# Micropropagation of *Acacia mangium* and nitrogen fixation

Antoine Galiana, Yves Prin, Jeanne Ahée,
Emile Duhoux

*Acacia mangium* is a tree legume which originated in the Australasian region. It is one of the most productive species in the wet tropical zone and is intensively cultivated for the paper industry in Indonesia, Malaysia and Thailand. This species also has a nitrogen-fixing symbiosis with a bacteria of the genus *Bradyrhizobium*.

Relatively few studies have been made of micropropagation of tropical woody varieties that fix nitrogen from the atmosphere. Since this species is of industrial interest, it was important to master these techniques both in laboratory studies and in the production of clones selected for symbiotic performance and satisfactory growth in the field.

## Selection of plants

Five plants produced from 600 seeds of four different sources, aged seven months, were selected on the basis of height (29 to 73 cm) and identified: RR-G1, IR-M2, L-G1, L-P1 and L-P2. All plants had been previously inoculated with the specific strain Aust 13c of *Bradyrhizobium*.

# Micropropagation of selected plants

## Multiplication

Explants (knots) were cultured on a Murashige and Skoog (MS) medium containing 2% sucrose, 5 mg $l^{-1}$ FeEDTA, Nitsch and Nitsch vitamins, 1 mg $l^{-1}$ of benzylaminopurine (BAP) and 0.8% Difco Agar (pH: 5.7). After six weeks, the majority of explants showed tufts of 14 to 18 axillary stems. The highest multiplication rate (40) was observed during the initial tranfer and decreased to 10-20 after the second transfer.

## Rooting

Microcuttings were rooted on half-strength MS medium and with added indole-3-butyric acid (0.05 mg $l^{-1}$). The rooting capacity of juvenile explants depended to a large extent on the concentration of BAP in the multiplication medium. Thus explants from seven-month-old plants, multiplied on MS medium with 1 mg $l^{-1}$ of BAP and transferred on half-strength MS medium with 0.05 mg $l^{-1}$ of IBA, had a rooting rate of 70% whereas in the same conditions explants from two-week-old plants had a rooting rate of only 10%.

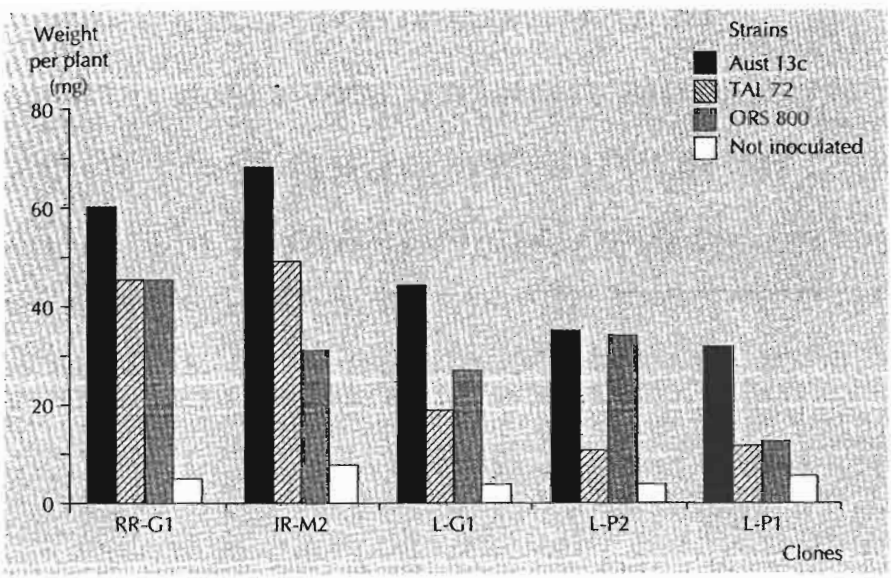
## Inoculation of vitroplants

After rooting, the five clones were transferred aseptically to a polypropylene fiber support on a BROUGHTON and DILWORTH (1971) medium without nitrogen and diluted by half. Each of the clones was inoculated with one of the following three strains of *Bradyrhizobium:* Aust 13c, TAL 72 or ORS 800.

# Evaluation of nitrogen-fixing potential of the plants

One month after inoculation, 100% of the plants displayed nodules (Plate II, 3). Four months after inoculation, the plants were harvested and the following parameters determined: number and dry weight of nodules, dry weight of aerial parts of the plant (see figure below), and acetylene-reducing activity. Statistical analysis of the results obtained showed that the effect of the clones and of the bacterial strains was significant. The best clones were RR-G1 and IR-M2 and the best strain was Aust 13c.

These results show that there is no clone/strain interaction and that both the plant and the bacteria can be selected separately.




*Effect on dry weight of aerial parts of the plant of inoculation with three strains of* Bradyrhizobium *spp. of five clones of* Acacia mangium *obtained by micropropagation after four months growth* in vitro.

# Bibliography

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