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Response of directly seeded high-value timber species to microorganisms, fertiliser and a water retention polymer: implications for reforestation of agricultural lands in Southeast Asia

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ABSTRACT: High-value native timber species are being promoted in reforestation in Southeast Asia. However, slow growth during early establishment, coupled with poor soil fertility, poses challenges for promoting tree planting programs. Field trials were undertaken on agroforestry land in Thailand to examine the efficacy of reforestation treatments. The first trial examined the effect of applying microorganisms and fertiliser on directly seeded *Acacia mangium* Willdenow, *Dalbergia cochinchinensis* Pierre and *Xylia xylocarpa* (Roxburgh) Theobald. After 20 months, a mixed inoculum of arbuscular mycorrhizal and ectomycorrhizal fungi improved survival and basal diameter of *D. cochinchinensis* by 15 and 43%, respectively. The co-inoculation of arbuscular mycorrhizal fungi and N_2-fixing bacteria improved survival by 17%. The second trial investigated the effects of fertiliser and a water retention polymer on directly seeded *A. mangium*, *Afzelia xylocarpa* (Kurz) Craib, *D. cochinchinensis*, *Eucalyptus camaldulensis* Dehnhardt, *Sindora cochinchinensis* Baillon and *X. xylocarpa*. Height was improved by 40% at 20 months. Our findings suggest that reforestation is viable, provided that suitable treatments are used. These principles can be applied for reforestation of nutrient-impoverished soils of continental Southeast Asia.

Keywords: forestry; mycorrhizal fungi; rehabilitation; root nodule bacteria; Rose woods

In Southeast Asia, many high-value, slow-growing timber species from the legume family such as *Afzelia xylocarpa* (Kurz) Craib and *Dalbergia cochinchinensis* Pierre (Soerianegara, Lemmens 1993; Vũ 1996) are threatened or endangered in their natural habitats (IUCN 2009; Pakkad et al. 2014), and are placed on top of the priority species list of many countries (Cambodia, Lao PDR, Thailand and Vietnam) (Nghia 2004; Phongoudome, Mounlamai 2004; Sumantakul 2004; FLD 2006). The listing indicates the significant role of the species in economic development and conservation, as well as the need for promoting them in reforestation programs (Luoma-Aho et al. 2004). However, apart from a few small-scale plantations managed for conservation purposes (Nghia 2004; Phongoudome, Mounlamai 2004; FA 2007), these species have not been widely used in tree plantings. The slow-growing habit during establishment (Soerianegara, Lemmens 1993; Lee 2005) is the main constraint in promoting these species, as the young seedlings are vulnerable to early competition with weeds and damage from forest fire. One of the practical solutions in reforestation when using slow-growing species is to plant them beneath the canopy of fast-growing nurse crops, mainly *Acacia mangium* Willdenow and *A. auriculiformis* A. Cunningham ex Bentham, when the grasses have been outcompeted

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(Otsamo 1998; McNamara et al. 2006). Another potential solution is the application of fertiliser, which is widely used to facilitate the early growth of newly planted seedlings to help them overcome the harsh conditions of some planting sites (Kalinganire, Pinyopusarerk 2000; Noor et al. 2002).

The majority of legume trees form symbiotic associations with N_2-fixing bacteria and mycorrhizal fungi (Faria et al. 1987; Herrera et al. 1993; Rasolomampianina et al. 2005). However, the knowledge of the status of N_2-fixing capacity or mycorrhizal fungal association or dependence of high-value timber species of continental Southeast Asia is limited. In addition, the value of microorganisms in promoting the early establishment and growth from direct seeding of high-value timber legumes has not been investigated.

Soil solution, a mixture of soil water and dissolved nutrients, is a key medium for supporting plant and soil biota growth (Bardgett 2005). In bare ground, such as agricultural land, the soil moisture content in the surface horizon is significantly lower than that under forest cover (Uhl et al. 1988). Therefore, maintaining soil moisture availability is essential to prevent plant mortality and to sustain growth. Traditionally, soil moisture can be maintained by application of organic matter including covering the soil surface with mulch (Russell 1973). These methods, however, are time and resource consuming. Application of water retention polymers for conserving soil moisture for promoting plant growth has been tested, particularly in arid regions (Duangpatra, Attanandana 1992; Hayat, Ali 2004; Bhat et al. 2009). However, the effect of water retention polymers on direct seeding in reforestation has not been tested. It is likely that water retention polymers not only retain soil water during intermittent dry periods in the wet season, but also extend soil water availability into the dry season. Such products are worth testing together with direct seeding as newly emerged seedlings are susceptible to drought. Sustaining water availability during the first growing season may enhance the growth of newly emerged seedlings and thus they are better able to withstand the ensuing dry period.

The aim of this study is to explore the effects of microorganisms, fertiliser and a water retention polymer on directly seeded high-value timber species of Southeast Asia: *A. xylocarpa*, *D. cochinchinensis*, *Sindora cochinchinensis* Baillon and *Xylia xylocarpa* (Roxburgh) Theobald. Two fast-growing exotic species, *A. mangium* and *Eucalyptus camaldulensis* Dehnhardt, were included in the study as reference species, but they are not the main focus. Consideration of the growth characteristics of the high-value timber trees and possibilities for promoting early establishment and growth led to the following hypotheses:

(*i*) Direct seeding of high-value timber species is a viable option for reforestation of a former agricultural land;

(*ii*) Beneficial microorganisms, inorganic fertiliser and a water retention polymer enhance the survival and early growth of directly seeded, high-value timber species.

To test these hypotheses, two separate experiments were established at a site in Sakeaw Province, Thailand, adjacent to the Thai-Cambodian border. This site was chosen because: (*i*) it is the natural habitat of all indigenous species used in this study, (*ii*) the climatic conditions represent a large region of continental Southeast Asia.

MATERIAL AND METHODS

Study site. The study site (13°33’54”N, 102°10’20”E) was located in Wang Nam Yen District, Sakaew Province, Thailand, ca. 200 km east of Bangkok. It was located within the forest plantation concession of the Forest Industry Organization, a state-owned forest enterprise under the Ministry of Natural Resources and Environment, Thailand. The area is flat with an altitude of 80 m a.s.l. The climate is governed by the monsoon and characterized by two distinct seasons, the wet season from May to October, and the dry season from November to April. In 2008, the area received an accumulative rainfall of 1939 mm and the mean annual temperature was 27°C (Thai Meteorological Department 2008). The trial site was previously covered with a mixed deciduous forest, dominated by *Pterocarpus macrocarpus* Kurz, *A. xylocarpa*, *X. xylocarpa* and *D. cochinchinensis*, which was cleared in the late 1970s. Since then, the area has been managed for crop production, six-year rotation of *E. camaldulensis* and cassava agroforestry for the past 30 years before the establishment of the experimental plots (Chaiya Junsawang 2008, pers. comm.).

The soil is a sandy loam derived from a red-yellow Podzol (Moormann, Rojanasoonthon 1967). The topsoil, which varies from 5 to 20 cm, is grey in colour. Gravels and stones constitute around 20–30% and 40–50% of the soil composition in the top soil and subsoil, respectively. Two soil samples were randomly collected and sent to the Laboratory of Forest Soil at the Faculty of Forestry, Kasetsart University, Thailand, for physical and chemical analyses (Table 1).

Table 1. Physical and chemical properties of soils in the experimental trials

	Experiment 1		Experiment 2	
	0–20 (cm)	21–50 (cm)	0–20 (cm)	21–50 (cm)
Sand (%)	65	78	66	71
Silt (%)	19	9	18	16
Clay (%)	16	13	16	13
pH (H_2O ratio 1:1)	6.04	5.54	5.32	5.9
Total C (%)[1]	1.73	0.58	1.16	0.45
Total N (%)[1]	0.16	0.07	0.11	0.05
P (mg kg^{-1})[2]	8.91	2.03	4.17	1.8
K (mg kg^{-1})[3]	174	63.94	70.3	28.16
CEC (cmol kg^{-1}) [4]	10.24	4.99	8.25	5.56
OM (%)[5]	3.41	0.93	2.08	0.58
Ca (mg kg^{-1})[3]	1,663.6	854.8	1,337.6	107
Mg (mg kg^{-1})[3]	177.36	72.66	99.16	50.4

[1]total C and N: combustion method (Dumas method), [2]bray II, [3]extracted with NH_4OAc, pH 7.0 and analysed with an atomic absorption spectrophotometer, [4]1M NH_4OAc, pH 7.0, [5]Walkley-Black acid digestion, the values are single measurements
CEC – cation exchange capacity, OM – organic matter

Seed sources. Four indigenous tree species, *A. xylocarpa*, *D. cochinchinensis*, *S. cochinchinensis* and *X. xylocarpa*, were selected for this study based on availability, socio-economic value, conservation value and potential N_2-fixing capability. Seeds were provided by the Royal Forest Department of Thailand. Seeds of *A. xylocarpa* and *S. cochinchinensis* were collected in 2003, those of *D. cochinchinensis* in 2006, and those of *X. xylocarpa* in 2008. Seeds of *A. mangium* and *E. camaldulensis* were collected in 2006 by the Australian Tree Seed Center, Canberra. Seeds of uniform size and no signs of physical damage or insect attack were selected by visual examination (except for *E. camaldulensis* due to the small seed size). The seeds were then pretreated, if required, to promote quick and uniform germination. Seed coats of *A. xylocarpa* and *S. cochinchinensis* were scarified at the distal end opposite to the hilum and then soaked in tap water overnight. Seeds of *D. cochinchinensis* and *X. xylocarpa* were soaked in tap water overnight. Seeds of *A. mangium* were immersed in hot water for 1 min, whereas those of *E. camaldulensis* required no pretreatment (Gunn 2001).

Experimental design and treatment. Two experiments were established at the same site.

Experiment 1: Through direct seeding, this experiment investigated the effect of microorganisms with or without inorganic fertiliser on survival and growth of three tree species, *A. mangium*, *D. cochinchinensis* and *X. xylocarpa*. The layout was a split-plot design involving three tree species as subplot treatments in a 2 × 6 (12 plots) factorial experiment with two levels of inorganic fertiliser and six levels of microorganism treatments (Table 2) as main plots. Within each of the 3 replicated blocks there were 12 main plots, arranged in a randomized complete block design (RCBD), six of which were randomly allocated to "with fertiliser" and the other six were allocated to "without fertiliser". Each main plot was then subdivided into six planting rows to which three tree species were randomly allocated to two adjacent rows. The spacing between two adjacent seeding spots within a row or between two adjacent rows was 1 m. There were 3 m wide buffer strips, without trees, between adjacent plots. This design resulted in 20 seeding spots per species or 60 planting spots per plot.

Experiment 2: Through direct seeding, this experiment investigated the effect of inorganic fertiliser and a water retention polymer on survival and growth of six species, the three species used in experiment 1, plus *A. xylocarpa*, *E. camaldulensis* and *S. cochinchinensis*. The trial was set up using a split-plot design involving six tree species in the subplots and four levels of treatments in the main plots (Table 2). Within each of the 3 replicated blocks there were four main plots, arranged in RCBD. The arrangement of subplots within a main plot and the spacing within and between rows were the same as in experiment 1, except that the main plot had 12 planting rows. This design resulted in 20 direct seeding holes per subplot or 120 holes per main plot. Buffer strips were used between adjacent plots as in experiment 1.

Trial establishment. The trial site was prepared in mid-April 2008. A small bulldozer was used to push over the stumps of eucalypts, which remained following clear cutting, and the area was then disc-ploughed twice to 20 cm depth. Remaining debris was removed by hand. The trial was established in mid-

Table 2. Descriptions of the treatments used and methods of application in experiments 1 and 2

Treatment	Method of treatment application
Experiment 1	
Microorganism	
Root nodule	Crushed root nodule solution was applied 2 cm below the seed (ca. 1 nodule per seeding spot). Root nodules of *Acacia mangium*, *Dalbergia cochinchinensis* and *Xylia xylocarpa* were collected from mature trees from planted or native remnant vegetation near the trial site. Freshly collected root nodules (two days old) were used. Nodules were used because no pure tested inoculum was available for these species at the time the experiments were established
AMF	Mixed AMF inoculum (ca. 170 spores per seeding spot) was applied 2 cm below the seed. The AMF inoculum contained *Scutellospora* sp. + *Glomus etunicatum* W.N. Becker & Gerd (from Department of Biology, Chiang Mai University, Thailand); *Glomus* sp. + *Acaulospora* sp. (from Department of Soil Science and the Rhizobium Research Center, Kasetsart University, Thailand) and unknown species from a commercial inoculum (Myco Star; Amputpong Co. Ltd., Bangkok, Thailand). All AMF inocula were mixed, with equal numbers of spores from each inoculum, into one batch, giving a total of ca. 24 spores g^{-1} soil
AMF + ECMF	The same amount of mixed AMF inoculum + 10 ml of ECMF spore solution (ca. 3×10^8 spores per seeding spot) were placed 2 cm below the seeds. The ECMF inoculum contained 99% of *Pisolithus alba* spores, a species that fruits beneath eucalypt plantations in Thailand
Root nodule + AMF	A dual application of root nodule and AMF. The same amount of crashed root nodule solution mixed with the same amount of mixed AMF inoculums. The mixture was place 2 cm below the seeds
Root nodule + AMF + ECMF	A tripartite application of root nodule, AMF and ECMF. The same amount of crashed root nodule solution mixed with the same amount of mixed AMF inoculums and the same amount of ECMF spore solution. The mixture was place 2 cm below the seeds
Control	No inoculum
Fertiliser	
With fertiliser	One set of microorganism treatments above received 5 g of inorganic fertiliser (14:14:14 $N:P_2O_5:K_2O$; Osmocote Controlled Release Fertiliser, Sotus International Co. Ltd., Nonthaburi, Thailand) + micronutrient mix (0.675 mg MgO, 0.225 mg S, 0.3 mg Fe, 0.3 mg Mn, 0.113 mg Cu, 0.113 mg B, 0.038 mg Zn and 0.008 mg Mo per seeding spot; Sahan Kaset Co. Ltd., Bangkok, Thailand) applied at 10 cm from the seeding spot to a depth of 10 cm at the time of seeding
Without fertiliser	Another set of six microorganism treatments received no inorganic fertiliser
Experiment 2	
Fertiliser	The same amount of fertiliser used in experiment 1 was applied in the same way and at the same time
Polymer	A solution of 2.5 g of water retention polymer (WaterSave, Polymer Innovations Pty Ltd, Singleton, Australia) dissolved in 1 l of water was placed 20 cm below the seed
Fertiliser + polymer	A dual application of fertilizer and polymer. The same amount of fertilizer used in experiment 1, applied in the same way and at the same time, and a solution of 2.5 g of water retention polymer dissolved in 1 l of water was placed 20 cm below the seed
Control	Neither fertiliser nor polymer

AMF – endomycorrhizal fungi, ECMF – ectomycorrhizal fungi

May 2008 after the soil had received some monsoonal rain. Each seeding spot was marked with a 0.5 m stick to facilitate seeding, monitoring and maintenance. Three seeds were placed in each seeding spot [ca. 10 × 10 × 2–5 cm (depth, depending on seed size)] and covered with a soil layer to the depth of the seed. The number of seeds of *E. camaldulensis* per seeding spot was not consistent as it was not practical to separate the seed from the chaff. Large seeding holes, 20 × 20 × 20 cm, were made to accommodate the 1 l volume of water retention polymer. Treatments and methods of treatment applications are shown in Table 2.

Maintenance of the field trial. Two weeks after trial establishment in both experiments, seeding spots without germinants were replaced with three seeds of the same species. Seeding spots that contained one or more germinants were not reseeded. The refilling rates were ≤ 5% for *A. mangium*, *A. xylocarpa*, *D. cochinchinensis*, *S. cochinchinensis* and *X. xylocarpa* and ca. 60% for *E. camaldulensis*. No repeat treatments

Table 3. Survival rate, height and diameter growth at 20 months in experiment 1 as effects of microorganism, fertiliser and tree species

	Survival rate (%)[1]			Height (m)[1]		Diameter (cm)[1]	
Microorganism							
Root nodule		97.50 ± 0.93		2.59 ± 0.56		3.61 ± 0.56	
AMF		97.22 ± 0.92		2.61 ± 0.49		3.64 ± 0.48	
AMF + ECMF		99.44 ± 0.56		2.59 ± 0.52		3.58 ± 0.48	
Root nodule + AMF		96.39 ± 1.39		2.33 ± 0.50		3.26 ± 0.52	
Root nodule + AMF + ECMF		93.06 ± 3.16		2.43 ± 0.55		3.34 ± 0.55	
Control		96.67 ± 1.76		2.40 ± 0.50		3.31 ± 0.48	
Fertiliser							
With fertiliser		95.65 ± 1.17^{b}		2.57 ± 0.30		3.52 ± 0.29	
Without fertiliser		97.78 ± 0.74^{a}		2.42 ± 0.29		3.40 ± 0.29	
Tree species							
Acacia mangium		98.33 ± 0.66^{a}		5.43 ± 0.14^{a}		6.26 ± 0.18^{a}	
Dalbergia cochinchinensis		92.50 ± 1.78^{b}		1.13 ± 0.07^{b}		1.81 ± 0.09^{c}	
Xylia xylocarpa		99.31 ± 0.29^{a}		0.91 ± 0.05^{c}		2.30 ± 0.07^{b}	
Analysis of variance	*df*	*F*	Sig.	*F*	Sig.	*F*	Sig.
Main-plot analysis							
Block	2	1.471	0.251	6.213	0.007	3.653	0.043
Microorganism	5	2.299	0.080	1.251	0.320	1.339	0.285
Fertiliser	1	5.285	**0.031**	1.082	0.309	0.696	0.413
Microorganism × fertiliser	5	2.190	0.092	1.682	0.181	1.433	0.252
Error	22						
Subplot analysis							
Tree species	2	21.332	**0.000**	746.489	**0.000**	560.274	**0.000**
Microorganism × tree species	10	2.713	**0.010**	1.967	0.059	2.537	**0.015**
Fertiliser × tree species	2	0.317	0.730	0.161	0.852	1.773	0.181
Microorganism × fertiliser × tree species	10	1.386	0.215	0.965	0.486	0.710	0.711
Error	48						

[1]values are replicated block means (n = 3) ± SE, significant ANOVA probabilities are marked in bold, means followed by the same letters are not significantly different at $P \leq 0.05$

AMF – endomycorrhizal fungi, ECMF – ectomycorrhizal fungi

were applied to the refilling spots. At two months after trial establishment, thinning was undertaken where the seeding spots contained more than one seedling. The trial site was fenced, using barbwire, to a height of 1.5 m to reduce the entry of cattle and humans. Weeding in plots was carried out two months after seeding using hand tools. Weeding was then carried out every two months until the seedlings were six-months-old (December 2008). A final weeding was carried out in the middle of the second rainy season, August 2009. The buffer zones between plots were treated once with herbicide at three months using glyphosate isopropylammonium mixed with a surfactant (brand name: Mixer) at a respective rate of 300 ml and 200 ml in 20 l of water, as directed by the label.

Data collection and analysis. Survival rate, height and basal diameter (at 2 cm above the ground level) were recorded at 20 months after trial establishment. Data analyses were carried out using SPSS statistical package Version 15.0 (SPSS Inc., Chicago, USA). For all tests, the probability $P \leq 0.05$ was used to determine significance. For both experiments, Linear (Mixed Models) analysis was used. For post hoc analyses, a multiple comparison test, Sidak test, was used to detect differences among means. ANOVA with Type III sums of squares was used, as this adjusts the treatment effects for any differences between blocks. The survival rate was calculated based on the number of direct seeding holes with the presence of a seedling at the time of assessment as percentage of the total

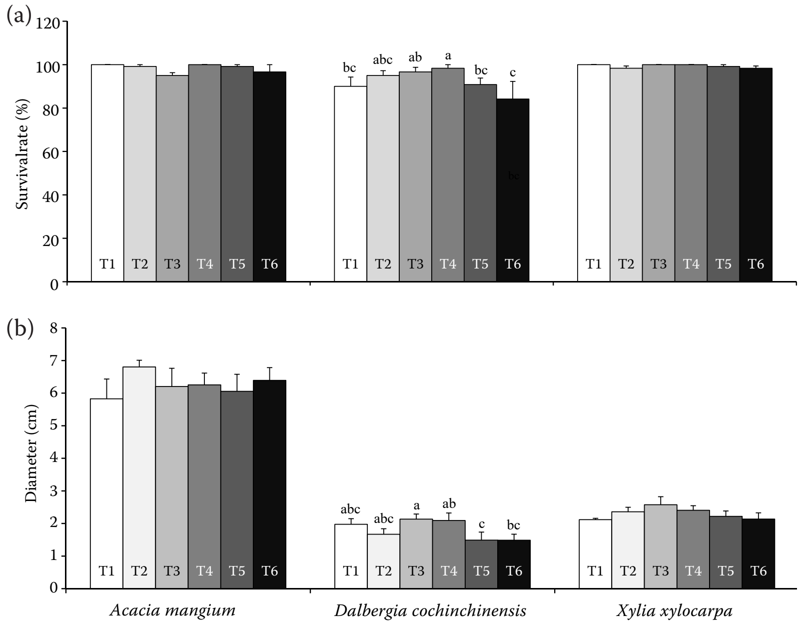

Fig. 1. Effect of microorganism treatments on survival rate and diameter growth of three tree species at 20 months in experiment 1

in the same species, columns with the same letters are not significantly different at $P \leq 0.05$
T1–T6 – microorganism treatments, T1 – root nodule, T2 – endomycorrhizal fungi, T3 – endomycorrhizal + ectomycorrhizal fungi, T4 – root nodule + endomycorrhizal fungi, T5 – root nodule + endomycorrhizal fungi + ectomycorrhizal fungi, T6 – control

direct seeding holes per plot (n = 20). Percentages of survival rates are replicated block means across three blocks. They were transformed to arc-sin square root transformation (Gomez, Gomez 1984) prior to statistical analysis. Height and diameter data in both experiments were Napierian logarithms transformed before analysis to fit the normal distribution. The descriptive statistics presented in graphs and tables are untransformed data.

RESULTS

Experiment 1

Overall, *A. mangium*, *D. cochinchinensis* and *X. xylocarpa* exhibited remarkably high survival rates (> 92%) after 20 months of growth (Table 3). The difference between the highest, *X. xylocarpa*, and the lowest, *D. cochinchinensis*, was only 7%. Among the three species, only *D. cochinchinensis* responded positively to the co-inoculation of endomycorrhizal fungi (AMF) + ectomycorrhizal fungi (ECMF) and root nodule + AMF (Fig. 1a). The differences in the survival rate of these two treatments from the control were 15 and 17%, respectively.

Height was not affected by microorganisms or fertiliser (Table 3) but it differed among tree species with *D. cochinchinensis* being 24% higher than *X. xylocarpa*. Diameter growth of all species was not affected by fertiliser. Among the three species, *D. cochinchinensis* responded positively to co-inoculation with AMF + ECMF resulting in 43% larger diameters than the control trees (Fig. 1b). The differences in diameter growth among tree species were not affected by the fertiliser used.

Experiment 2

After 20 months, treatments did not promote the survival (Table 4) of any of the 6 species. As for ex-

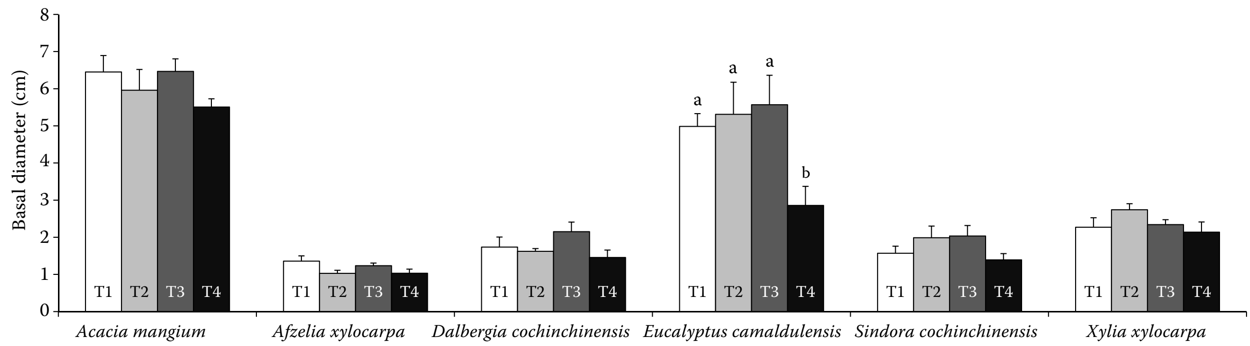

Fig. 2. Effect of treatments on diameter growth of *Acacia mangium, Afzelia xylocarpa, Dalbergia cochinchinensis, Eucalyptus camaldulensis, Sindora cochinchinensis* and *Xylia xylocarpa* at 20 months in experiment 2

for the same tree species, columns followed by the same letters are not significantly different at $P \leq 0.05$
T1–T4 – treatments, T1 – fertiliser, T2 – polymer, T3 – fertiliser + polymer, T4 – control

Table 4. Survival rate, height and diameter growth at 20 months in experiment 2 as effects of treatments and tree species

		Survival rate (%)[1]		Height (m)[1]		Diameter (cm)[1]	
Treatment							
Fertiliser		90.28 ± 5.37		2.14 ± 0.49[ab]		3.06 ± 0.48[ab]	
Polymer		91.94 ± 3.69		2.29 ± 0.49[ab]		3.11 ± 0.48[ab]	
Fertiliser + polymer		92.50 ± 3.51		2.40 ± 0.48[a]		3.30 ± 0.50[a]	
Control		84.72 ± 5.92		1.72 ± 0.40[b]		2.40 ± 0.38[b]	
Tree species							
Acacia mangium		99.58 ± 0.42[a]		5.19 ± 0.16[a]		6.10 ± 0.21[a]	
Afzelia xylocarpa		91.67 ± 3.39[a]		0.48 ± 0.03[d]		1.17 ± 0.06[e]	
Dalbergia cochinchinensis		97.50 ± 0.97[a]		1.10 ± 0.10[b]		1.74 ± 0.12[d]	
Eucalyptus camaldulensis		64.58 ± 8.84[b]		4.25 ± 0.33[a]		4.68 ± 0.43[b]	
Sindora cochinchinensis		97.92 ± 1.44[a]		0.86 ± 0.10[c]		1.75 ± 0.13[d]	
Xylia xylocarpa		87.92 ± 6.47[a]		0.95 ± 0.09[bc]		2.38 ± 0.11[c]	
Analysis of variance	*df*	*F*	Sig.	*F*	Sig.	*F*	Sig.
Main-plot analysis							
Block	2	0.008	0.992	4.959	0.054	4.183	0.073
Treatment	3	0.346	0.794	4.907	**0.047**	5.845	**0.033**
Error	6						
Subplot analysis							
Tree species	5	9.672	**0.000**	271.031	**0.000**	178.773	**0.000**
Treatment × tree species	15	0.945	0.526	1.833	0.064	1.922	**0.050**
Error	40						

[1]values are replicated block means (n = 3) ± SE, significant ANOVA probabilities are marked in bold, means followed by the same letters are not significantly different at $P \leq 0.05$

periment 1, the survival rate of all indigenous species was high, being ≥ 87%. There was a significant difference among tree species, *A. mangium* had the highest survival rate and *E. camaldulensis* had the lowest rate.

Treatments had a significant effect on the height of all species tested (Table 4). The combination of fertiliser and water retention polymer resulted in a 40% increase in height over the control. Height also significantly differed among tree species. Among the high-value indigenous trees, *D. cochinchinensis* had the highest height growth, being more than double that of *A. xylocarpa.* Treatments had a significant effect on the diameter growth of *E. camaldulensis* (Fig. 2). Fertiliser, polymer and the combination of fertiliser and polymer increased the diameter of *E. camaldulensis* by 74, 86 and 95%, respectively.

DISCUSSION

The survival rate for the indigenous species was very high after 20 months (> 87%). Therefore, the hypothesis that direct seeding of high-value timber species is a viable option for reforestation of a former agricultural land was established. These survival rates are much higher than in previous direct seeding trials in the region, for example, the Cambodia Tree Seed project conducted a direct seeding trial of *A. xylocarpa* on degraded land in Kampong Speu province, Cambodia, and the survival rate at three months was only 30% (CTSP 2005). Tunjai (2005) obtained a survival rate of 52% and height growth of 29.3 cm after one year for *A. xylocarpa* that was directly seeded in Chiang Mai province, Northern Thailand. In the latter, weeding was undertaken every two months. The high survival rate achieved in the current study could have been enhanced by a range of factors including the method of seeding, post seeding maintenance, species characteristics and environmental conditions (e.g. early rainfall, fencing, low seed/seedling predation).

Pretreatment of seeds to accelerate seed germination improves the efficiency of direct seeding as individual seed is ready to grow (Schmidt 2008). In this study, seeds were buried to the seed width, therefore, they were not exposed to desiccation prior to germination. This method of direct seeding was reported by Doust et al. (2006) for 18 species in tropical Queensland. They showed that burying seeds resulted in higher survival rates than broadcasting seeds. All of the four indigenous tree species used in the current study originate from deciduous forests (Vũ 1996; Dy Phon 2000) and thus can be presumed to have adapted themselves to the harsh conditions of those forest lands. These species can regenerate in natural habitats when optimal conditions are met (Kaewkrom et al. 2005; Koonkhunthod et al. 2007). In addition, the trial was established at the beginning of the wet season, in the second half of May, after the soil had received some rain. Thus, the newly emerged seedlings had enough time to develop a rooting system in the subsoil in order to access stored water during the dry season. The trial site had been disc-ploughed twice before seeding, and the plots were kept free of weeds until the middle of the second rainy season (August 2009). The results may have been significantly different if weeding was not undertaken. Non-weeded observational plots established adjacent to experiment 2 were fully covered with vigorous weeds to a height of > 1 m at eight months. During the second year, none of the four indigenous and two fast-growing species, *A. mangium* and *E. camaldulensis*, had emerged from the weeds, even though germination was evident shortly after seeding (data not presented). Weed competition and the requirement of frequent weeding is a great disadvantage of direct seeding (Schmidt 2000). Consequently, some researchers have treated weeding frequency as a set of treatments, not only in direct seeding experiments (Willoughby, Jinks 2009), but also in the planting of nursery-raised seedlings (Günter et al. 2009).

One of the objectives of experiment 1 was to look at the effects of microorganisms on direct seeding of high-value timber species. Only *D. cochinchinensis* responded to inoculation with microorganisms, both in increased survival and diameter growth. Dual inoculation of AMF either with ECMF or N_2-fixing bacteria (crushed root nodules) significantly improved the survival rate. Dual inoculation of AMF with ECMF also improved the diameter growth of this species. It is unclear why the other inoculation treatments were not efficacious (see also So et al. 2011). It was not possible to check mycorrhizal infection and root nodule formation in the field and any preference of *D. cochinchinensis* for particular mycorrhizal fungi is unknown. Limited observations to date suggest that *D. cochinchinensis* can form symbiotic associations with ECMF (Chalermpong, Bunthaveekun 1982), but the compatible species are unknown. The genus *Dalbergia* is known for its symbiotic association with AMF (Khan 2001; Mridha, Dhar 2007; Bargali 2011). Bisht et al. (2009) reported growth improvement in *D. sissoo* Roxburgh ex de Candolle under glasshouse conditions when the species was inoculated with AMF (*Gigaspora albida* Schenck & Smith, *Glomus intraradices* Smith & Schenck and *Acaulospora scrobiculata* Trappe), and a synergistic effect occurred when the fungi were co-inoculated with *Rhizobium leguminosarum* bv. *viciae*. *Xylia xylocarpa* and *A. mangium* did not respond to inoculation with bacteria or fungi. *Xylia xylocarpa* is reported to form symbiotic associations with AMF (Lakshman et al. 2001; Youpensuk et al. 2004), ECMF (Chalermpong, Bunthaveekun 1982) and slow-growing *Bradyrhizobium* spp. (Manassila et al. 2007).

The combination of fertiliser and water retention polymer (experiment 2) resulted in a 40% increase in the height growth of all species over the control. It is likely that the polymer helped retain the fertiliser on site and thus increased its availability for plant growth. The inorganic fertiliser had no effect on plant growth in experiment 1 where the polymer was not applied. That *E. camaldulensis* was more responsive than the other five large-seeded species in experiment 2 is likely to be related to the small seed reserves and hence greater dependence on soil nutrients immediately after emergence (Milberg et al. 1998). Previous studies have shown an improvement in the growth of planted seedlings when fertilisers were used in conjunction with water retention polymers (Duangpatra, Attanandana 1992; Hayat, Ali 2004; Bhat et al. 2009; Ruthrof et al. 2012). Duangpatra and Attanandana (1992) reported that, in sandy soil in the northeast of Thailand, the application of 70 g of a polymer (Acryhope) to each planting hole, together with 120 g of rock phosphate, increased the height of cashew (*Anacardium occidentale* Linnaeus), mango (*Mangiferra indica* Linnaeus cv. Keo-Sawei) and para-rubber (*Hevea brasiliensis* Muell, Arg) planted as seedlings in the dry season (January to May). Improving height growth is a desirable characteristic when planting slow-growing indigenous species, so seedlings can better compete with weeds for sunlight.

It is widely accepted that direct seeding significantly reduces establishment costs (Bullard et al. 1992; Engel, Parrotta 2001; Cole et al. 2011). Establishment cost may vary between species and methods of site preparation; therefore, a cost/benefit analysis is needed, particularly if a species with expensive seed is to be used in direct seeding. Reforestation by direct seeding has not been widely practiced in Southeast Asia. The availability of seeds of the target species could be a limiting factor for this practice. Currently, finding sufficient seeds of high-value timber trees, like *A. xylocarpa* and *D. cochinchinensis*, is a challenge in countries such as Cambodia, where there is a limited number of seed sources and the seed distribution system is in the early stage of development. In addition to scarcity, seed of *D. cochinchinensis* is expensive, being USD·200 kg^{-1} in Cambodia in 2010 (Uon Samol 2009, pers. comm.). Traditionally, many slow-growing indigenous species need to be raised and maintained in a nursery for about one year before outplanting in the field (FORRU 2006). With a minimum survival rate of 87% in the second year in this study, the pertinent question is: are resources for nursery activities essential for these species in reforestation? This study confirms that direct seeding of high-value timber species is practical for local communities and biodiversity conservation practitioners to restore community forests and conservation areas.

In conclusion, we found that reforestation of agricultural land through direct seeding of high-value timber species, *A. xylocarpa*, *D. cochinchinensis*, *S. cochinchinensis* and *X. xylocarpa*, is viable, provided that good site preparation and intensive weeding are undertaken, particularly during the first six months of growth. Dual inoculation of AMF either with ECMF or N_2-fixing bacteria substantially improved the survival of *D. cochinchinensis*, whereas the co-inoculation of AMF with ECMF improved the diameter growth of the same species after 20 months of direct seeding. In addition, the co-inoculation of inorganic fertiliser and a water retention polymer promoted the height growth of *A. xylocarpa*, *D. cochinchinensis*, *S. cochinchinensis* and *X. xylocarpa*.

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