*	
Shorea robusta	Height (cm)	105a	107a	106.5a	104a	110a	60b
	Girth (cm)	3.6a	3.5a	3.4a	3.5a	3.7a	2.1b
Dipterocarpus turbinatus	Height (cm)	125a	128a	70b	123a	75b	65b
	Girth (cm)	10.5a	11.1a	4.5b	10.4a	5.2b	4.4b
Hopea odorata	Height (cm)	153a	155a	78b	151a	85b	73b
	Girth (cm)	7.5a	7.9a	3.8b	7.7a	4.2b	3.6b

Means in row followed by the same letter are significantly different by DMRT at $P \leq 0.05$

Fig. 1. Growth of *Shorea robusta* saplings in ECM fungi inoculated and non-inoculated condition

Discussion

Host specificity: In this study it was found that ECM fungi *Russula* and *Suillus* were not colonize in association with *Dipterocarpus turbinatus* and *Hopea odorata* saplings but colonize with *Shorea robusta* saplings which indicate these ECM fungi were host specific (Table 1). Newton and Haigh, (1998) observed the ecological specificity and host ranges of a variety of ECM fungi. Different studies have examined ECM fungal host specificity at various host-taxon levels using molecular approaches (Walker *et al*. 2005 at species level; Cullings *et al*. 2000 at generic level; Richard *et al*. 2005 and Nara, 2006 at family level). These studies examined host specificity (species, genus or family) using two or three host species. Using sporocarps study Massicotte *et al.* (1999) have suggested that host specificity at higher levels of the host taxon (i.e. genus or family) is relatively common. Ishida *et al.* (2007) tested 55 different ECM fungal species to eight coexisting different host trees and they found most of the ECM fungi were host specific. In Bangladesh, Huda *et al.* (2006) also found the host specificity of ECM fungi to dipterocarp plantations by sporocarps survey. These studies also support the host specificity of ECM fungi to dipterocarps in Bangladesh ecological condition.

Host growth: Significantly vigorous growth was observed in the ECM fungi colonized dipterocarps saplings compared to non-inoculated and un-colonized saplings of this study (Table 2, Figs. 1-3). ECM fungi improve host performance by increasing nutrients and water availability to the soil and protecting host roots from pathogens and toxic compounds (Smith and Read, 1997).

Fig. 2. Growth of *Dipterocarpus turbinatus* saplings in ECM fungi inoculated and non-inoculated condition

Reduced ECM fungi abundance could result in decreased plant performance and reduced competitive ability (Parke *et al*. 1983). Lower ECM fungi abundance on trees reduces the amount of ECM inoculum in the soil, where EM host plant density is low (Haskins and Gehring, 2005). So, ECM fungal inoculation could enhance the growth of dipterocarps and other plantation especially in nutrients deficient condition.

Fig. 3. Growth of *Hopea odorata* saplings in ECM fungi inoculated and non-inoculated condition

This study conclude that *Russula* and *scleroderma* ECM fungal genus can colonize with *Shorea robusta* but can't with *Dipterocarpus turbinatus* and *Hopea odorata* i. e. ECM fungi are host specific and ECM fungal inoculation can improve the growth of dipterocarps saplings.

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