Forest Ecology and Management 376 (2016) 67–73

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Arbuscular mycorrhizal fungal inoculation improves *Albizia saman* and *Paraserianthes falcataria* growth in post-opencast coal mine field in East Kalimantan, Indonesia

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ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form 2 June 2016
Accepted 3 June 2016

Keywords:
Opencast mining
Arbuscular mycorrhizal fungus
Albizia saman
Paraserianthes falcataria
Reforestation
Indonesia

ABSTRACT

Opencast mining carried out in the natural forests of Indonesia has resulted in difficulty of reforestation due to soil removal. Arbuscular mycorrhizal (AM) fungi can increase growth of tree species in disturbed fields. The objective of this study was to investigate the effect of inoculating three native AM fungi on the growth of *Albizia saman* and *Paraserianthes falcataria* in a nursery and a post-opencast coal mine field. Seeds of *A. saman* and *P. falcataria* were inoculated with three native AM fungi, *Rhizophagus clarus*, *Gigaspora decipiens*, and *Scutellospora* sp., and sown in sterilized compost under nursery conditions for six months. Non-inoculated seeds were used as control. Inoculated seedlings were transplanted in a post-opencast coal mine field and grown for seven months. AM colonization, shoot nitrogen (N) and phosphorus (P) concentration, stem diameter, and shoot dry weight were measured both in the nursery and in the field. AM colonization was 3–82% under nursery conditions and increased shoot P content and dry weight. Stem diameter, shoot N content, shoot P content, shoot dry weight, and survival rate under field conditions were higher in inoculated seedlings than in control seven months after transplanting. The results suggest that inoculating tree species with AM fungi promotes reforestation of post-opencast coal mine field.

1. Introduction

Exploitative logging, forest fire, opencast mining, and conversion forest into farmland such as oil palm and rubber plantations, are the leading causes of deforestation in tropical countries. In Indonesia, opencast mining is carried out for coal, nickel, tin, and bauxite as those raw materials are deposited in the surface layer of natural forests. The process of opencast mining consists of tree logging, topsoil stripping, removal of overburden, and exploitation of minerals (Ghose and Majee, 2000). Opencast mining is a major contributing factor to deforestation in Indonesia (Resosudarmo et al., 2009). In addition to deforestation, opencast mining also results in waste disposal, such as minestone or coal waste rock including fines produced during mining process (Bian et al., 2009), mine tailings (Dowarah et al., 2009), soil erosion, and decrease in soil fertility (Ghose, 2004).

The mining company has responsibility for reforestation in the area following mining, but most of company failed in reforestation. The degraded natural forests in Indonesia necessitate immediate, comprehensive, and systematic reforestation. Restoration of bare opencast mining land requires several hundred years and consists of the initial, middle, and climax stages (Burger and Zipper, 2002). Post-opencast mine land is physically, chemically, and biologically infertile habitat that hinders vegetation development (Sheoran et al., 2010). The utilization of beneficial soil microorganisms has been suggested as a possible approach for the restoration of post-opencast mine land (Juwarkar and Jambhulkar, 2008; Taheri and Bever, 2010).

It has been reported that AM fungal inoculation improved growth and nutrient uptake of tropical tree species in growth chambers and nurseries (Tawaraya and Turjaman, 2014). AM fungi increased seedling growth in 23 of 28 species from a lowland tropical rainforest in Costa Rica under nursery conditions (Janos, 1980). The inoculation of *Azadirachta indica* (Meliaceae) with AM fungi improved shoot growth compared with control seedlings under

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http://dx.doi.org/10.1016/j.foreco.2016.06.008
0378-1127/

nursery conditions (Muthukumar et al., 2001) *Clusia minor* and *Clusia multiflora* inoculated with *Scutellospora fulgida* in acidic soil showed increases of shoot and root biomass, leaf area and plant height in comparison to P-fertilized plants and non-mycorrhizal plants (Caceres and Cuenca, 2006). *Casuarina equisetifolia* seedlings inoculated with *Glomus geosporum* exhibited improved growth, nutrient acquisition, and quality under nursery conditions (Muthukumar and Udaiyan, 2010). *Araucaria angustifolia* seedlings inoculated with *Rhizophagus clarus* had higher shoot biomass and leaf P, K, Na, and Cu concentrations, and lower Ca, Mg, Fe, Mn, and B concentrations than controls (Zandavalli et al., 2004). Inoculation with soil-containing AM fungi increased shoot growth and nutrient contents of *Macaranga denticulata* when P was limited but N was abundant (Youpensuk et al., 2004). Inoculation of non-timber forest product species *Dyera polyphylla* and *Aquilaria filaria* with *R. clarus* and *Gigaspora decipiens* increased shoot N and P uptake under greenhouse conditions, indicating that AM fungi can reduce the need for chemical fertilizer applications (Turjaman et al., 2006). Inoculation with mycorrhizal roots of individual or a mixture of tree species (*Inga acreana*, *Tabebuia chrysantha*, *Cedrela montana*, *Heliocarpus americanus*) improved growth of six-month-old *C. montana* and *H. americanus* (Urgiles et al., 2009).

It has been reported that the utilization of one AM fungal species enhanced growth of some tropical tree species. AM fungi promoted the growth of *Acacia nilotica* and *Leucaena leucocephala* (Leguminosae) at 12 weeks after transplantation under greenhouse conditions (Michelsen and Rosendahl, 1990). AM fungi increased the growth of three multipurpose fruit tree species *Parkia biglobosa*, *Tamarindus indica*, and *Zizyphus mauritiana* at 2 months after inoculation (Guissou et al., 1998). *Glomus aggregatum* stimulated the growth of seventeen leguminous plants (Duponnois et al., 2001). *Glomus macrocarpum* promoted the growth of *Sesbania aegyptiaca* and *Sesbania grandiflora* (Giri et al., 2004). The inoculation of *D. polyphylla* with *R. clarus* and *G. decipiens* increased N and P contents when the plant was grown in peat swamp forest in Central Kalimantan, Indonesia (Graham et al., 2013).

Albizia saman adapts well to a wide range of soil types and soil pH values. The high adaptability of this species to dry sites (Wishnie et al., 2007) as evidenced by its consistently high survival rate makes it a potential candidate for the remediation of degraded land (Hall et al., 2011). *Paraserianthes falcataria* is a fast-growing pioneer species and has potential for afforestation due to its high survival rate (Otsamo et al., 1997). This species grows in a wide range of soils, including Latosol, Andosol, luvial and red-yellow podzolic soil (Krisnawati et al., 2011). Furthermore, *P. falcataria* is a profitable species for mixed plantation or agroforestry (Siregar et al., 2007).

We have high expectations that AM fungal inoculation would improve P and N uptake and shoot biomass of two tropical tree species grown under nursery and post-opencast coal mine field conditions. The purpose of the present study was to test the effect of inoculating three native AM fungi on the growth of *A. saman* and *P. falcataria* under nursery and post-opencast coal mine field conditions.

2. Materials and methods

2.1. Substrate

Chicken manure compost was collected from a local area in Binungan, Tanjung Redeb, Berau Regency, East Kalimantan, Indonesia. The compost was sterilized in a drum by heating over wood fire for three hours and further stored at room temperature. The properties of the compost were as follows: pH (H_2O), 5.25; total N, 26.5 g kg^{-1}; total carbon, 372.1 g kg^{-1}; available P, 622 mg P_2O_5 kg^{-1}.

2.2. Arbuscular mycorrhizal fungal inoculum and inoculation

R. clarus CK001 Nicholson & Schenck and *G. decipiens* CK003 Hall & Abbott were isolated from peat soil in Kalampangan, Palangkaraya, Central Kalimantan, Indonesia (Turjaman et al., 2006). *Scutellospora* sp. was isolated from a post-opencast coal mine field in Tanjung Tabalong Regency (S02°14′13.8″; E115°28′49.5″), South Kalimantan. AM fungal inoculum consists of zeolite containing spores and mycorrhizal roots from a pot culture of *Pueraria javanica* Benth grown under greenhouse conditions in the Forest Research and Development Centre, Bogor, West Java, Indonesia for 90 days. Twenty grams of AM fungal inoculum was inoculated by mixing with 800 g of sterilized compost in a polyethylene bag (10 cm diameter × 15 cm height). Twenty gram of sterilized zeolite was put into non inoculated pots as control.

2.3. Seedling preparation

Seeds of *A. saman* (Jacq.) Merr. and *P. falcataria* (L.) Nielsen were obtained from a local seed company in Bogor, West Java, Indonesia. Behavior of both species is orthodox. The seeds were soaked in boiled water at 80 °C for 1–2 min. Five seeds were sown and one seedling per polyethylene bag was allowed to grow after germination. The seedlings were transferred into a larger polyethylene bag (15 cm diameter × 20 cm height) filled with the same sterilized compost four months after sowing due to rapid growth. The seedlings in the polyethylene bags were arranged randomly on the bench 1 m above the ground in a nursery. Tap water was applied once every two days. The seedlings were grown under 50% shade for six months from 19 September 2012 to 8 March 2013 in the nursery in Binungan (N02°02′, E117°27′), Tanjung Redeb, Berau Regency, East Kalimantan, Indonesia. This growth period was determined to grow seedlings of appropriate size for transplanting in the field. The seedlings were subjected to one of four treatments: (1) control, (2) inoculation with *Scutellospora* sp., (3) inoculation with *G. clarum*, and (4) inoculation with *G. decipiens*. Each treatment had 20 replications.

2.4. Transplanting in the field

The field was located in a post-opencast coal mine area 5 km from the nursery in Binungan, Tanjung Redeb, Berau Regency, East Kalimantan, Indonesia. Mining has ceased in the area six months prior to this study, had no vegetation, and was covered with overburden or mine tailing and disposal waste from mining activity. Binungan area has a tropical climate and average annual rainfall in the last five years is approximately 4290 mm yr^{-1}. The average daily minimum temperature is 30 °C and maximum temperature is 40 °C. The average daily minimum humidity is 35% and maximum humidity is 90%. The properties of the post-opencast coal mine field were as follows: pH (H_2O), 4.31; pH (KCl), 3.57; available P, 4.15 mg P_2O_5 kg^{-1}; exchangeable Al, 1738.4 mg kg^{-1}.

The field experiment was conducted using a randomized complete block design with four treatments replicated four times. Four 25 m × 33 m blocks with similar soil conditions and a flat slope were prepared in the post-opencast coal mine field. The distance between the blocks was 15 m. Each block was subjected to four treatments: (1) control, (2) *Scutellospora* sp. inoculation, (3) *R. clarus* inoculation, and (4) *G. decipiens* inoculation. Each treatment area measured 10 m × 14 m. The distance between treatment areas was 5 m.

Planting holes (20 cm × 20 cm × 20 cm) with 2 m distance between holes were prepared. Two hundred grams of chicken manure compost was applied to planting hole. Six six-month-old seedlings each of *A. saman* and *P. falcataria* were transplanted in the holes for each treatment. Tap water was applied to the