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Effect of mycorrhizal inoculations on the growth of *Shorea robusta* seedlings

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Abstract. *Tapwal A, Kumar R, Borah D. 2015. Effect of mycorrhizal inoculations on the growth of Shorea robusta seedlings. Nusantara Bioscience 7: 1-5. Shorea robusta* is one of important timber yielding tree species of northeast India and known to have both ectomycorrhizal (EcM) and endomycorrhizal (AM) associations. It is hypothesized that under favorable conditions different mycorrhizal fungi present in soil develop symbiotic association with fine roots of trees. In present investigations, mycorrhizal inoculum of EcM and AM fungi applied to *S. robusta* seedlings raised in polyethylene bags in nursery. Observations on growth characters and mycorrhizal colonization were recorded at the interval of three months. The results revealed that irrespective of type of mycorrhizal inoculation, growth of the seedlings increased significantly in comparison to control. Maximum growth was observed for the seedlings inoculated with EcM alone, followed by dual inoculations (EcM+AM), seedlings inoculated with AM fungi and minimum in control.

Keywords: Ectomycorrhiza, endomycorrhiza, *Shorea robusta*, nursery trials.

INTRODUCTION

Shorea robusta Gaertn. f. is an important timber-yielding species of northeastern India and belongs to family Dipterocarpaceae that dominates the rain forests in South and Southeast Asia. With reference to South Asia, the members of family are distributed in India, Nepal, and Srilanka (Ashton 1982). Most of dipterocarps occur in evergreen and well-drained tropical rain forests of the Indo-Malayan region and most of them are equipped with wings which aid in the dispersal of fruit by wind (Shukla et al. 2012). The members of Dipterocarpaceae are known to have ectomycorrhizal association but few species possess dual association with ectomycorrhizal (EcM) and arbuscular mycorrhizal (AM) fungi (Lee 2006). Besides this, some other tree genera like *Alnus*, *Eucalyptus*, *Casuarina*, *Cupressus*, *Juniperus*, *Tilia*, *Ulmus*, and *Arbutus* also associate with ectomycorrhizal and endomycorrhizal fungi depending on soil conditions and trees' age (Harley 1969; Marks and Kozlowski 1973; Harley and Smith 1983).

Mycorrhizae are the widespread symbiotic association involving root-inhabiting fungi and roots. Symbiosis was initially used to define both lichens and parasites (DeBary 1887), but many workers now use this term to describe beneficial associations only (Lewis 1985; Paracer and Ahmadjian 2000). Mycorrhizae are the very important fungal symbionts in the forest ecosystem. They act as natural barriers to the soilborne pathogens and help host plants for the absorption of nutrients; besides this the fruit bodies of many ectomycorrhizal fungi are edible. Fungal symbiosis has been defined as an association in which fungi come into contact with living host establishing mutual nutrient exchange (Cook 1977). The mycorrhizal fungi also play an important role in the process of plant adaptation when transplanted to new habitats (Księżniak 2007). Increased survival and growth were also observed in micropropagated plants and their rootstocks inoculated with mycorrhizal fungi (Grange et al. 1997; Borkowska et al. 2008).

In temperate and boreal forests, up to 95% of the short roots form ectomycorrhizae (Smith and Read 1997). Ectomycorrhizae have a helpful impact on plant growth in natural and agroforestry ecosystems. In addition to absorbing and transferring nutrients, minerals, and water from the external environment into the plants, many ectomycorrhizal fungi can degrade recalcitrant organic sources (Smith and Read 1997) and some are also involved in the dissolution of soil minerals (Landeweert et al. 2001) to get access to nutrients and minerals. The AM fungi are also common symbionts in terrestrial ecosystems, associating with about 80% of plant families worldwide. The importance of AM mycorrhizae as a tool for improving the growth and productivity in diverse groups of plants was recognized only after pioneer work of Gerdemann (1968) and Baylis (1972). Arbuscular mycorrhizal associations are the most frequent symbiosis found in nature because of their broad association with plants and cosmopolitan distribution (Harley and Smith 1983) and are one of the beneficial soil microorganisms that play an important role in the mineral nutrition of forest trees (Koide and Mosse 2004). Numerous studies in tropical rain forest mycorrhizae have indicated the dominance of arbuscular mycorrhizae (Janos 1980; Bereau and Garbaye 1994).

Most of earlier studies revealed the ectomycorrhizal association with the roots of Dipterocarpaceae but comparatively little work has been one on the endomycorrhizal associations. AM colonization was

approximately 40% in tree species in tropical heath forests and mixed Dipterocarpaceae forest in Brunei (Moyersoen et al. 2001). Shi et al. (2002) have recorded *Acaulospora* and *Glomus* as dominant genera associated with different dipterocarps species with varying rates of colonization. Kumar et al. (2013) also observed dominant association of *Glomus* followed by *Acaulospora* species in dipterocarps of northeast India. Considering the importance of *S. robusta* in the northeastern region of India, the present study was carried out to investigate the effect of ectomycorrhizal and endomycorrhizal inoculations on the growth of *S. robusta* seedlings under net house conditions.

MATERIALS AND METHODS

The regions of Assam, northeast India dominated by *S. robusta* were surveyed in different seasons for the collection of associated ectomycorrhizal fungi and rhizosphere soil for the isolation of dominant AM fungi. Diversity of EcM and AM association with dipterocarps was worked out earlier by the authors (Tapwal et al. 2013; Kumar et al. 2013).

Mass inoculum production of mycobiont. Dominant ectomycorrhizal associate of *S. robusta* (*Russula amoena*) was cultured on potato dextrose agar and mass inoculum was raised on wheat grains (Stoller 1962). The AM spores were collected by wet sieving and decanting technique of Gerdemann and Nicolson (1963) and Singh and Tiwari (2001) from the rhizosphere soil of healthy trees and mass inoculum of dominant species (*Glomus* spp.) was raised with wheat seedlings in earthen pots.

Seed sowing and mycorrhizal inoculation. Nursery bags (20 x 21 cm^2) filled with sieved and fumigated soil were sown with freshly collected seeds of *S. robusta*. At the time of seed sowing the nursery, bags were inoculated with mycorrhizal fungi (wheat spawn @ 2g/bag; 50 AM spores/ bag) and placed in agro shed nets. Four sets of experiments were laid: (i) inoculated with ectomycorrhizal fungi, (ii) inoculated with both ecto- and endomycorrhizal fungi, (iii) inoculated with endomycorrhizal fungi, (iv) control (not inoculated).

Observation in nursery. At the interval of three months, the plants were observed for growth parameters like shoot height, collar diameter, root and shoot volume, root and shoot fresh and dry weight and % mycorrhizal colonization. The comparisons were made with control and among treatments.

Analysis of mycorrhizal association. The percentage of EcM infection was calculated by using the following formula (Huda et al. 2006):

$$\text{ECM association (\%)} = \frac{\text{Total number of infected root tips}}{\text{Total number of root tips studied}} \text{X100}$$

AM colonization was studied by rapid clearing and staining method of Phillips and Hayman (1970). The percentage mycorrhizal root colonization was determined by following formula:

$$\text{AM colonization (\%)} = \frac{\text{Total number of infected root segiments}}{\text{Total number of root segments examined}} \text{X100}$$

RESULTS AND DISCUSSIONS

Our earlier study confirmed that *Russula amoena* was dominant ectomycorrhizal and *Glomus* spp. were dominant endomycorrhizal associates of *S. robusta* (Tapwal et al. 2013; Kumar et al. 2013). They were mass multiplied and applied in nursery as described in material and methods. Five seedlings from each treatment were uprooted at the interval of three months and observed for presence of mycorrhizal colonization, number of AM spores/ 50g of soil and growth characters as described above.

Mycorrhizal colonization of *S. robusta* seedlings in nursery

Considerable root colonization was recorded in the roots of *S. robusta* seedlings inoculated with mycorrhizal fungi. The root colonization and number of AM spores were increased consistently with the age of seedling (Table 1). Maximum EcM association (57.59%) was observed in twelve-months old seedlings followed by nine-months (54.14%), six months (46.15%) and minimum (38.16%) in three months old seedlings. Similarly, the seedlings inoculated with EcM+AM have recorded 53.29%, 46.67%, 30.26% root colonization and AM population of 37, 33, 20, 14 spores/50 g soil respectively in twelve, nine, six and three months old seedlings. While the seedlings inoculated with AM only have recorded comparatively higher spore count (31, 44, 61, 78 spores/50 g soil) and root colonization (17.95%, 25.26%, 36.97%, 44.14%) in three, six, nine and twelve months old seedlings respectively.

Effect of mycorrhizal inoculations on the root growth

The results of effect of mycorrhizal inoculations on the root growth of *S. robusta* are presented in Table 2. A significant increase in the root growth of inoculated seedlings was recorded in comparison to their respective controls. Maximum increase in root length was recorded in seedlings inoculated with EcM (11.60-18.82%) followed by the seedlings inoculated with EcM+AM (10.71-16.76%) and minimum by the seedlings inoculated with AM (9.91-15.60%). Similarly, the increase in the root volume was maximum for the seedlings inoculated with EcM (11.18-18.81%) followed by the seedlings inoculated with EcM + AM (9.40-14.15%) and minimum by the seedlings inoculated with AM fungi (8.67-13.14%). All treatments were found significant as compared to control. Likewise, the increase in the fresh root weight was also maximum for the seedlings inoculated with EcM (8.89-15.58%) followed by the seedlings inoculated with EcM+AM (8.69-14.63%) and minimum by the seedlings inoculated with AM (7.99-13.99%). Percent increase in fresh weight of the inoculated seedlings was significantly higher than the control. In similar trend, the increase in dry weight of root was higher in seedlings inoculated with EcM (8.19-13.54%) followed by the seedlings inoculated with EcM + AM (7.79-11.32%) and lowest by the seedlings inoculated with AM (7.55-10.17%). All treatments were recorded significant increase over control.

Table 1. Mycorrhizal colonization of inoculated seedlings of *S. robusta* in nursery.

Age of seedling	Treatment	No. of short roots	Non-mycorrhizal roots (No.)	Non-mycorrhizal root (%)	Mycorrhizal root (No)	EcM/ *AM (%)	AM spores/ 50 g of soil
3 month	Control	72	72	100.00	0	0.00	0.00
	EcM	76	47	61.84	29	38.16	0.00
	EcM + AM	76	53	69.74	23	30.26	14.00
	AM	78	64	82.05	14	17.95	31.00
6 month	Control	93	93	100.00	0	0.00	0.00
	EcM	104	56	53.85	48	46.15	0.00
	EcM + AM	106	65	61.32	41	38.68	20.00
	AM	95	71	74.74	24	25.26	44.00
9 month	Control	115	115	100.00	0	0.00	0.00
	EcM	133	61	45.86	72	54.14	0.00
	EcM + AM	135	72	53.33	63	46.67	33.00
	AM	119	75	63.03	44	36.97	61.00
12 month	Control	136	136	100.00	0	0.00	0.00
	EcM	158	67	42.41	91	57.59	0.00
	EcM + AM	152	71	46.71	81	53.29	37.00
	AM	145	81	55.86	64	44.14	78.00

Table 2. Effects of mycorrhizal inoculations on the root growth of *S. robusta* in nursery.

Growth parameter	Treatment	Age of seedling			
		3 month	6 month	9 month	12 month
Increase in root length (%)	EcM	17.19	18.82	15.81	11.60
	AM	13.96	15.60	13.98	9.91
	EcM + AM	15.20	16.76	15.15	10.71
		SEm± = 0.03, CD (p=0.05) = 0.09			
Increase in root volume (%)	EcM	18.81	14.81	11.18	13.41
	AM	13.14	10.69	8.67	10.23
	EcM + AM	14.15	13.14	9.40	12.76
		SEm± = 0.01, CD (p=0.05) = 0.03			
Increase in root fresh weight (%)	EcM	15.58	10.66	10.20	8.89
	AM	13.99	9.17	8.37	7.99
	EcM + AM	14.63	9.61	9.60	8.69
		SEm± = 0.004, CD (p=0.05) = 0.01			
Increase in root dry weight (%)	EcM	13.54	9.26	9.48	8.19
	AM	10.17	7.55	8.85	7.55
	EcM + AM	11.32	8.41	9.09	7.79
		SEm± = 0.002, CD (p=0.05) = 0.01			

Effect of mycorrhizal inoculations on the shoot growth

Like root growth, the seedlings inoculated with mycorrhizal fungi recorded significant increase in shoot growth (Table 3). The seedlings inoculated with only with EcM fungi had maximum increase (9.29-13.60%) in shoot height followed by seedlings inoculated with EcM+AM (8.56-11.18%) and minimum by the seedlings inoculated with AM (7.95-10.12%). The increase in shoot height was significant to the respective control seedlings. In similar trend, the percent increase in shoot volume was maximum for the seedlings inoculated with EcM (8.73-12.14%) followed by seedlings inoculated with EcM+AM (8.38-10.53%) and minimum by the seedlings inoculated with AM fungi (7.85-10.71%) and all treatments were recorded statistically significant with respective control. Likewise, the increase in shoot fresh weight was highest for the seedlings inoculated with EcM (7.19-11.00%) followed by the seedlings inoculated with EcM+AM (6.51-9.19%) and lowest for seedlings inoculated with AM alone (5.64-9.17%). All of the treatments were statistically significant. Similarly, a significant increase in the shoot dry weight was recorded in all treatments. It was also highest for the seedlings inoculated with EcM (8.58-12.08%) followed by the seedlings inoculated with EcM+AM (5.48-9.21%) and lowest for seedlings inoculated with AM only (6.35-10.03%). Similarly, the increase in the collar diameter was also maximum for the seedlings inoculated with EcM (3.17-4.98%) followed by the seedling inoculated with EcM+AM (3.10-4.39%) and AM (2.80-4.22%). All of the growth differences were recorded significantly higher in comparison to control.

Table 3. Effect of mycorrhizal inoculations on the shoot growth of *S. robusta* in nursery.

Growth parameter	Treatment	Age of seedling			
		3 month	6 month	9 month	12 month
Increase in shoot height (%)	EcM	13.60	14.77	9.29	9.84
	AM	10.12	12.30	7.95	8.54
	EcM + AM	11.18	13.40	8.56	8.94
		SEm± = 0.09, CD (p=0.05) = 0.27			
Increase in shoot volume (%)	EcM	12.14	12.05	8.73	11.32
	AM	9.19	10.71	7.85	8.09
	EcM + AM	10.53	11.49	8.38	9.73
		SEm± = 0.01, CD (p=0.05) = 0.02			
Increase in shoot fresh weight (%)	EcM	11.00	10.04	7.19	9.14
	AM	8.54	9.17	5.64	7.38
	EcM + AM	9.63	9.19	6.51	8.74
		SEm± = 0.01, CD (p=0.05) = 0.02			
Increase in shoot dry weight (%)	EcM	8.58	12.08	8.71	10.76
	AM	5.48	8.26	6.49	9.21
	EcM + AM	6.35	10.03	7.73	9.86
		SEm± = 0.004, CD (p=0.05) = 0.01			
Increase in shoot collar diameter (%)	EcM	4.98	4.62	3.17	3.79
	AM	4.22	3.88	2.80	2.46
	EcM + AM	4.31	4.39	3.10	3.35
		SEm± = 0.004, CD (p=0.05) = 0.01			

Discussions

Mycorrhizae play a significant role in plant nutrition, growth improvement, successful afforestation, reforestation, bio-control of pathogens and land reclamation programs (Marx 1977; Rawat et al. 2003). Harley and Smith (1983) recognized seven types of mycorrhizae: Ectomycorrhizae, Endomycorrhizae, Ectendomycorrhizae, Arbutoid, Monotropoid, Ericoid and Orchidaceous types. The members of family Dipterocarpaceae are known as obligatory ectomycorrhizal (Bakshi 1974; Smits 1994; Lee et al. 2008; Soni et al., 2011; Pyasi et al. 2011, 2013), but studies also recorded AM colonization (Chalermpongse 1987; Shi et al. 2002; Kumar et al. 2013). Species of *Amanita*, *Boletus*, and *Russula* are common ectomycorrhizal associates of dipterocarps (Natarajan et al. 2005) and species *Acaulospora* and *Glomus* are common AM fungi with varying degree of colonization (Shi et al. 2002; Kumar et al. 2013). In agreement with earlier studies, the inoculum of *Russula amoena* (EcM) and *Glomus* species (AM) was applied to *S. robusta* seedlings in nursery bags containing sterilized soil. The mycorrhizal fungi reproduced in nursery bags and colonized the roots of growing seedlings. A significant increase in the growth of colonized seedlings and mycorrhizal colonization was recorded in inoculated seedlings. It was highest for the seedlings inoculated with EcM, followed in dual inoculations, seedlings inoculated with AM alone and least in control. The higher growth in inoculated seedlings may be due to mobilization of additional nutrients to the roots by associated mycorrhizal fungi. Mycorrhizal fungi are known to enhance the uptake of low mobility minerals such as phosphorus and micronutrients (Smith and Read 1997). Mycorrhizae also improve plant health by protecting them from pathogens (Morin et al. 1999). Earlier studies on nursery experiments also reported improvement in growth of dipterocarps seedlings and nutrient uptake inoculated with mycorrhizal fungi (Lee and Alexander 1994; Tawaraya et al. 2003; Lee et al. 2008). *S. robusta* is one of important tree species of northeast India and by applying the mycorrhizal inoculum in nursery, the seedling establishment and performance can be improved during field transplantations.

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