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ORIGINAL PAPER

Arbuscular mycorrhizal fungi increased early growth of two nontimber forest product species *Dyera polyphylla* and *Aquilaria filaria* under greenhouse conditions

Maman Turjaman · Yutaka Tamai · Erdy Santoso · Mitsuru Osaki · Keitaro Tawaraya

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Abstract Nontimber forest products (NTFPs) represent an important source of income to millions of people in tropical forest regions, but some NTFP species have decreased in number and become endangered due to overexploitation. There is increasing concern that the planting stocks of *Dyera polyphylla* and *Aquilaria filaria* are not sufficient to sustain the yield of NTFPs and promote forest conservation. The objective of this study was to determine the effect of two arbuscular mycorrhizal (AM) fungi, *Glomus clarum* and *Gigaspora decipiens*, on the early growth of two NTFP species, *D. polyphylla* and *A. filaria*, under greenhouse conditions. The seedlings of both species were inoculated with *G. clarum* or *G. decipiens*, or uninoculated (control) under greenhouse conditions. Percentage of AM colonization, plant growth, survival rate, and nitrogen (N) and phosphorus (P) concentrations were measured after 180 days of growth. The percentage of AM colonization of *D. polyphylla* and *A. filaria* ranged from 87 to 93% and from 22 to 39%, respectively. Colonization by *G. clarum* and *G. decipiens* increased plant height, diameter, and shoot and root dry weights. Shoot N and P concentrations of the seedlings were increased by AM colonization by as much as 70–153% and 135–360%, respectively. Survival rates were higher in the AM-colonized seedlings at 180 days after transplantation than in the control seedlings. The results suggest that AM fungi can accelerate the establishment of the planting stocks of *D. polyphylla* and *A. filaria*, thereby promoting their conservation ecologically and sustaining the production of these NTFPs economically.

Keywords AM fungi · Tropical forest · NTFP · *Dyera polyphylla* · *Aquilaria filaria* · Rehabilitation

M. Turjaman · E. Santoso
Forest Microbiology Laboratory, Forest and Nature Conservation Research and Development Centre (FNCRDC),
Ministry of Forestry,
Bogor 16610, Indonesia

K. Tawaraya (✉)
Faculty of Agriculture, Yamagata University,
Tsuruoka 997-8555, Japan
e-mail: tawaraya@tds1.tr.yamagata-u.ac.jp

Y. Tamai · M. Osaki
Graduate School of Agriculture, Hokkaido University,
Sapporo 060-8589, Japan

Introduction

Nontimber forest products (NTFPs) are regarded as a means of subsistence and an income generation resource for people living in or near forests, and are thought to reduce the depletion of natural tropical forests by humans (Donovan and Puri 2004). NTFPs are obtained from forest resources, including resins, latex, bark, roots, seeds, flowers, fruits, leaves, mushrooms and other nonwood plant parts. The families Thymelaeaceae and Apocynaceae are important as they provide NTFPs and timber for the forest community in Southeast Asia (Lemmens et al. 1998; Oyen and Dung 1999). Thymelaeaceae consists of 50 genera with *Gyrinops*, *Enkleia*, *Gonystylus*, *Wikstroemia*, and *Aquilaria* producing NTFPs (Ding Hou 1960). *Aquilaria* species are used extensively as NTFPs and traded internationally. The NTFPs of *Aquilaria* species are used in the manufacture of incense, perfume, traditional medicine, and other commercial products by Asian Buddhists and Moslems. *Aquilaria filaria* is common in primary and secondary lowland tropical forests of Indonesian West Papua, Papua New Guinea, Peninsular Malaysia, and the Philippines. The Apocynaceae consists of 164 genera with

Tabernaemontana, *Secamone*, *Ochrosia*, *Alstonia*, and *Dyera* producing NTFPs. *Dyera* species are common in the peat swamp forests of Sumatra, Kalimantan, and Malaysia. Valuable latex is harvested from the stem of *Dyera polyphylla* (Lemmens et al. 1998). However, many NTFP species remain uncultivated, making it difficult not only to measure the conservation status of NTFP species, but also to sustain extraction and harvest.

Tropical forests are disappearing at the rate of 13.5 million hectares each year due largely to logging, burning, and clearing for agriculture and shifting cultivation (Kobayashi 2004). Timber harvesting has resulted in the transformation of more than five million hectares of tropical forest annually into overlogged, poorly managed, and degraded forests. Degraded tropical forests require wide-scale rehabilitation. It is necessary to improve the biological diversity of tropical forests and to enhance the commercial value of timber, pulp, and NTFPs. In addition, *Aquilaria* species, which are listed in Appendix II of CITES (2005), are considered to be threatened according to the International Union for Conservation of Nature and Natural Resources Red List (CITES 2005; Soehartono and Newton 2000). The rapid production of forest seedlings of high quality in nurseries is important for replenishing degraded tropical forests. Indigenous NTFP tree species, such as *D. polyphylla* and *A. filaria*, often grow slowly in the early growth stage compared with such exotic species as *Acacia mangium*, *Acacia crassicarpa*, and *Gmelina arborea*, which can be transplanted to the field within 3 months, and are difficult to improve in the nursery. Moreover, many soils of tropical forests are nutrient-poor and most Indonesian soils are ultisols, which are typically acidic and low in available phosphorus.

Arbuscular mycorrhizal (AM) fungi were reported to increase the growth of some tropical trees. They increased seedling growth of 23 of 28 species from a lowland tropical rain forest in Costa Rica under nursery conditions (Janos 1980). AM colonization of the tropical tree *Oubanguia alata* (Scytopetalaceae) was positively correlated with increase in phosphorus (P) uptake despite low P availability in Cameroon (Moyersoen et al. 1998). AM fungi also improved the growth of the Brazilian pine *Araucaria angustifolia* (Araucariaceae) (Zandavalli et al. 2004). There are also some reports on the improved growth of NTFP tree species after AM fungal inoculation in tropical forests. Muthukumar et al. (2001) reported that the inoculation of *Azadirachta indica* (Meliaceae) with AM fungi improved plant growth compared with control seedlings. Furthermore, the combination of inoculation of AM fungi with P-solubilizing and nitrogen (N)-fixing bacteria increased the growth of *A. indica*. Conversely, *Azadirachta excelsa* inoculated with AM fungi (without fertilizer) grew more slowly than controls at low P availability (Huat et al. 2002). Kashyap et al. (2004) showed that the inoculation of *Morus alba* (Moraceae) with AM fungi, *Azotobacter*, and indole butyric acid increased the survival of saplings.

Little is known about the effect of AM inoculation on the growth of Apocynaceae species in tropical forests. Weber et al. (1995) reported that the inoculation of three Apocynaceae species (*Adenium obesum*, *Pachypodium lamerei*, and *Plumeria obtusa*) with AM fungi almost doubled plant growth compared with control seedlings. Guadarrama et al. (2004) showed that the inoculation of the late pioneer tropical tree species *Stemmadenia donnell-smithii* (Apocynaceae) with AM fungi increased survival rate and biomass. To our knowledge, there are no reports on the improved growth of Thymelaeaceae tree species after AM fungal inoculation. Tawaraya et al. (2003) found the high natural AM colonization of native species including *Gonystylus bancanus* (Thymelaeaceae) in the peat swamp forests of Kalimantan, suggesting a possibility of growth improvement of these species by AM fungal inoculation. However, no information is available regarding the effect of AM fungi on the early growth of *Dyera* and *Aquilaria* tree species. The objective of this study was to determine whether two AM fungi, *Glomus clarum* Nicholson & Schenk and *Gigaspora decipiens* Hall & Abbott, increase the early growth of two NTFP species, *D. polyphylla* (Miq.) v. Steenis and *A. filaria* (Oken) Merr, under greenhouse conditions. Isolates of *G. clarum* and *G. decipiens* are native to the peat swamp forests of Central Kalimantan.

Materials and methods

Seed germination

Seeds of *D. polyphylla* were collected from a peat swamp forest in Kalampangan, Palangka Raya, Central Kalimantan (2° 13′ S, 113° 56′ E) and seeds of *A. filaria* were collected from a mountain forest in Ciapus, Bogor, West Java (6° 36′ S, 106° 47′ E). The seeds were soaked in water for about 2 h and then surface-sterilized by shaking in 5% NaClO solution for 5 min. They were thoroughly rinsed twice in sterile distilled water. The seeds were sown in a plastic flat containing autoclave-sterilized zeolite and grown under a 55% shading intensity net to control solar radiation because these species require shady conditions. The seeds of *D. polyphylla* and *A. filaria* were allowed to germinate for 14 and 22 days after sowing, respectively.

Soil preparation

Soil used in the experiment was an ultisol collected from Haurbentes Experimental Forest, Jasinga, West Java (6°

Table 1 AM colonization, shoot and root growth, and shoot nitrogen and phosphorus concentrations of *D. polyphylla* and *A. filaria* inoculated with or without *G. clarum* and *G. decipiens*

Species	Treatment	Plant growth				AM	Shoot nutrient concentrations	
		Height (cm)	Stem diameter (mm)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Colonization (%)	N (mg/g)	P (mg/g)
D. polyphylla	Control	11.2 a	2.9 a	0.79 a	0.54 a	10 a	12.13 a	0.34 a
	G. clarum	16.7 b	3.6 b	1.67 b	0.90 b	93 b	25.55 b	1.58 b
	G. decipiens	14.1 b	3.4 b	1.53 b	0.83 b	87 b	20.65 b	0.80 b
A. filaria	Control	13.2 x	1.8 x	0.29 x	0.15 x	0 x	12.76 x	0.95 x
	G. clarum	16.6 y	2.2 y	0.75 y	0.30 y	39 y	32.23 y	2.56 y
	G. decipiens	14.8 xy	2.0 xy	0.77 y	0.32 y	22 y	23.32 y	2.27 y

Values with the same letter are not significantly different ($P<0.05$)

32′–33′ S, 108° 26′ E) and stored in a greenhouse. It was passed through a 5-mm sieve and then mixed with river sand (3:1, v/v) to improve drainage. The pH (H_2O) of the soil mixture was 4.8 and available P (Bray-1) was 0.17 mg kg^{-1}. The soil mixture was sterilized at 121°C for 30 min.

Arbuscular mycorrhizal inoculum preparation

AM fungi *G. clarum* and *G. decipiens* were isolated from peat soil at Kalampangan, Palangka Raya, Central Kalimantan by trap culture. The pot cultures began as mixed spore cultures. They were propagated in pot cultures of *Pueraria javanica*. Plastic pots were filled with 175 g of sterilized zeolite and 5 g of AM fungal inoculum was placed in the planting hole. A preliminary experiment showed that AM fungal inoculum was effective without a microbial filtrate application. Two 6-day-old *P. javanica* seedlings were transplanted into the pots and grown under natural light greenhouse conditions with no temperature and humidity control. After 90 days, spores, external hyphae, and colonized roots of *G. clarum* and *G. decipiens* were observed in the zeolite.

Inoculation and maintenance

Polyethylene pots (15×10 cm) were filled with 500 g of sterilized soil mixture. AM inoculation was achieved by placing 5 g of inoculum of each species 1–3 cm below seedlings. One 14-day-old *D. polyphylla* or one 22-day-old *A. filaria* seedling was transplanted into the pots. Control seedlings were not mock-inoculated because a preliminary experiment showed that the sterilized inoculum did not affect the growth of the seedlings. Seedlings were watered daily with tap water to field capacity. No fertilizer was applied during the course of the experiment. Weeds and pests were removed manually. The seedlings were grown for 180 days in a greenhouse at the Forest and Nature Conservation Research and Development Center, Bogor, West Java (6° 36′ S, 106° 45′ E). Temperature varied between 26 and 35°C, relative humidity was 80–90%, and the photoperiod was about 12 h.

Growth parameters

The experiment consisted of three treatments of *D. polyphylla* and *A. filaria* seedlings: (1) control (no inoculum); (2) *G. clarum*; and (3) *G. decipiens*. There were four replications per treatment. Shoot height and stem diameter at 1 cm from the soil surface were measured 180 days after transplantation. After harvest, shoots and roots were separated. They were oven-dried at 70°C for 72 h before weighing. Ground shoots were digested with H_2SO_4 and H_2O_2 solution (3:1, v/v). N and P concentration in the digested solution were determined by the semi-micro Kjeldahl method and vanadomolybdate yellow assay (Olsen and Sommers 1982), respectively.

An additional 30 seedlings each of *D. polyphylla* and *A. filaria* inoculated with *G. clarum* or *G. decipiens*, or uninoculated, were grown under the same conditions as those of the seedlings in the above experiment. Numbers of viable seedlings were counted 180 days after transplanting. Survival rate was calculated as follows: survival rate (%)=number of viable seedlings/number of initial seedlings ×100.

Arbuscular mycorrhizal colonization

Roots of *D. polyphylla* and *A. filaria* were washed gently over a 2-mm sieve under running tap water to separate them from soil particles. The roots were cleared in 100 g l^{-1} of KOH for 1 h, acidified with diluted HCl, and stained with 500 mg l^{-1} trypan blue in lactoglycerol (Brundrett et al. 1996). The roots were destained in 50% glycerol and 30 1-cm segments were viewed under a compound microscope at ×200 magnification.

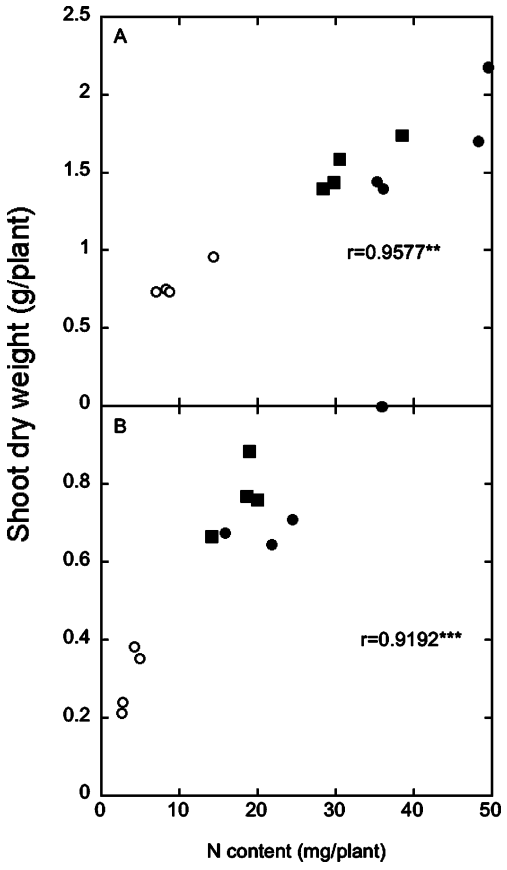

Fig. 1 Correlation between shoot N uptake and shoot dry weight of *D. polyphylla* (**a**) and *A. filaria* (**b**) inoculated with or without *G. clarum* and *G. decipiens*. Control (*open oblong*), *G. clarum* (*filled oblong*), and *G. decipiens* (*filled rectangle*). ***P<0.001 and **P<0.01

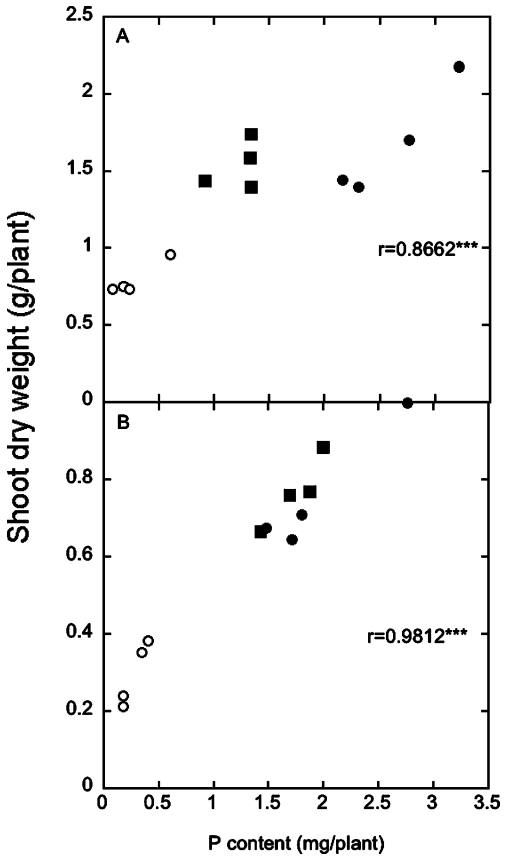

Fig. 2 Correlation between shoot P uptake and shoot dry weight of *D. polyphylla* (**a**) and *A. filaria* (**b**) inoculated with or without *G. clarum* and *G. decipiens*. Control (*open oblong*), *G. clarum* (*filled oblong*), and *G. decipiens* (*filled rectangle*). ***P<0.001

Percentage of mycorrhizal colonization was examined using the gridline intersect method (Giovannetti and Mosse 1980).

Statistical analyses

Data were statistically analyzed using analyses of variance with the statistical software StatView 5.0 (Abacus Concepts). Comparison of means was done using the least significant difference method at 5% probability level where the F value was significant.

Results

Arbuscular mycorrhizal colonization

D. polyphylla roots were more highly colonized by both *G. clarum* and *G. decipiens* than roots of *A. filaria* 180 days after transplantation under greenhouse conditions (Table 1). There was no difference in percentage colonization between *G. clarum* and *G. decipiens*. The control seedlings of *D. polyphylla* were colonized by indigenous AM fungi. No colonization was observed in the control seedlings of *A. filaria*.

Shoot nutrient concentrations and contents

N and P concentrations were higher in shoots of *D. polyphylla* inoculated with *G. clarum* and *G. decipiens* than control seedlings (Table 1), and *D. polyphylla* seedlings inoculated with these two AM fungi were not different in shoot N and P concentrations. AM colonization by *G. clarum* and *G. decipiens* increased shoot N and P content of *D. polyphylla* (Fig. 1). There was no difference in shoot N and P contents of *D. polyphylla* between *G. clarum* and *G. decipiens*.

Shoot N and P concentrations of *A. filaria* were higher in AM-colonized seedlings than in that of control seedlings (Table 1). There was no difference in shoot N and P contents of *A. filaria* between *G. clarum* and *G. decipiens*.

Correlation analyses revealed a positive relationship between shoot N content and shoot dry weight of *D.*

polyphylla or *A. filaria* (Fig. 1). Similarly, P content was positively correlated with shoot dry weight of *D. polyphylla* or *A. filaria* (Fig. 2).

Plant growth

AM colonization by *G. clarum* and *G. decipiens* increased shoot height, stem diameter, and root and shoot dry weight of *D. polyphylla* 180 days after transplantation (Table 1), although there was no difference between *D. polyphylla* seedlings inoculated with *G. clarum* and *G. decipiens*. AM colonization by *G. clarum* increased shoot height and stem diameter of *A. filaria* (Table 1). By contrast, *G. decipiens* did not increase shoot height or stem diameter of *A. filaria*. Furthermore, inoculation with *G. clarum* and *G. decipiens* increased shoot dry weight and root dry weight of *A. filaria*, although there was no difference between *A. filaria* seedlings inoculated with *G. clarum* and *G. decipiens*.

Survival rate

AM colonization by *G. clarum* and *G. decipiens* increased the survival rates of *D. polyphylla* 180 days after transplantation under greenhouse conditions. The survival rates of *D. polyphylla* inoculated with *G. clarum* (92%) and *G. decipiens* (100%) were higher than control seedlings (75%). The survival rates of *A. filaria* inoculated with both AM fungi (100%) were also higher than that of the control seedlings (90%). The two seedlings inoculated with *G. clarum* and *G. decipiens* were not different in survival rate.

Discussion

Inoculation of two AM fungi increased the early growth and nutrient concentrations of *D. polyphylla* and *A. filaria*. Although AM colonization was observed in native tree species grown in the tropical rain forest of Southeast Asia (Smits 1994; Moyersoen et al. 2001), studies about the role of AM in the tropical rain forest of Southeast Asia are rare (Alexander et al. 1992) compared to those of Africa and Latin America. *D. polyphylla* and *A. filaria* are important tree species in rain forests of Southeast Asia because these species provide NTFPs. Shoot height and stem diameter were also increased by inoculation of AM fungi. These parameters can determine the value of these species on the NTFPs. Improvement of early growth of these species would increase production of the NTFPs. Moreover, inoculation of AM fungi would be useful for conservation of *A. filaria* because this species is among the list of species that are considered to be threatened (Appendix II in CITES 2005).

Shoot growth response to the AM colonization was different between two plant species. Mycorrhizal dependency in *D. polyphylla* was 53 and 48% when inoculated with *G. clarum* and *G. decipiens*, respectively; and 61 and 62% in *A. filaria*. *A. filaria* responded more to the AM colonization than *D. polyphylla*. The AM colonization of *A. filaria* was lower than *D. polyphylla* but no explanation in the difference can be found. Different mycorrhizal dependency of tree species is related to characteristics of root and root hair (Pope et al. 1983; Manjunath and Habte 1991; Jasper and Davy 1993). The present study did not measure any root characteristics due to the limited amount of root. Further investigation needs to clarify a mechanism of different response to AM colonization between two plant species.

AM colonization increased contents of N and P of *D. polyphylla* and *A. filaria*. Chemical fertilizer was commonly applied to these species in nurseries on the commercial scale. Heavy application of chemical fertilizer decreases AM colonization and thereafter diminish growth improvement by AM fungi. If AM fungal inoculum could be produced at small cost, inoculation of AM fungi can reduce the application of chemical fertilizer without decrease in AM colonization.

The survival rate of seedlings is a key measure of success in reforestation and afforestation. In Indonesia, loss of tropical forests was extensive due to population growth, logging, and land demand for various development projects. Today, reforestation and afforestation programs aim to cover three million hectares during a 5-year period to convert degraded forest into sustainable forest production units. This means that more than three billion seedlings (1 ha=1,000 seedlings) are needed. There is a 10% difference in survival rate between AM-colonized seedlings and control seedlings. The estimated profit from this 10% difference is US $ 90 million (price of one seedling=US $ 0.3). This high profit is tantamount to saving in seedling stocks, labor cost, and fertilizer cost in nurseries. Increased survival rates were noted in tropical tree species with AM colonization, such as *Ochroma pyramidale*, *Luehe seemanii* (Kiers et al. 2000), *Heliocarpus appendiculatus*, and *S. donnell-smithii* (Guadarrama et al. 2004).

In conclusion, colonization by *G. clarum* and *G. decipiens* increased plant growth, shoot nutrient concentrations, and survival rates of *D. polyphylla* and *A. filaria* seedlings 180 days after transplantation under greenhouse conditions. Field trials with AM fungal inoculation are required to monitor the growth and survival rates of both species. Inoculation techniques may be adopted by a large-scale nursery jointly with reforestation programs, thereby aiding in the recovery of the population of such endangered tropical tree species as *A. filaria*. From the results of this study, we suggest that AM fungi can accelerate the establishment of the planting stocks of *D. polyphylla* and *A. filaria*, thereby ecologically promoting nature conserva-

tion and economically sustaining the harvest and production of NTFPs.

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