EFFECTS OF ARBUSCULAR MYCORRHIZAL INOCULATION AND FERTILISATION ON THE GROWTH OF *ACACIA MANGIUM* SEEDLINGS

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JEYANNY V, LEE SS & WAN RASIDAH K. 2011. Effects of arbuscular mycorrhizal inoculation and fertilisation on the growth of *Acacia mangium* seedlings. *Acacia mangium* is a popular species for forest plantation and is widely used for the production of furniture, particleboard, pulp and paper and wood chips. Proper fertilisation and soil enhancement will further increase its growth and yield for sustainable forest plantation. Arbuscular mycorrhizas (AM) and fertilisation have shown promising results in increasing plant growth and biomass through increased nutrient uptake. However, the combined use of AM inoculation and fertilisation in fast-growing species such as *A. mangium* needs to be further assessed. The effects of the treatment on the growth of *A. mangium* seedlings, chemical contents of the growth media and shoot nutrient concentrations were investigated. Pot trials of uninoculated control without fertilisation (control), uninoculated with fertilisation (+NPK), inoculated without fertilisation (+AM) and inoculated with fertilisation (+AM+NPK) were laid out in a complete randomised design. Seedling growth was monitored for three months in the nursery. Shoot height, stem diameter and number of phyllodes were recorded monthly. Root and shoot biomass, root:shoot ratio, mycorrhizal infection rates, nutrient concentrations in media and shoot were determined at the end of the experiment. Treatments with +NPK and +AM+NPK significantly increased plant growth and media nutrient contents of *A. mangium* seedlings. Shoot nutrient concentrations of +AM fungi were relatively increased with inoculation.

Keywords: Mycorrhizas, fertiliser, seedling establishment, nursery, forest plantation, yield

JEYANNY V, LEE SS & WAN RASIDAH K. 2011. Kesan inokulasi mikoriza arbuskular dan pembajaan terhadap tumbesaran anak benih *Acacia mangium*. *Acacia mangium* merupakan spesies yang popular untuk perladangan hutan dan digunakan secara meluas untuk pengeluaran perabot, papan serpai, pulpa dan kertas serta serpai kayu. Pembajaan dan rawatan tanah yang sesuai akan meningkatkan lagi tumbesaran dan hasil kayu untuk pengurusan hutan secara mampan. Mikoriza arbuskular (AM) dan pembajaan telah menunjukkan kesan yang baik dalam meningkatkan prestasi tumbesaran pokok serta biojisim melalui penyerapan nutrien. Bagaimanapun, rawatan kombinasi inokulasi AM dan pembajaan untuk spesies yang cepat membesar seperti *A. mangium* perlu dikaji selidik. Kesan rawatan yang berlainan terhadap tumbesaran anak benih *A. mangium*, kandungan kimia media pertumbuhan dan kepekatan nutrien dalam pucuk telah dikaji. Kajian yang dijalankan dalam pasu ini terdiri daripada rawatan yang tidak mengandungi inokulasi dan pembajaan (kawalan), pembajaan tanpa inokulasi (+NPK), inokulasi tanpa pembajaan (+AM) dan kombinasi inokulasi serta pembajaan (+AM+NPK) dan kesemuanya disusun dalam reka bentuk rawak lengkap. Pertumbuhan anak benih telah dipantau untuk tiga bulan di tapak semaian. Ketinggian anak benih, diameter batang dan bilangan daun direkod setiap bulan. Biojisim akar dan pucuk, nisbah akar:pucuk, kadar jangkitan mikoriza serta kepekatan nutrien dalam medium dan pucuk telah ditentukan pada akhir eksperimen. Rawatan +NPK dan +AM+NPK telah meningkatkan tumbesaran anak benih dan kandungan nutrien medium *A.mangium* secara signifikan. Secara relatif inokulasi telah meningkatkan kandungan nutrien pucuk bagi rawatan +AM.

INTRODUCTION

Acacia mangium is a popular species for large-scale forest plantations in Malaysia and many other countries in South-East Asia (Harwood 2011). A member of the Leguminosae, this species was first introduced to Malaysia in the early 1960s (Yap 1986). *Acacia* species are known to form arbuscular mycorrhizas (AM). There are reports confirming the presence of spores of *Gigaspora* and *Glomus* in potted plants of *A. mangium* in the nursery (Reddell & Warren 1987). In another study, the inoculation levels of AM persisted into the field even after two years of outplanting (Azizah et al. 1994).

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Arbuscular mycorrhizas are known to enhance soil nutrient uptake, particularly phosphorus (P), and increase plant yield. These microbes have also been reported to improve drought resistance (Sieverding 1991), plant water retention (Kyllo et al. 2003) and carbon sequestration in soil (Treseder & Turner 2007). Arbuscular mycorrhizas have shown promising results in increasing plant height and biomass of forest species in China through increased nutrient uptake (Brundrett et al. 1995).

In Malaysia, it was reported that *A. mangium* planted on degraded ex-tin mining areas showed better plant growth when inoculated with AM (Azizah et al. 1994). Later investigations showed that incorporation of AM during outplanting trials for three-month-old *A. mangium* seedlings increased relative growth rates (diameter and height) for a period of up to six months in the field (Lee et al. 2006). The beneficial effect of AM on P uptake in poor soils has also been well documented (Martensson & Carlgren 1994, Ghosh & Verma 2006). It has also been reported that P fertilisers combined with mycorrhizas have enhanced growth of *Albizia falcataria, Parkia speciosa* (Norani 1989), *Hopea* spp. (Lee & Alexander 1994) and *Azadirachta excelsa* (Ong et al. (2002).

Establishing productive stands of forest tree species for forest plantation is vital, whereby management strategies should start from the nursery. Seedling stocks which are nutrient enriched, viable and productive in all soil types, particularly marginal areas, will have an upper hand in withstanding adverse environmental conditions to produce substantial yield (Xu & Timmer 1999). Incorporation of fertiliser with AM inoculation in forest tree nurseries has been successful in increasing the efficiency of plant mineral uptake and growth (Singh 1998). However, information on the combined effects of AM and fertilisation on *A. mangium* in the nursery is still scarce. Therefore, this preliminary study was carried out to investigate the effectiveness of a combination of AM inoculum and fertilisation on *A. mangium* seedlings in the nursery. The outcome from this investigation will further assist nursery managers in the decision-making process of whether to include this technique in their goal of achieving maximum production of viable planting stocks.

MATERIALS AND METHODS

The study was conducted at the Forest Research Institute Malaysia (FRIM), Kepong from July till October 2008. The temperature of the shadehouse where the experiment was carried out ranged between 29 and 32 °C. Light penetration was 32% and relative humidity, between 80 and 82%. Polyethylene bags (15 × 20 cm) were filled with 1.2 kg of 3:2:1 ratio of unsterile soil:organic matter:sand prior to transplanting. The mixture contained 0.14% N, 2.8% organic C, 35.5 mg kg^{-1} available P, 0.89 cmol kg^{-1} Ca, 0.14 cmol kg^{-1} Mg and 0.29 cmol kg^{-1} K. The pH, cation exchange capacity and electrical conductivity of the media were 5.0, 7.9 cmol kg^{-1} and 0.02 mS cm^{-1} respectively.

Acacia mangium seeds were obtained from the FRIM Seed Technology Laboratory. The seeds were collected from Karak, Pahang, Malaysia in July 2007. Seeds were germinated on river sand beds before transplanting. The nutrient content of seedlings prior to experiment was not determined due to the very low (less than 0.5 g) dry weight obtained.

In treatment with AM fungi alone (+AM), one-month-old seedlings were inoculated using 50 g Rhizagold™ (commercially produced native inocula) for each polybag. For treatment with NPK fertiliser (+NPK), a liquid nutrient solution comprising 120 mg N, P, K and 2 mg Mg was prepared by adding NPK Blue fertiliser (12% N:12% P_2O_5:7% K_2O:2% MgO) and distilled water. Seedlings were fertilised one week after transplanting to avoid transplanting shock. The rate and type of fertiliser selected were according to FRIM standard nursery practices. The +AM+NPK treatments consisted of 50 g Rhizagold™ and 1 g NPK Blue fertiliser. No AM and NPK fertiliser were added to the control treatment.

The factorial experiment had six replications for each treatment and was laid out in a complete randomised design on a clean sterilised canvas sheet to avoid contamination from the shadehouse flooring. Irrigation was carried out using an automated sprinkler system once daily. The effects of the treatments were evaluated three months later based on stem diameter, seedling height, number of phyllodes, root dry weight, shoot dry weight, root to shoot ratio and percentage of AM root infection. At harvest, the seedlings were separated into roots and shoots.

The roots were washed under running water to remove the soil. Fine roots (approximately 5 g) were collected using a 1-mm sieve to determine the extent of mycorrhizal inoculation. The remaining roots and shoots were dried at 70 °C in a ventilated oven for 48 hours and their biomass determined thereafter. Soil samples from each treatment were collected and air dried at room temperature (27 °C) until constant weight and passed thorough a 2-mm sieve. The pH, electrical conductivity, concentrations of C, N, P, exchangeable K, Ca and Mg of the growth media were measured based on standard chemistry laboratory practice—the dry pH of the soil was determined in a 1:2.5 soil:water slurry; electrical conductivity was determined using an EC meter; organic carbon was analysed according to Walkley and Black (1934) method; soil nitrogen by Kjeldahl digestion; available P by Bray and Kurtz Method No. 2 (Sharifuddin & Dynoodt 1981); exchangeable K, Ca and Mg were extracted using 1 M ammonium acetate (NH_4OAc) calibrated at pH 7.

Oven-dried shoot portions were ground to fine powder before analysis. Due to the small size of plants at three months after transplanting (less than 3 mm in diameter) only samples amounting to more than 1 g were analysed. Total nitrogen was determined by dry combustion method (Carter & Gregorich 2008), carbon by Walkley and Black (1934) method and total P, K, Ca and Mg values were determined using the dry ashing and nitric acid digestion method (MS 1980) followed by determination of element concentration using inductively coupled plasma (ICP) spectrometer.

Variables were analysed using one-way ANOVA. Tukey's test was used for means comparison using Statistical Analysis System version 9.13.

RESULTS

Treatments +NPK and +AM+NPK were significant for seedling height and stem diameter compared with control (Table 1). Seedling height increased by 60 and 77% whereas stem diameter increased by 93 and 134% for +NPK and +AM+NPK respectively. The combined treatment of inoculation and fertilisation (+AM+NPK) also significantly increased phyllode number (25%), root:shoot ratio (92%), root weight (21 folds), shoot weight (9 folds) and mycorrhizal infection (195 folds) over the control.

The mean effects of +AM fungi for pH, EC, exchangeable Ca and Mg were all significantly higher compared with the control at 12, 67, 37 and 400% respectively for growing media (Table 2). Nitrogen levels were significantly elevated (13%) in +NPK but organic C content was the same for all treatments. Levels of P for +NPK and +AM+NPK were higher than the control, i.e. 33 and 27 folds respectively. The same treatments showed similar increased levels of K at about 100%.

Table 1 Effects of arbuscular mycorrhizal inoculation and fertilisation on the growth, biomass and root infection *of Acacia mangium* seedlings three months after transplanting

Treatment	Height (cm)	Stem diameter (mm)	Phyllode number	Root weight (R) (g)	Shoot weight (S) (g)	R/S ratio	Mycorrhizal infection (%)
Control	9.9 b	1.10 b	2 a	0.01 b	0.10 b	0.13 a	0.40 b
	(1.15)	(0.13)	(0.48)	(0.02)	(0.01)	(0.05)	(0.33)
+NPK	15.8 a	2.12 a	2 a	0.25 a	0.81 a	0.29 a	24.4 b
	(1.54)	(0.21)	(0.61)	(0.06)	(0.16)	(0.04)	(6.26)
+AM	10.1 b	1.55 b	2 a	0.08 ab	0.51 ab	0.13 a	70.7 a
	(1.52)	(0.12)	(0.82)	(0.03)	(0.20)	(0.05)	(3.13)
+AM+NPK	17.5 a	2.57 a	2.5 b	0.21 a	0.87 a	0.25 a	77.9 a
	(0.71)	(0.08)	(0.91)	(0.05)	(0.17)	(0.03)	(5.10)

Values followed by the same letter(s) in the same column are not significantly different by Tukey's test ($p \leq 0.05$); values in parentheses represent standard errors; control: no AM and no NPK fertiliser, +NPK: fertilised with NPK at 1g per 1.2 kg soil, +AM: inoculated with 50 g Rhizagold™ per 1.2 kg soil, +AM+NPK: combined AM and NPK

Foliar samples were scarce and were not analysed statistically as some of the replicates did not yield sufficient samples for standard laboratory analysis. Overall, the shoot nutrient concentrations for +AM+NPK were relatively better than the control. Treatment +AM+NPK showed highest P, C, Ca, Mg and K concentrations compared with the rest of the treatments (Table 3). Inoculation and fertilisation generally increased P concentration in shoots (eight folds) compared with the control. Shoot nutrient concentrations in +AM fungi plants were comparable with +NPK and were relatively better than those of the control. It was also noted that shoot nutrient concentrations in +AM fungi plants were almost five folds higher than in the control (Table 3), although there was no fertilisation in both treatments.

DISCUSSION

Fertilisation is a common practice in tropical nurseries to increase yield. However, the combined effects of fertiliser with inoculation gave an added advantage in improving the overall growth and nutrient content of seedlings. The high inoculation rate (50 g plant^{-1}) combined with fertilisation improved the physical growth of *A. mangium* seedlings in this study. Improved seedling growth and biomass have also been reported in *Hopea helferi* after nutrient addition

Table 2 Effects of arbuscular mycorrhizal inoculation and fertilisation on chemical properties of growing media planted with *Acacia mangium* seedlings three months after transplanting

Treatment	pH	EC (mS cm^{-1})	N (%)	C (%)	Av P (mg kg^{-1})	Ca (cmol kg^{-1})	Mg (cmol kg^{-1})	K (cmol kg^{-1})
Control	5.5 c	0.03 b	0.15 b	2.7 a	13.05 c	1.9 b	0.2 c	0.1 b
	(0.02)	(0.03)	(0)	(0.04)	(0.38)	(0.02)	(0)	(0)
+NPK	5.2 d	0.03 ab	0.17 a	2.6 a	430.5 a	1.4 c	0.2 c	0.2 a
	(0.02)	(0.03)	(0)	(0.05)	(20.77)	(0.08)	(0)	(0)
+AM fungi	6.2 a	0.05 a	0.14 c	2.5 a	24.41 c	2.6 a	1.0 a	0.1 b
	(0.02)	(0.03)	(0)	(0.06)	(1.43)	(0.06)	(0.01)	(0)
+AM+NPK	5.8 b	0.04 ab	0.14 c	2.4 a	351.67 b	2.0 b	0.8 b	0.2 a
	(0.07)	(0.08)	(0)	(0.12)	(20.73)	(0.11)	(0.08)	(0)

Values followed by the same letter(s) in the same column are not significantly different by Tukey's test ($p \leq 0.05$); values in parentheses represent standard errors; control: no AM and no NPK fertiliser, +NPK: fertilised with NPK at 1g per 1.2 kg soil, +AM: inoculated with 50 g Rhizagold™ per 1.2 kg soil, +AM+NPK: combined AM and NPK

Table 3 Effects of arbuscular mycorrhizal inoculation and fertilisation on total shoot nutrient content of *Acacia mangium* seedlings three months after transplanting

Treatment/fertiliser	Total shoot nutrient content (mg g^{-1})					
	N	P	C	Ca	Mg	K
Control	2.48	0.17	38.01	0.72	0.19	1.42
	(0.00)	(0.07)	(0.19)	(0.22)	(0.04)	(0.05)
+NPK	11.49	0.76	163.02	2.65	0.76	6.39
	(0.42)	(0.01)	(2.73)	(0.12)	(0.02)	(0.11)
+AM	13.49	0.84	166.94	2.97	0.62	6.22
	(0.11)	(0.13)	(3.76)	(0.12)	(0.06)	(0.30)
+AM+NPK	12.98	1.48	195.32	4.28	1.24	7.73
	(0.51)	(0.34)	(1.05)	(0.24)	(0.19)	(0.77)

Values in parentheses represent standard errors; control: no AM and no NPK fertiliser, +NPK: fertilised with NPK at 1g per 1.2 kg soil, +AM: inoculated with 50 g Rhizagold™ per 1.2 kg soil, +AM+NPK: combined AM and NPK and mycorrhizal infection (Lee & Alexander 1994). The application of fertiliser either alone or with AM also increased the contents of N, P, K and Mg in shoots of seedlings.

Contrary to reports that optimal root colonisation requires low soil fertility (e.g. Hamel 1996, Chen et al. 2006), fertilisation did not hamper root infection rates. The higher root:shoot ratios of +NPK and +AM+NPK might be due to higher nutrient absorption by seedlings from a greater pool of nutrient availability, which simultaneously increased shoot and root weight (Kung'u 2010).

Arbuscular mycorrhizas promote plant growth by absorbing P with its hyphae and in turn obtain C from the photosynthate of the plant (Saito et al. 2000). Although effects on physical variables were not apparent when AM was applied without fertiliser, the high P content in +AM shoots compared with control proved that AM had an important role in P transfer (Mosse 1981). In addition, shoot concentrations of the rest of the shoot nutrients in +AM treatment were higher compared with the control. These elevated levels were attributed to the indirect effect of elimination of P deficiency using AM which simultaneously enhanced uptake of other nutrients such as N and K (Sieverding 1991). The comparable shoot nutrient concentrations in +NPK and +AM also indicated that AM was effective in nutrient transfer even with the absence of fertilisation.

From results of this study, we concluded that nutrient addition was the primary factor in improving the growth of *A. mangium* seedlings in the nursery as demonstrated by +NPK treatment. Similar results have been obtained by Ong et al. (2002). The fact that natural N fixation may have also taken place to further boost the symbiotic effects and growth of *A. mangium* cannot be ignored (Azizah et al. 1994). Elevated levels of N, P, Mg and K in shoots of fertilised seedlings (+NPK and +AM+NPK) were also closely related to the greater root biomass of the seedlings (Table 3), which in turn, provided a bigger surface area for nutrient transport.

The pH, EC, available P, exchangeable Ca and Mg contents were higher in +AM treatment compared with the untreated control and the initial soil chemical properties. Thus, it was not certain if AM played a significant role in altering the soil chemical status of the growing media. It is also difficult to ascertain the reason behind these results as other soil abiotic and biotic interactions may have taken place during the study. However, it has been reported that pH 6–7 was the best for mycorhizal development and has an important role in determining the nutrient availability of media for plant growth (Kumar et al. 2010).

Generally, the effects of AM inoculation and fertilisation on the early growth of *A. mangium* seedlings were found to be significant for most of the variables tested. However, inoculation alone was not able to give a better yield in terms of physical growth because its effectiveness was dependent on competitive interactions with native mycorrhizal species (Ong et al. 2002), mycorrhizal dependency (Hamel 1996) and host specificity (Bever et al. 2001). Thus, the results of this nursery study are important to clarify if AM positively affected *A. mangium* yield before field trials involving large numbers of seedling stocks using AM inoculum is implemented. Further research is needed in testing the Rhizagold™ inoculum with different types of fertiliser and age of seedlings and a longer duration to verify the benefits of AM in promoting plant growth. In the meantime, compound fertilisers such as NPK Blue fertiliser can be used in the nursery to enhance seedling growth.

CONCLUSIONS

Fertilisation alone and the combined effects of fertilisation with AM inoculation positively increased plant growth and biomass, nutrient contents in growing media and shoot nutrient concentrations. Arbuscular mycorrhiza is important in elevating shoot nutrient concentration of seedlings to promote better seedling growth if fertilisation is not an option.

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