MICROPROPAGATION OF *ACACIA MANGIUM* FROM ASEPTICALLY GERMINATED SEEDLINGS

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DARUS HAJI AHMAD. 1991. Micropropagation of *Acacia mangium* from aseptically germinated seedlings. Micropropagation technique for 1-*mth*-old aseptically germinated *Acacia mangium* seedlings was developed. Nodal explants from different stem positions of germinated seedlings were segmented to about 2 to 4 *mm* long and cultured on Murashige and Skoog (MS) basal medium supplemented with 6-benzylamino purine (BAP) and kinetin (Kin) at different concentration levels. MS basal medium with 0.5 $mg\,l^{1}$ BAP was the best combination to induce a higher shoot multiplication with an average of 25.4 shoots per explant. For root formation, excised shoots treated with Seradix 3 (commercial rooting powder) produced 85.0% rooting percentage.

Key words: *Acacia mangium* - nodal explant - multiplication - root initiation

Introduction

Acacia mangium is native to Australia, New Guinea and the Moluccas islands of Indonesia. It was introduced to Sabah, Malaysia, in the 1960s and first planted as fire breaks in pine plantations. Currently, it is one of the five fast growing species chosen for intensive planting for general utility timbers. Although, it is a fast growing species, it has shown very poor form and suffers from heart rot disease. Thus, vegetative propagation by tissue culture is essential for genetic improvement as well as for mass production of selected plus trees for future clonal plantations.

Tissue culture techniques have been successfully developed by micropropagation and callus culture techniques for the following acacias: *Acacia koa* (Skolmen & Mapes 1976), *Acacia nilotica* (Mathur & Chandra 1983), *Acacia albida* (Dohoux & Davies 1985) and *Acacia melanoxylon* (Meyer & Staden 1987).

The aim of this study was to vegetatively propagate *A. mangium* by micropropagation techniques as a means of producing healthy and superior stock plants for plantation establishment.

Materials and methods

A. mangium seeds were first treated to break dormancy by pouring 50 *ml* hot water (80-90°*C*) into a 60 *ml* bottle containing 300 to 500 seeds and left at room temperature for 24 *h*. After pretreatment, the seeds were surface-sterilised with

10% (v/v) Domestos for 30 *min* followed by three washings with sterile distilled water. The seeds were then sown into a 175 *ml* bottles containing 40 *ml* half-strength Murashige and Skoog's basal medium (MS) (Murashige & Skoog 1962) supplemented with 3% sucrose (w/v) and 0.6% bacteriological agar (w/v). Each bottle contained ten seeds.

The nodal explants were taken from different stem positions of 1-*mth*-old aseptically germinated seedlings. Each germinated seedling was cut into four nodal segments and one shoot tip. They were cut to 2 to 4 *mm* in length and cultured into 60 *ml* bottles containing 25 *ml* MS basal medium supplemented with 3% sucrose (w/v), 0.6% bacteriological agar (w/v) and 6-benzylamino purine (BAP) at concentrations of 0.1, 0.25, 0.5, 1.0, 2.0, and 4.0 $mg\ l^{1}$ or kinetin (Kin) at concentrations of 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 $mg\ l^{1}$.

Each treatment consisted of 20 explants and was incubated in a tissue culture growth room, with the temperature of 20±3 °*C* and a photoperiod of 18 *h* at a light intensity of 30000 *lux* from white fluorescent tubes. After one month, these explants were transferred onto fresh medium of the same composition. Observations were carried out on 2-*mth*-old cultures, and the developed shoots were carefully excised from original explants and placed in petri dishes. The height of developed shoots were measured and their number recorded.

Root initiation

The excised shoots which were more than 0.5 *cm* long were used for *in vivo* rooting experiments. Three different types of auxins, namely IBA (Indole-butyric acid) and NAA (Alpha-naphthalene acetic acid) at 100, 250, 500 and 1000 ppm and Seradix 3 (commercial rooting powder) were used. Excised shoots were treated with NAA and IBA by dipping the base into freshly prepared solution for about 5 *s* before inserting them into the misted rooting chambers containing a 1:1 mixture of Irish sphagnum peat moss and sand. Some shoots were treated with Seradix 3 (commercial rooting powder) and for the control treatment, the shoots were dipped into distilled water for about five seconds before planting them into the same rooting medium. The treatments were set out in randomised manner with five replicates of eight shoots per replicate.

Observations were carried out one month after planting. A shoot was considered to have rooted when one newly developed root was clearly visible (at least 0.2 *cm* in length). All shoots were carefully lifted and number of rooted shoots counted. The number of non-rooted and dead shoots were also counted. A shoot was considered to have died when it turned brown in colour.

Results

Shoot multiplication and elongation

Shoot growth occurred within two weeks of inoculation. The shoots were

healthy, green in colour and with small, normal juvenile compound leaves. Of the tree cytokinins tested for their ability to induce multiple shoot formation, 6-benzylamino purine (BAP) was the most effective (Table 1). The highest number of shoots per explant was obtained from basal medium supplemented with 0.5 $mg\ l^{1}$ BAP, with an average of 25.4 shoots per explant (Table 1). Media containing less than 0.5 $mg\ l^{1}$ and higher than 1.0 $mg\ l^{1}$ BAP produced fewer shoots per explant. For kinetin, the optimum concentration for multiple shoot induction was 4.0 $mg\ l^{1}$, with an average of 12.3 shoots per explant (Table 2). The average length of developed shoots was normally more than 0.5 *cm* except adventitious shoots cultured in MS basal medium supplemented with higher concentrations of BAP and kinetin (Tables 1 & 2).

Table 1. Effect of BAP concentration on shoot multiplication and shoot length (*cm*) after two months in culture

BAP concentration ($mg\ l^{1}$)	Average shoot length (*cm*) ± S.E	Average number of shoots per explant ± S.E.
0.0	0.67 ± 0.06	1.2 ± 0.43
0.1	0.61 ± 0.06	4.6 ± 0.33
0.25	0.62 ± 0.03	7.1 ± 0.53
0.5	0.68 ± 0.03	25.4 ± 1.90
1.0	0.59 ± 0.03	24.6 ± 1.28
2.0	0.55 ± 0.03	19.3 ± 0.78
4.0	0.43 ± 0.03	15.8 ± 1.46

Table 2. Effect of kinetin concentration on shoot multiplication and shoot length (*cm*) after two months in culture

Kinetin concentration ($mg\ l^{1}$)	Average shoot length (*cm*) ± S.E	Average number of shoots per explant ± S.E.
0.5	0.76 ± 0.11	2.0 ± 0.65
1.0	0.95 ± 0.14	2.6 ± 0.27
2.0	0.61 ± 0.02	4.5 ± 0.89
4.0	0.44 ± 0.02	12.3 ± 1.46
6.0	0.35 ± 0.02	6.0 ± 0.28
8.0	0.38 ± 0.02	4.8 ± 0.36

Rooting percentage of excised shoots

Different types of growth substances and their concentrations influenced root formation of the excised shoots (Table 3). Seradix 3 was found to be the best in inducing root formation and giving the highest rooting percentage. Excised shoots treated with NAA at higher concentrations, 500 and 1000 ppm or with a lower concentration of IBA, 100 ppm or without any hormone treatment produced very low rooting percentages.

Table 3. Rooting percentage of excised shoots (n=40) with and without growth substance in misted rooting chambers

Treatment	Total shoots rooted	Total shoots non-rooted	% rooted shoots ± S.E
Control	7	18	17.5 ± 3.1
Seradix 3	34	3	85.0 ± 2.5
100 ppm NAA	16	14	40.0 ± 2.5
250 ppm NAA	14	14	35.0 ± 4.7
500 ppm NAA	9	17	22.5 ± 2.5
1000 ppm NAA	5	22	12.5 ± 0.0
100 ppm IBA	7	18	17.5 ± 3.1
250 ppm IBA	13	18	32.5 ± 5.0
500 ppm IBA	16	15	40.0 ± 6.1
1000 ppm IBA	15	12	37.5 ± 5.6

Discussion and conclusion

The results show that BAP is more effective than kinetin for multiple shoot induction. Like nodal cultures of *Lagerstroemia flos-regina* (Paily & D'Souza 1986), cultured nodal explants from aseptically germinated *A. mangium* seedlings required a lower concentration of BAP for optimum shoot multiplication. In general, BAP has been frequently reported to induce better shoot multiplication than other cytokinins, particularly in tree species. Its effectiveness has been demonstrated in juvenile as well as mature tissues of *Calophyllum inophyllum, Eugenia grandis, Fragraea fragrans* (Rao & Lee 1982), *Bougainvillea glabra* (Sharma *et al.* 1981), *Prunus serotina* (Tricoli *et al.* 1985) and *Eucalyptus* spp. (Gupta *et al.* 1981).

This study also demonstrates that excised shoots are rooted easily *in vivo* but different forms of growth substances and their concentrations influenced the rooting percentage and the survival rate. Of the three hormones tested, Seradix 3 was the best. This may be due to: 1) it is composed of 0.8% indole butyric acid (IBA) and fungicide to protect the shoot from fungal attack; and 2) when the shoots are dipped into the powder, the bottom parts remain covered and this may give a longer lasting effect for root formation. The advantages of the rooting technique reported here compared to *in vitro* rooting techniques of other *Acacia* species like *A. koa* (Skolmen & Mapes 1976), *A. melanoxylon* (Meyer & Staden 1987) and *A. albida* (Duhoux & Davies 1985) are that: 1) more shoots can be easily rooted in a misted rooting chamber; 2) aseptic techniques are not required; and 3) it is easy to transplant rooted shoots into pots.

In conclusion, this study demonstrates the possibility of mass production of *A. mangium* using: 1) nodal segments of 1-*mth*-old aseptically germinated seedlings; 2) MS basal medium with lower concentration of BAP for higher shoot multiplication; and 3) non-sterile misted rooting chambers for rooting.

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