EFFECT OF INOCULATION WITH SELECTED BRADYRHIZOBIUM SPP. ON THE SURVIVAL AND GROWTH OF ACACIA MANGIUM SAPLINGS AFTER 20 MONTHS IN THE FIELD

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MARTIN-LAURENT, F., FREMONT, M., LEE, S. K., THAM, F. Y., PRIN, Y., TAN, T. K. & DIEM, H. G. 1999. Effect of inoculation with selected Bradyrhizobium spp. on the survival and growth of Acacia mangium saplings after 20 months in the field. This work was designed to test the long-term effect of the inoculation of Acacia mangium seedlings with 10 selected strains of Bradyrhizobium spp. The percentage of survival of seedlings inoculated with any of the Bradyrhizobium strains was increased

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by 10% as compared to the control plants. However, out of the 10 *Bradyrhizobium*
strains tested, only 3 strains, Aust 13c, Lu 4 and Tel 8, belonging to the phylogenetic
group 1, significantly enhanced the growth of *A. mangium* after 20 months in the
field. For the first time, inoculation with indigenous Malaysian strains *Bradyrhizobium*
such as Tel 8 and Lu 4 at the seedling stage is reported to produce enhanced and
sustained growth and development of *A. mangium* in the field. We propose that in the
future, it may be best to isolate and select local strains from each reforestation plot
using a simple screening to determine their phylogenetic group, and conducting a
simple nursery inoculation test to assess their competitiveness and efficiency when
associated with *A. mangium* seedlings.

**Key words:** *Acacia mangium - Bradyrhizobium* spp. - N<sub>2</sub> fixation - tree saplings - *Imperata
cylindrica*

**MARTIN-LAURENT, F., FREMONT, M., LEE, S. K., THAM, F. Y., PRIN, Y.,
terpih terhadap kemandirian dan pertumbuhan anak pokok *Acacia mangium*
selepas 20 bulan di lapangan. Kajian ini bertujuan untuk menguji kesan jangka-
panjang penginokulatan anak benih *Acacia mangium* dengan 10 saringan terpilih
*Bradyrhizobium* spp. Peratus kemandirian anak benih yang diinokulat dengan mana-
mana saringan *Bradyrhizobium* bertambah sebanyak 10% berbanding dengan tumbuhan
yang dikawal. Bagaimanapun, daripada 10 saringan *Bradyrhizobium* yang diuji, hanya 3
saringan, Aust 13c, Lu 4 dan Tel 8, yang dimiliki oleh kumpulan 1 filogenetik,
menggalakkan pertumbuhan *A. mangium* selepas 20 bulan di ladang. Buat kali
pertama, penginokulatan dengan saringan *Bradyrhizobium* Malaysia yang asli seperti Tel
8 dan Lu 4 di peringkat anak benih dilaporkan meningkat dan mengekalkan
pertumbuhan dan perkembangan *A. mangium* di ladang. Kami mencadangkan
supaya pada masa hadapan, lebih baik untuk mengasing dan memilih saringan
tempatan daripada setiap petak penghutanan semula menggunakan penyaringan
yang mudah bagi menentukan kumpulan filogenetiknya, serta menjalankan ujian
penginokulatan di tapak semai untuk menilai daya saing dan kecekapannya
apabila bersekuatu dengan anak benih *A. mangium*.

**Introduction**

Several million hectares of tropical forest are lost every year due to excessive
industrial exploitation, clearing for agricultural purposes and collection of fire-
wood. One of the most critical issues of deforestation in the Southeast Asian region
is the risk of erosion and landslides, both of which have significant effect on soil
fertility and water catchment. Up to now, degraded land does not attract potential
investors for plantation as the soil is poor and therefore the plantation outputs are
very limited. However, in the last two decades, fast-growing legume trees have
gained increasing popularity for reforestation. *Acacia mangium* Willd. has been
used successfully for reforestation programmes in the humid tropics on degraded
land where it competes well with noxious grasses such as *Imperata cylindrica*
(National Academy of Sciences 1983, Turnbull 1986). Due to its nitrogen-fixing
ability, *A. mangium* possesses the remarkable growth potential of pioneer tree
legumes and will grow well even on very acid and infertile soil. Moreover, it presents
good properties for pulp and paper, and is commonly able to reach a height of 20
to 25 m in 10 to 15 years in Sabah, with a wood production averaging 25 to 30 m$^3$ ha$^{-1}$ y$^{-1}$ (Sim 1986). Its introduction is, however, not always successful due to growth limitations induced by detrimental factors in the soil of the site of introduction such as acidity and aluminium stress (Lesueur et al. 1993). In fact, it needs association with symbiotic soil organisms including rhizobia and/or mycorrhiza to survive and grow in natural forest ecosystems (Dela Cruz & Garcia 1991). Acacia mangium is specifically associated with slow-growing Rhizobium strains belonging to the Bradyrhizobium group (Dreyfus & Dommergues 1981, Souvannavong & Galiana 1991). However, many strains naturally present in the soil have low nitrogen fixation efficiency (Dart et al. 1991). In order to improve their growth, the seedlings should be inoculated with the appropriate Bradyrhizobium strain which is effective in fixing atmospheric nitrogen and aggressive enough to compete with the less efficient strains present naturally in the soils (Galiana et al. 1990). Several inoculation experiments carried out in the field in different countries on different types of soils have demonstrated a positive effect of inoculation with some Bradyrhizobium strains on tree growth (Mallet & Gnahoua 1989, Galiana 1990, Mallet 1990, Souvannavong & Galiana 1991). The more efficient strains, in particular Aust 13c which has been widely used, were isolated from A. mangium nodules collected in Australia (Galiana 1990). It has also been demonstrated that they are able to survive many years in the nodules of inoculated plants (Galiana et al. 1994). However, most experiments have been done in Africa where indigenous Bradyrhizobium strains able to form the N-fixing symbiosis with A. mangium do not compete well with the introduced strain. In the Southeast Asian tropics, indigenous Bradyrhizobium strains are present in abundance in the soil. Therefore, there is a high competition pressure on the introduced strains.

The objective of this work was to assess the effect of the inoculation of A. mangium seedlings with different Bradyrhizobium strains in the nursery and their growth and development 20 months after planting into the field. Bradyrhizobium strains well adapted to the conditions encountered in Sabah (Malaysia) could be selected for use in the production of nodulated tree saplings for reforestation purposes.

**Materials and methods**

**Seed germination**

Acacia mangium seeds collected from trees planted in the Luasong Forestry Centre (CIRAD Foret/Inoprise Corporation, Tawau, Sabah, Malaysia) chosen for their superior growth were mixed in order to diminish the natural genetic variability due to open field pollination and sexual reproduction. Seeds were scarified by soaking for one minute in boiling water and left overnight in fresh water. They were then sown in sterile sand. Two weeks after germination, the seedlings were potted in 1 litre plastic tubes containing sandy clay nursery soil sterilised by boiling for 1 h and moistened with rain water for one week to avoid possible toxicity problems.
**Nursery trial**

The experiment consisted of 5 blocks (locations in the nursery) and 11 treatments (1 non-inoculated control and 10 inoculated with different *Bradyrhizobium* strains) set up in a randomised block design. For each treatment, 45 plants were grown in 5 different blocks (9 plants per treatment per block). A total of 495 plants were grown for the trial. After four months of growth in the nursery, the stem diameter (base of the plant), height and mortality were recorded before transplantation to the field.

**Field trial**

The site chosen was provided by Inoprise Corporation (PISP project) in Luasong Forestry centre (Sabah, East Malaysia). It was initially occupied by a very degraded forest that had been logged in the early 1970s and subsequently destroyed by fires which damaged an important part of the Sabah forest in 1983-84. Before the plantation, the site was colonised by *Imperata cylindrica* growing on very poor and acidic soils. The soil is a fine sandy clay, moderately deep and well drained. The soil had pH values (1:2 soil:liquid ratio) between 4.8 and 5.8 and a conductivity of 14 to 18 mS. Total soil nitrogen and phosphorus contents (Table 1) were estimated by Kjeldahl's digestion of 1 g of dried soil in concentrated sulfuric acid using a Kjeltec auto 1030 analyser according to Allen (1989). For each block, there were 10 replicates. The climate is tropical, characterised by high temperatures (26 °C to 28 °C), high relative humidity (above 85%) and high annual rainfall (over 2000 mm). Similar to the nursery trials, the experiment was conducted in 5 different blocks or locations in the field site with 11 treatments (Figure 1). The four-month-old saplings were planted at three-metre intervals. Fertilisation with 100 g phosphate fertiliser was provided for each seedling at planting time (Xu & Yang 1992). Four months after transplanting, the height of the plants was measured. Two years after the plantation was established, the stem diameter and height were measured. The total foliar nitrogen, phosphorus and chlorophyll contents were determined (data presented here are from treatments where a significant difference in height was recorded).

<table>
<thead>
<tr>
<th>Block number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH (H$_2$O)</strong></td>
<td>5.4</td>
<td>5.2</td>
<td>4.8</td>
<td>5.5</td>
<td>5.8</td>
</tr>
<tr>
<td><strong>Conductivity (mS)</strong></td>
<td>18</td>
<td>15</td>
<td>17</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td><strong>Total nitrogen content (%)</strong></td>
<td>1.63</td>
<td>1.23</td>
<td>1.49</td>
<td>1.30</td>
<td>1.24</td>
</tr>
<tr>
<td><strong>Total phosphorus content (%)</strong></td>
<td>0.0286</td>
<td>0.0215</td>
<td>0.0280</td>
<td>0.0189</td>
<td>0.0237</td>
</tr>
</tbody>
</table>

Table 1. Analysis of soils from five blocks (locations) in Luasong Forestry Centre, Sabah, East Malaysia; soil:liquid ratio of 1:2 (v/v)
Nitrogen and phosphorus contents

Nitrogen and phosphorus contents were determined by Kjeldahl's digestion of 0.2 g of dried leaves in concentrated sulphuric acid using a Kjeltac auto 1030 analyser and the stannous chloride reaction method respectively (Allen 1989). For each treatment, there were six replicates from independent bulked samples and each bulk was constituted from four young fully expanded leaves randomly chosen from the whole plant (Sun et al. 1993).

Inoculum production and inoculation

Ten *Bradyrhizobium* strains were selected from the CIRAD-Foret collection according to their nitrogen-fixing ability (Fremont 1994). They were grouped in two different phylogenetic groups according to polymerase chain restriction fragment length polymorphism (PCR-RFLP) of the intergenic spacer (IGS) between the 16S and 23S rRNA genes of the ribosomal operon (Prin 1993). Group I included Australian strains (Aust 11c and Aust 13c), Singaporean strain (But 1) and closely-related Sabah strains (Lu 4, Was 3, Was 9, Tel 2 and Tel 8); Group II included only Sabah strains (Nlu 3, Tel 6) (Table 2). Strains were cultured for one
week in liquid YM (yeast extract-mannitol) medium (mannitol 1%, K$_2$HPO$_4$ 0.05%, MgSO$_4$.7H$_2$O 0.2%, NaCl 0.01%, yeast extract 0.05%, pH 6.8), at 28 °C with agitation. The concentration of the bacteria inoculant was adjusted to 10$^9$ bacteria/ml according to the relationship between the number of bacteria and the optical density at 650 nm determined by a series of counting of bacterial populations as described by Cooper (1979) and Hoben and Somasegaran (1982). Acacias were inoculated two weeks after germination by applying 2 ml of bacterial inoculum at the base of the seedlings.

Table 2. _Bradyrhizobium_ strains used to inoculate the acacia seedlings

<table>
<thead>
<tr>
<th>Name</th>
<th>Origin</th>
<th>Phylogenetic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aust 11c</td>
<td>Bloomfield, Australia</td>
<td>Group I</td>
</tr>
<tr>
<td>Aust 13c</td>
<td>Daintree, Australia</td>
<td>Group I</td>
</tr>
<tr>
<td>But 1</td>
<td>Bukit Timah Forest, Singapore</td>
<td>Group I</td>
</tr>
<tr>
<td>Lu 4</td>
<td>Luasong Forestry Centre, Sabah, East Malaysia</td>
<td>Group I</td>
</tr>
<tr>
<td>Was 3</td>
<td>Luasong Forestry Centre, Sabah, East Malaysia</td>
<td>Group I</td>
</tr>
<tr>
<td>Was 9</td>
<td>Luasong Forestry Centre, Sabah, East Malaysia</td>
<td>Group I</td>
</tr>
<tr>
<td>Tel 2</td>
<td>TV Relay, Sabah, East Malaysia</td>
<td>Group I</td>
</tr>
<tr>
<td>Tel 8</td>
<td>Telupid, Forest Department, Malaysia</td>
<td>Group I</td>
</tr>
<tr>
<td>Nlu 3</td>
<td>Luasong Forestry Centre, Sabah, East Malaysia</td>
<td>Group II</td>
</tr>
<tr>
<td>Tel 6</td>
<td>Sejati Plantations, Telupid, Malaysia</td>
<td>Group II</td>
</tr>
</tbody>
</table>

**Chlorophyll content determination**

Per plant, four leaf discs of 1 cm diameter each were cut from four leaves, randomly harvested from the whole plant as described above, and ground in 2 ml 80% acetone solution. The supernatant was collected, adjusted to 10 ml with 80% acetone and centrifuged for 10 min at 4000 rpm. The clear supernatant was decanted into a 3-ml glass cuvette and the absorbance was recorded at 663 and 645 nm using a UV-visible recording spectrometer UV-160A (model Shimadzu, Japan). Chlorophyll content was calculated according to the method of Arnon (1949) and expressed in micromoles per unit leaf area (mg m$^{-2}$). For each treatment, six replicates were used as described for nitrogen and phosphorus contents analysis.

**Statistical analysis**

The data set was analysed using MINITAB program (Ryan _et al_. 1985). A single factor analysis of variance (one-way ANOVA) was used to test for significant differences in treatments. A multiple range analysis was used to test for significant difference between treatments using Duncan’s procedure at $p<0.5$. A double factor analysis of variance (two-way ANOVA) was used to test for significant interaction between treatment and blocks.
Results

Effect of Bradyrhizobium inoculation on the growth of Acacia mangium

In the nursery (Figure 2A), plants inoculated with Bradyrhizobium strains Aust 13c or Aust 11c showed a significant increase in mean height compared to control plants (Table 3). The growth of plants inoculated with other Bradyrhizobium strains was also greater than the control although the difference was not significant.

Four months after planting in the field (Figure 2B), the nodulation was very successful and well established in plants inoculated with any of the Bradyrhizobium spp. Small nodules could also be observed in some of the controls (Fremont 1994). The percentage survival of seedlings inoculated with any of the Bradyrhizobium strains was higher than non-inoculated seedlings; the percentages of survival were 95 ± 3% and 85 ± 2% respectively.

Significant increase in mean height of plants inoculated with Tel 8, a Sabah strain, was recorded four months after transplanting to the forest site (Table 3). On the other hand, Aust 13c and Aust 11c, both Australian strains which were observed in the nursery trials to produce a significant increase in height of plants, when out in the field site did not perform as well as Tel 8.

Figure 2. (2A) Acacia mangium saplings grown under the shade in the nursery in Luasong Forestry Centre (Sabah, East-Malaysia); A. mangium saplings at 4 months (2B) and 20 months (2C) after their transfer to the field in Luasong Forestry Centre (Sabah, East Malaysia)
Twenty months after planting (Figure 2C), acacias inoculated with Sabah strains Tel 8 and Lu 4, and Australian strain Aust 13c showed statistically significant increases in mean height (Table 3), Tel 8 inoculated plants being the tallest. The other *Bradyrhizobium* strains did not significantly increase the height growth of the acacias. There was also no significant increase in acacia stem diameter in response to inoculation with the *Bradyrhizobium* strains; mean stem diameters were in the region of 8.9 ± 0.7 cm (Table 3).

**Effect of planting location on the growth of Acacia mangium**

Measurements of height made four months after the plants were transferred to the field site showed that the location of planting (blocks) also significantly affected growth (Table 4). Plants inoculated with strain Was 3 in Blocks 2 and 5 had significantly greater heights than Was 3 inoculated plants in other blocks. Similarly, plants inoculated with Nlu 3 or Tel 6 growing in Block 5 were taller than corresponding plants growing in other blocks (Table 4). In contrast, control plants which were not inoculated and growing in Block 2 were significantly lower in height than those growing in the other blocks. Likewise, a negative effect on height was recorded for Aust 11c inoculated acacias in Blocks 2 and 3 as well as But 1 inoculated acacias in Block 3.

After twenty months, it was observed that acacias inoculated with But 1, Lu 4, Was 3, Tel 2 and Tel 6 planted in Block 5 were all significantly greater in height than their counterparts planted in the other four blocks (Table 4). On the other hand, a negative effect on growth was recorded for plants inoculated with Aust 11c growing in Blocks 2 and 3, and plants inoculated with Aust 13c growing in Block 5.

The results of the two-way ANOVA demonstrated that there was a significant and positive interaction between different *Bradyrhizobium* treatments and planting location on the height of the acacias (data not shown).

**Effect of *Bradyrhizobium* inoculation on nitrogen, phosphorus and chlorophyll contents of Acacia mangium**

Twenty months after transplanting into the field, acacias inoculated with Aust 13c, Lu 4 and Tel 8 were taller than the control plants by 8, 7 and 12 % respectively (Table 5). In addition, leaf chlorophyll content was significantly higher in plants inoculated with Aust 13c, Lu 4 and Tel 8. However, for leaf nitrogen and phosphorus contents, there were significant increases only in plants inoculated with Lu 4.

**Discussion**

In the last few decades, several groups have demonstrated that inoculation of *A. mangium* with *Bradyrhizobium* have significantly positive effects on growth and development in the field (Mallet & Gnaboua 1989, Galiana *et al.* 1990, Mallet *et al.*
Table 3. Effect of different *Bradyrhizobium* strains on height and diameter growth of *Acacia mangium* in the nursery and in the field

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Aust 11c</th>
<th>Aust 13c</th>
<th>But 1</th>
<th>Lu 4</th>
<th>Was 3</th>
<th>Was 9</th>
<th>Tel 2</th>
<th>Tel 8</th>
<th>Nlu 3</th>
<th>Tel 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average height (m) after 4 months of growth in the nursery</td>
<td>0.4a</td>
<td>0.6b</td>
<td>0.6b</td>
<td>0.5a</td>
<td>0.4a</td>
<td>0.5a</td>
<td>0.5a</td>
<td>0.4a</td>
<td>0.5a</td>
<td>0.5a</td>
<td>0.4a</td>
</tr>
<tr>
<td>Average height (m) at 4 months after planting in the field</td>
<td>1.2c</td>
<td>1.3c</td>
<td>1.4c</td>
<td>1.4c</td>
<td>1.3c</td>
<td>1.3c</td>
<td>1.3c</td>
<td>1.4c</td>
<td>1.5d</td>
<td>1.4c</td>
<td>1.3c</td>
</tr>
<tr>
<td>Average height (m) at 20 months after planting in the field</td>
<td>8.1e</td>
<td>8.3e</td>
<td>8.8f</td>
<td>8.3e</td>
<td>8.7f</td>
<td>8.5e</td>
<td>8.3e</td>
<td>8.6e</td>
<td>9.0f</td>
<td>8.5e</td>
<td>8.5e</td>
</tr>
<tr>
<td>Average diameter (cm) at 20 months after planting in the field</td>
<td>8.4g</td>
<td>8.1g</td>
<td>9.0g</td>
<td>8.7g</td>
<td>8.7g</td>
<td>8.9g</td>
<td>8.2g</td>
<td>8.0g</td>
<td>8.9g</td>
<td>9.0g</td>
<td>8.9g</td>
</tr>
</tbody>
</table>

Values followed by the same letter do not differ significantly at $p < 0.5$. 
Table 4. Effect of planting sites (blocks) on the height of *Acacia mangium* after 4 months (1993) and 20 months (1995) of growth in the field

<table>
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<tbody>
<tr>
<td></td>
<td>Block 1</td>
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<td>Block 3</td>
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<td>Block 4</td>
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<td>Block 5</td>
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</tr>
<tr>
<td></td>
<td>1.3b</td>
<td>8.2e</td>
<td>1.5c</td>
<td>9.1f</td>
<td>1.4b</td>
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<td>8.3e</td>
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<td>8.8f</td>
<td>1.2b</td>
<td>8.7f</td>
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<tr>
<td></td>
<td>0.9a</td>
<td>8.2e</td>
<td>1.1a</td>
<td>7.7d</td>
<td>1.5c</td>
<td>8.6e</td>
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<td>8.3e</td>
<td>1.5c</td>
<td>8.5e</td>
<td>1.4b</td>
<td>8.0d</td>
<td>1.2b</td>
<td>7.8d</td>
<td>1.3b</td>
<td>8.7f</td>
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<td></td>
<td>1.2b</td>
<td>7.8d</td>
<td>1.2b</td>
<td>7.7d</td>
<td>1.5c</td>
<td>8.8f</td>
<td>0.9a</td>
<td>7.6d</td>
<td>1.2b</td>
<td>8.4e</td>
<td>1.2b</td>
<td>7.6d</td>
<td>1.3b</td>
<td>8.2e</td>
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<td>9.7g</td>
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<td>1.2b</td>
<td>8.3e</td>
<td>1.6c</td>
<td>8.9f</td>
</tr>
</tbody>
</table>

Values followed by the same letter do not differ significantly at p < 0.5.

Table 5. Effect of *Bradyrhizobium* strains Aust 13c, Tel 8 and Lu 4 on height, diameter, foliar nitrogen, foliar phosphorus and chlorophyll contents of *Acacia mangium* after 20 months of growth in the field

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Aust 13c</th>
<th>Tel 8</th>
<th>Lu 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean height ± se (m)</td>
<td>8.10 ± 0.49a</td>
<td>8.77 ± 0.97b</td>
<td>9.06 ± 0.49b</td>
<td>8.71 ± 0.50b</td>
</tr>
<tr>
<td>Mean diameter ± se (cm)</td>
<td>8.40 ± 0.80c</td>
<td>9.01 ± 0.70c</td>
<td>8.94 ± 0.80c</td>
<td>8.68 ± 0.70c</td>
</tr>
<tr>
<td>Mean chlorophyll content ± se (mg m²)</td>
<td>225 ± 27d</td>
<td>413 ± 38c</td>
<td>337 ± 60c</td>
<td>339 ± 44e</td>
</tr>
<tr>
<td>Mean nitrogen content ± se (%)</td>
<td>4.97 ± 0.25f</td>
<td>4.65 ± 0.10f</td>
<td>4.33 ± 0.19g</td>
<td>5.57 ± 0.50g</td>
</tr>
<tr>
<td>Mean phosphorous ± se (%)</td>
<td>0.0140 ± 0.0010i</td>
<td>0.0093 ± 0.0010h</td>
<td>0.0123 ± 0.0040i</td>
<td>0.0210 ± 0.0040j</td>
</tr>
</tbody>
</table>

Values followed by the same letter do not differ significantly at p < 0.5.
1990, Souvannanong & Galiana 1991). However, most of these studies have been carried out in the Ivory Coast in Africa, using *Bradyrhizobium* strains isolated from nodules collected in the natural area of distribution of *A. mangium*, Australia. Galiana *et al.* (1994) showed that *Bradyrhizobium* strains from Australia, such as Aust 13c, are able to survive and persist in the nodules of *A. mangium* even many years after its introduction. Although *A. mangium* is not an indigenous tree of the Malaysian region, it has been reported that in Malaysia, some local *Bradyrhizobium* strains are able to associate symbiotically with this tree species (Fremont *et al.* 1994). *Acacia mangium* has thus been used successfully outside of its natural range for reforestation purposes in the Southeast Asian region, notably Sabah in East Malaysia (Sim 1986, Gunn & Midgley 1991). This paper reports for the first time that growth of acacia planted in sterile soil and inoculated at the seedling stage with both Australian and Malaysian *Bradyrhizobium* strains is not only enhanced but sustained in the field in the Luasong Forestry Centre, Sabah, East Malaysia.

In the present study, it was observed that irrespective of the strain used, inoculation of acacia seedlings with *Bradyrhizobium* enhanced their percentage of survival in the field by 10% as compared to control plants. The establishment of a functional nitrogen-fixing symbiosis between the *Bradyrhizobium* strains used and the acacia host seedlings in the nursery increased the amount of nitrogen available to the plant prior to planting in the field, and therefore, improved survival rates. However, out of the 10 *Bradyrhizobium* strains tested, only 3 strains, Aust 13c, Lu 4 and Tel 8, significantly enhanced the growth of *A. mangium* in the field. The response of the seedlings to inoculation with different *Bradyrhizobium* strains varied between nursery and field trials. This may be due to a change in the physical environmental conditions between nursery and field sites or, may be related to stages in the development of the plant. The effects of the Australian strains, Aust 11c and Aust 13c, in enhancing the development of the plants were significantly greater in the nursery trials than in the field after four months; significant positive inoculation effects were observed in the field only with Aust 13c after 20 months in the field. In contrast, Malaysian strains, Lu 4 and Tel 8, both of which produced little effect on plants in the nursery, significantly enhanced growth of saplings after transfer onto degraded land in the field. Contrary to a previous study by Galiana *et al.* (1996), inoculation of young acacia seedlings with *Bradyrhizobium* strains did not yield any significant increase in their mean diameter. None of the *Bradyrhizobium* strains belonging to phylogenetic Group II was observed to significantly affect the development of *A. mangium*. Only *Bradyrhizobium* strains Aust 13c, Lu 4 and Tel 8 which belong to the phylogenetic Group I significantly increased the growth of *A. mangium* in the field. The effects of the growth were also sustained only in acacia inoculated with these strains. It would appear that the *Bradyrhizobium* strains belonging to phylogenetic Group I are more efficient in association with acacia.

There was a significant effect of planting site on acacia growth in the five locations selected in the present field study. Analysis of the soils from the different locations showed that there was no correlation to different chemical composition of the sites. It would appear that other parameters are responsible in part for the
effect of planting blocks or locations in the present study. These could be topographical (e.g. slope of the land which would affect drainage) as well as whether the site was on the leeward or windward side (which would determine the extent of exposure of the plot to sunlight and wind). Furthermore, microorganisms such as endomycorrhizal fungi and other native \textit{Bradyrhizobium} strains naturally present in the soil of the Luasong Forestry Centre are probably different between sites. This could produce the significant effect on growth between planting blocks. Block 5 is geographically isolated from the other planting blocks and in general appeared to be more favourable for growth of acacia than the other sites.

Despite the obvious effect of planting site on growth, and the indication that there was a significant interaction between planting blocks and inoculation treatments, an overall positive effect of inoculation with Aust 13c, Lu 4 and Tel 8 was clearly and consistently recorded throughout the different sites. This demonstrates that inoculation with the appropriate \textit{Bradyrhizobium} strains allowed the plants to homogeneously achieve enhanced growth irrespective of the site or soil heterogeneity. All plants inoculated with these three strains of \textit{Bradyrhizobium} had almost twice as much chlorophyll as the control. However, only plants inoculated with strain Lu 4 showed nitrogen and phosphorus contents in leaves higher than plants inoculated with other \textit{Bradyrhizobium} strains. \textit{Bradyrhizobium} strain Lu 4 may be more efficient in improving the general health of the plant, and good long-term effects of inoculation probably can be expected with this strain.

In conclusion, the results of this study show that inoculation with indigenous Malaysian strains of \textit{Bradyrhizobium} such as Tel 8 and Lu 4 in the nursery at the seedling stage can produce enhanced and sustained growth and development of \textit{A. mangium} in the field. The investment in inoculation of seedlings is viable, considering the 10\% increase in tree survival. Moreover, the inoculation of tree saplings with the appropriate \textit{Bradyrhizobium} strains homogenises growth of acacias in the plantation, masking partially the heterogeneity of the plot and the natural variation among the seedlings due to the use of seeds from open pollination. According to our results, indigenous \textit{Bradyrhizobium} strains may be more efficient in the long term due to ecological competition with other strains present in the site when the inoculated saplings are transferred to the field. Therefore, in future, it may be best to isolate and select local strains from each reforestation plot using simple screening to determine their phylogenetic group, and conducting a simple inoculation to assess their competitiveness and efficiency when associated with \textit{A. mangium} seedlings.

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References


