ESTABLISHING A PROTOCOL FOR COMMERCIAL MICROPROPAGATION OF *ACACIA MANGIUM* X *ACACIA AURICULIFORMIS* HYBRIDS

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AZIAH, M. Y., MCKELLAR, D., FADHILAH, Z., HALILAH, A. K. & HALIZA, I. 1999. Establishing a protocol for commercial micropropagation of *Acacia mangium* × *Acacia auriculiformis* hybrids. This study examined the multiplication and *in vitro* rooting of micropropagated *Acacia* hybrid clones and the subsequent acclimatisation survival of the plantlets. Shoot multiplication rates varied between 1.6 and 2.5 for the six hybrid clones tested. Of the shoot explants placed on *in vitro* rooting media, between 50 and 60% formed roots in culture. Shoots possessing *in vitro* formed roots exhibited far greater survival during the acclimatisation process than those without *in vitro* formed roots.

Key words: *Acacia* hybrid - micropropagation - acclimatisation

AZIAH, M. Y., MCKELLAR, D., FADHILAH, Z., HALILAH, A. K. & HALIZA, I. 1999. Perumusan kaedah mikroperambatan komersil untuk hibrid *Acacia mangium* × *Acacia auriculiformis*. Kajian ini merangkumi proses penambahan pucuk, pengakaran *in vitro* pucuk-pucuk terbitan mikro perambatan dan seterusnya kemandirian proses pengikliman anak pokok untuk beberapa klon hibrid *Acacia*. Nilai kadar pertambahan pucuk berbeza di antara 1.6 hingga 2.5 untuk enam klon yang dikaji. Di antara pucuk-pucuk yang dikulturkan dalam media pengakaran *in vitro*, 50% hingga 60% pucuk membentuk akar. Pucuk-pucuk yang telah membentuk akar secara *in vitro* mempaparkan kemandirian yang lebih tinggi pada peringkat pengikliman dibandingkan dengan pucuk–pucuk yang tidak membentuk akar secara *in vitro*.

Introduction

Acacia mangium Willd. x *A. auriculiformis* A. Cunn. *ex* Benth. hybrid is a very promising exotic plantation species for both timber and pulp in Southeast Asia. Other fast-growing species, investigated for wood-based industries in the region,

have had problems associated with them. *Eucalyptus* species are controversial because of their possible environment effects (Poore & Fries 1985), *Acacia mangium* is susceptible to heart rot (Zakaria *et al.* 1994) and *Paraserianthes falcataria* is highly susceptible to diseases and insect infestations (Kijkar 1992). The *Acacia* hybrid can grow on a wide range of soil types, is drought tolerant and resists insects and diseases (Kijkar 1992). Since the discovery of natural hybrids of *A. mangium* and *A. auriculiformis* in Sabah, East Malaysia, in the early nineteen seventies (Rufelds 1987), little has been done to develop a commercial hybrid plantation programme in Peninsular Malaysia. The genotypes offer many characteristics that are desirable for a short rotation plantation crop. Hybrids have been reported to grow more vigorously than trees of *A. mangium* (Tham 1979, Darus & Rasip 1989) and have greater stem volumes than the pure species (Le Dinh Kha 1996). Hybrids also show better stem form and longer clear bole than *A. auriculiformis* and lighter branching than *A. mangium* (Darus 1992). In addition, hybrid trees are significantly better in all important paper properties than *A. mangium* or *A. auriculiformis* (Yamada *et al.* 1992, Le Dinh Kha 1996).

Problems associated with commercial hybrid seed production are the low rates of spontaneous hybridisation in *A. mangium* and *A. auriculiformis* (Wickneswari & Norwati 1992) and the unsynchronised flowering pattern of the parent species (Zakaria & Awang 1992). Vegetative propagation offers an alternative solution for mass propagation of the hybrid. Haines and Griffin (1992) suggested the use of cuttings from young seedlings of advanced generation families, whereas Kijkar (1992) and Chia (1993) used cutting material rejuvenated from coppices of mature trees. Micropropagation, though the more expensive propagation option, has the advantage of higher multiplication rates and can be used for producing selected individuals until a family-based macropropagation programme is developed.

The Forest Research Institute of Malaysia (FRIM) together with the New Zealand paper producer, Fletcher Challenge Pulp and Paper Ltd., have been investigating micropropagation for the production of selected *Acacia* hybrid clones. This paper reports some aspects of this micropropagation work.

Materials and methods

Clones 1, 2 and 3 were initiated from 2-y-old *Acacia* hybrid trees, growing on a tin tailing site near Malim Nawar, Perak. These particular hybrids were derived from seeds collected from *A. auriculiformis* trees. Clones 4 to 9 were initiated from 8-y-old *Acacia* hybrid trees growing at Ulu Sedili, Johore. The seed origin of these hybrids were from *A. mangium* mothers. These hybrids were selected using morphological characteristics and further verified by isoenzyme techniques. In the initiation procedure, the shoot tips at the ends of the lower branches were removed and then immediately soaked in tap water, containing 1% boric acid and 1 g/l Benlate fungicide, for approximately five minutes. The moist shoots were then wrapped in paper towelling and packed in an ice chest for transportation to the laboratory. The shoots were placed into culture within 24 h of being collected.

Before being surface sterilised, using a procedure modified from Darus (1992), the shoot stems were further trimmed to ± 4 cm lengths and the leaves to half their surface area. Following a thorough rinse in sterile distilled water, the shoots were cut into nodal segments (± 1.0 cm) under aseptic conditions and then placed on basal MS (Murashige & Skoog 1962) medium in test tubes (Figure 1).

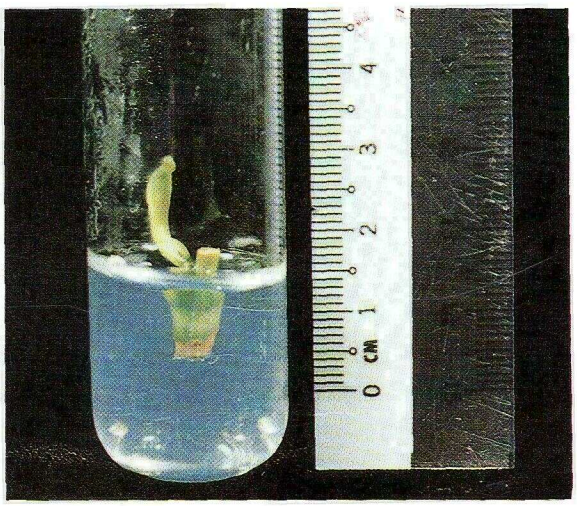

Figure 1. Nodal segment explants of *Acacia* hybrid with the emergence of a new bud in establishment medium

After two weeks, contaminated cultures were discarded. Uncontaminated explants were left on the basal MS medium until a new shoot appeared from the axillary meristem of the nodal explants. When the new bud shoot had grown to approximately 1.0 cm, it was aseptically removed from the original explant and placed on multiplication medium (modified from Darus 1992). All cultures were in 150-ml baby food glass jars, each with a transparent polypropylene cap. Growth room conditions were 23 ± 2 °C with a 16-h photoperiod (22.2 µmol m^{-2} sec^{-1}).

After four weeks, new shoots had developed from the transferred shoot explants and these new shoots were ready to be sub-divided into single stem segments (Figure 2). The freshly cut stem segments were placed on multiplication medium for the next four week subculture cycle. Once adequate micropropagation stocks of each clone had been obtained, the largest shoots (> 2.5 cm), developed from each shoot explant, were transferred to modified MS *in vitro* rooting medium at the end of the multiplication subculture cycle. Root initials started to appear at the base of the single shoots after approximately three weeks. At four weeks, rooted and non-rooted shoots were removed from culture for nursery acclimatisation (Figure 3). In both the multiplication and rooting experiments, 30 shoots per clone were utilised and both experiments were repeated three times.

Figure 2. Clusters of *Acacia* hybrid shoots on multiplication medium

Figuree 3. Rooted *Acacia* hybrid shoots in rooting medium prior to outplanting

Shoots, removed from the *in vitro* rooting medium, were rinsed under running tap water and separated into rooted and non-rooted shoots. The *in vitro* formed roots were trimmed so that *c.* 2.0 cm of roots remained attached to the stem. All shoots were kept moistened with frequent misting from a hand-held spray bottle. The shoots were then immediately taken to the acclimatisation nursery for setting in Plantek 63F (Transplant Systems Ltd. Christchurch, New Zealand), moulded

plastic nursery containers. The nursery growing medium comprised shredded coconut husks. Shoots, with trimmed roots, were set directly in the moistened growing medium. To induce rooting by an *in vivo* method, the bases of the non-rooted shoots were first dipped in Seradix (May & Baker Ltd., England) rooting compound before being set in the growing medium. All shoots were placed under a misting system (10 min every hour between 0800 h and 1900 h) and with 50% shade. The roof of the acclimatisation house was clad with transparent plastic sheeting for protection against rain. After 3 - 4 weeks, shoots were transferred to another nursery area where once daily watering (30 min) was applied under 30% shade (Figure 4). After a month, plantlets were transferred to a hardening-off area, having 30% shade but no roof cover.

Figure 4. Plantlets after 4 months in the acclimatisation shed

Results and discussion

High incidences of bacterial and fungal contamination were observed with the field-collected shoot explants. Approximately 2 % of shoots placed in culture were uncontaminated after two weeks. Chia (1993) also experienced problems with contamination of hybrid material, collected from coppices in the open field.

The multiplication rate was calculated by dividing the number of shoots at the end of the four-week subculture period by the number of shoots at the beginning of the subculture. Shoots, used for the subsequent multiplication subculture, were selected on the basis that they were at least 1.5 cm in height. Variation was observed between the micropropagated hybrid clones at the multiplication stage (Figure 5).

This variation needs to be carefully noted when producing large numbers of micropropagated hybrid clones. If each clone multiplies at a different rate, larger numbers of the more prolific clones will emerge unless steps are taken to balance the clonal numbers at each transfer.

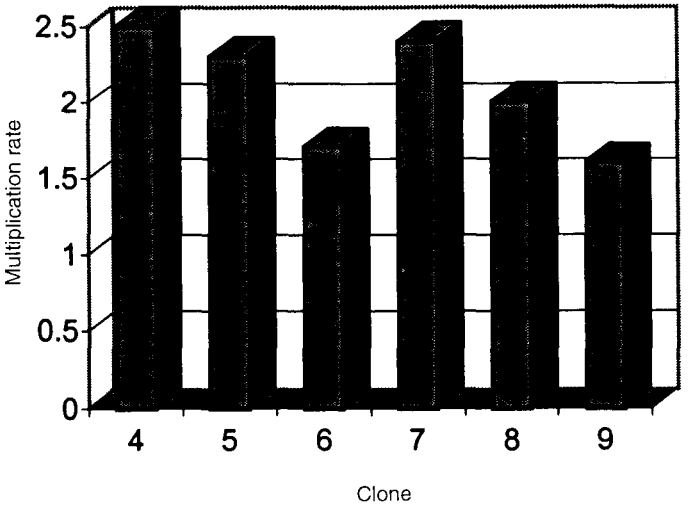

Figure 5. The multiplication rate of six hybrid clones after four weeks on multiplication medium

The multiplication rate value is used as the first selection criterion for eliminating unproductive hybrid clones from the commercial micropropagation system. Only clones having a multiplication rate of two or higher are kept for the *in vitro* rooting stage. The average multiplication rate, over at least five subculture cycles, is used in this selection.

In vitro rooting also varied between the hybrid clones tested (Figure 6). Clones 1, 2 and 3 derived from 2-y-old parent trees, rooted better than Clone 4, which was derived from an 8-y-old parent tree. The juvenility factor could play an important part in the rooting process. Further testing would be required before suggesting that shoots, derived from younger parent trees, are more likely to root than those collected from more mature trees. Another reason for the greater rooting ability of clones 1, 2 and 3 could be that they originated from an *A. auriculiformis* mother tree as opposed to an *A. mangium* mother tree for clone 4.

Similar rooting percentages, under *in vivo* conditions, were reported by Darus (1992) for *Acacia* hybrid cultures derived from aseptically germinated seedlings. Darus also noted that the rooting ability of hybrid shoots declined with increasing culture cycles. In the present study, the rooting ability remained constant, even after ten subculture cycles. The *in vitro* rooting percentages, obtained so far, are still too low for a cost effective commercial system. Rooting percentages should be 80% or higher to warrant the cost of the *in vitro* rooting and acclimatisation stages. At

present, the poorer rooting clones (with 50% rooting or less) are eliminated from the production system. Chia (1993) reported clonal variation in the rooting ability of *Acacia* cuttings and suggested that clones be first tested for their ability to root before being mass propagated.

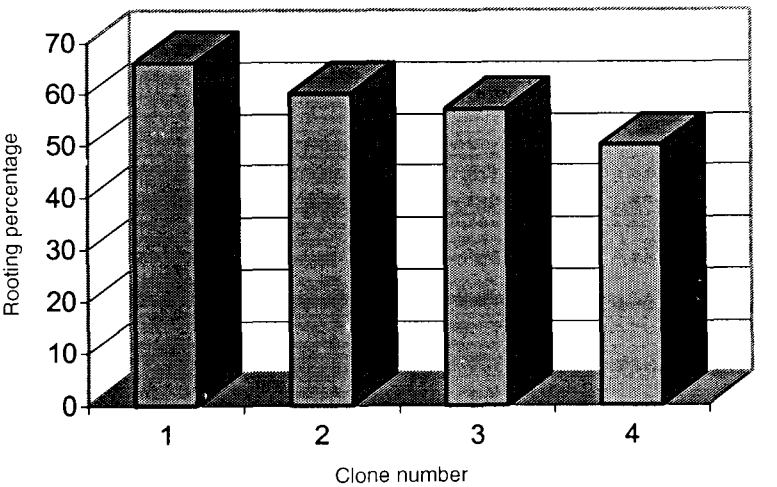

Figure 6. *In vitro* rooting percentage of four hybrid clones after four weeks on rooting medium.

As regards the survival of clones after one month of acclimatisation in the nursery, little variation existed between clone 4 (derived from an 8-y-old tree) and clones 2 and 3 (derived from 2-y-old trees) (Figure 7). Although high survival was recorded among clones having *in vitro* developed roots, those shoots that were placed in the nursery without any *in vitro* formed roots had a poor survival percentage. Those *in vivo* rooted shoots that did survive produced adventitious roots after approximately four weeks.

Darus (1992) achieved good survival and rooting (over 60%) with *in vivo* rooted hybrid shoots, due to the juvenile origin of the original explants (aseptically germinated seedlings) or the type of misted rooting chamber used. Previous trials, using similar rooting chambers as described by Darus, did not produce any better results than the *in vivo* rooting method described in this paper. Ensuring that a high proportion of shoots become successfully acclimatised is extremely important to the success of a commercial micropropagation system. Unless other means can be found to significantly increase the *in vivo* rooting percentages of those unrooted shoots removed from the *in vitro* rooting medium, it would not be cost effective to acclimatise the unrooted shoots.

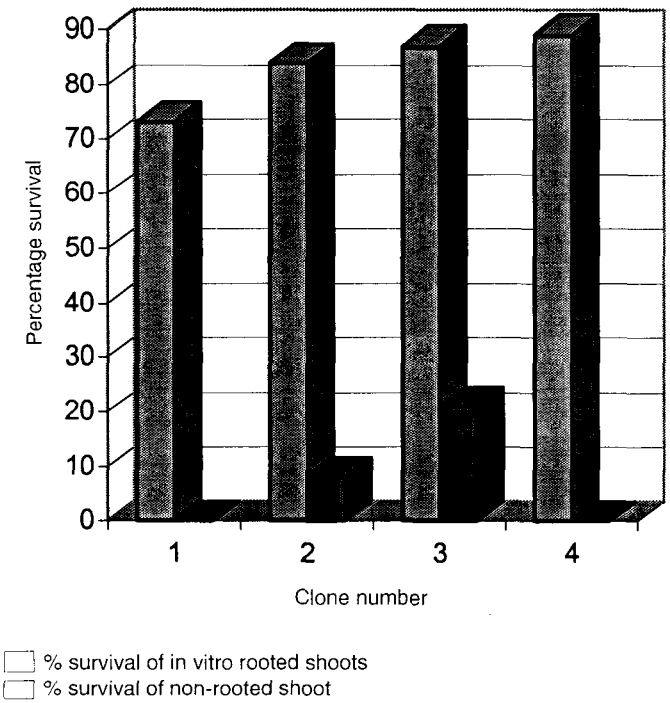

Figure 7. Survival of four hybrid clones after four weeks in the acclimatisation nursery.

Conclusion

Acacia hybrids can be successfully micropropagated from mature trees, thus providing a means of rapidly producing clonal material from superior trees. Clonal variation needs to be carefully monitored so that those clones, which do not have acceptable multiplication or rooting percentages, are removed from the commercial system as early as possible.

Genetically variable trees grown cheaply from seed are still largely preferred to genetically superior trees cloned at high cost (George 1996). Sound growth models, from field-tested micropropagated clones, need to be made available to forest managers so that any long-term gain from the micropropagated clones can be determined. The added value from the micropropagated clones needs to be large enough to warrant the initial high investment costs and subsequent operational costs of a micropropagation facility.

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