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In vitro Propagation from Axillary Buds of *Acacia mangium*, a Legume Tree in the Tropics

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Acacia mangium Willd. is a leguminous tree species well known for its fast growth and adaptability to a wide range of sites[1]. It is particularly noteworthy that *A. mangium* can grow even on acidic soils (pH 4 to 6)[2]. This is an important trait since acidic soils are widespread throughout the tropics. Therefore, *A. mangium* will become an important planting material in the tropics. However, in order to succeed in the reforestation of wastelands, it is necessary to breed varieties tolerant to adverse conditions.

Leguminous forest trees are recalcitrant to regeneration under *in vitro* condition[3], but their tissue cultures are frequently tested because of their economic and ecological importance. A few reports showed that plantlets were successfully regenerated *in vitro* in *Acacia* spp.[4-7] and *Albizia* spp.[3,4]. Aseptically germinated juvenile explants, which were younger than one month, were used in these experiments. For practical clonal propagations, it is advantageous to establish an *in vitro* regeneration and subculturing system from explants grown under field conditions.

The regeneration and subculturing systems of *A. mangium*[8-13] have been established already for aseptically germinated seedlings. While an *in vitro* regeneration method was reported by Galiana *et al.*[8] for 7-month-old seedlings, an effective subculturing method from explants originated under greenhouse conditions has not yet been reported. This is the first report dealing with a subculturing system from axillary buds of 6-month-old *A. mangium* seedlings grown under greenhouse conditions.

Establishment of in vitro culture

Six-month-old seedlings of *A. mangium*, growing on potting soil in a growth chamber kept at 30°C under fluorescent light of 400 μmol•m^{-2}•s^{-1} for a 16 h/day photoperiod, were used as the source of test materials. The seeds collected in Ceram, Indonesia were supplied by the Australian Tree Seed Center (CSIRO).

Nodal segments 1. 5 cm long with axillary buds were excised from the 6-month-old seedlings. The nodal segments were surface-sterilized with 70% ethanol for 1 min. and then with 1% sodium hypochloride for 6 min., and further with 3% hydrogen peroxide for 5 min. Finally they were washed with sterile water 3 times.

The sterilized nodal segments were placed onto solid nutrient medium (10 m*l*) in 25 mm × 120 mm test tubes. The nutrient medium was Murashige and Skoog (MS) medium[14] with 3% sucrose and 0. 8% agar, in addition, 6-benzylaminopurine (BAP) was added to the basic medium at different concentrations (0, 0. 5, 1, 5, 10 μM).

Throughout all experiments, cultures were kept at 25°C under fluorescent light of about 90 μmol•

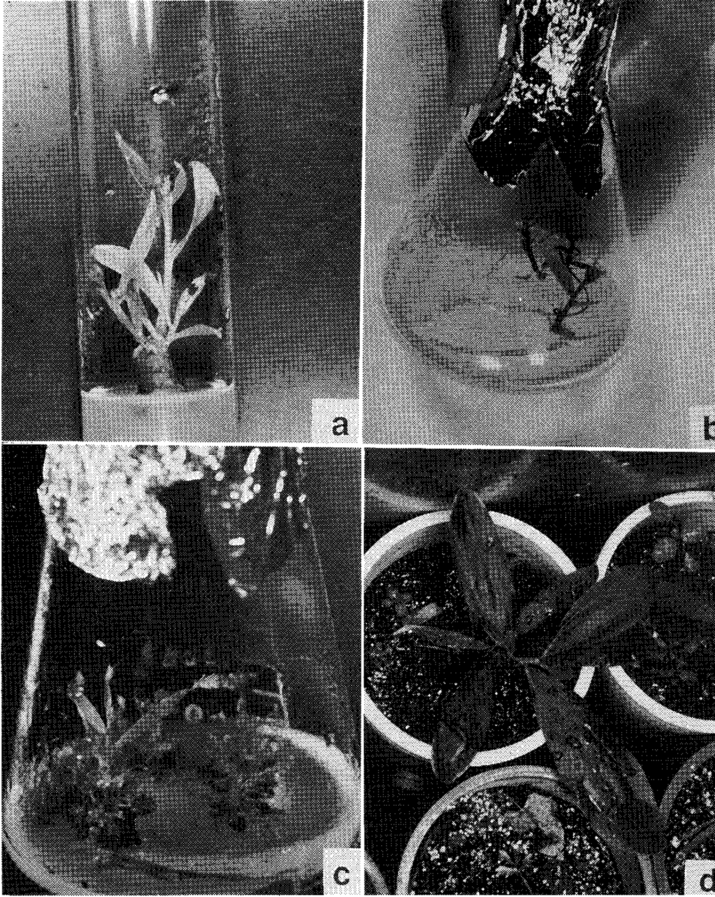

Fig. 1 a : Multiple shoots developed from an axillary bud of *A. mangium* after 35 days of culture on MS medium with 10 μM BAP. b : Rooted shoot on half-strength MS medium with 10 μM IAA after 20 days of culture. c : Subcultured multiple shoots on MS medium with 5 μM BAP for 45 days of culture. d : Acclimatized plantlets on soil.

$m^{-2} \cdot s^{-1}$ for a 16 h/day photoperiod. The medium was adjusted to pH 5. 6 before autoclaving at 120°C for 20 min.

After 35 days of culture, a few shoots with phyllodes developed at axillae as shown in **Fig. 1-a**. The media containing 1, 5, 10 μM BAP were effective for shoot davelopment. The percentage of explants producing long shoots was the highest on the medium with 10 μM BAP (**Fig. 2-a**). The number of shoots per explant increased with the BAP concentration (**Fig. 2-b**). Ten μM BAP was effective for inducing shoots from axillary buds.

In this experiment, an average of 2. 3 shoots were differentiated on MS medium from axillary buds of a 6-month-old tree. However, Ahmad[9] reported that an average of 25. 4 shoots were differentiated from nodal explants of 1-month-old aseptically germinated *A. mangium* seedling on MS medium containing 0. 5 mg/*l* (about 2. 5 μM) BAP[9]. Young nodal segments appear to regenerate new shoots easily with a low concentration of BAP.

Shoots, longer than 1. 5 cm, differentiated from the nodal segments were cut off and transferred onto the rooting medium. The rooting medium was a half-strength of MS medium, in which the major non-organic elements of MS medium were reduced to half concentrations, with 1% sucrose and 0. 8% agar. For the induction of roots, two methods were tested. Method 1 ; the bases of the

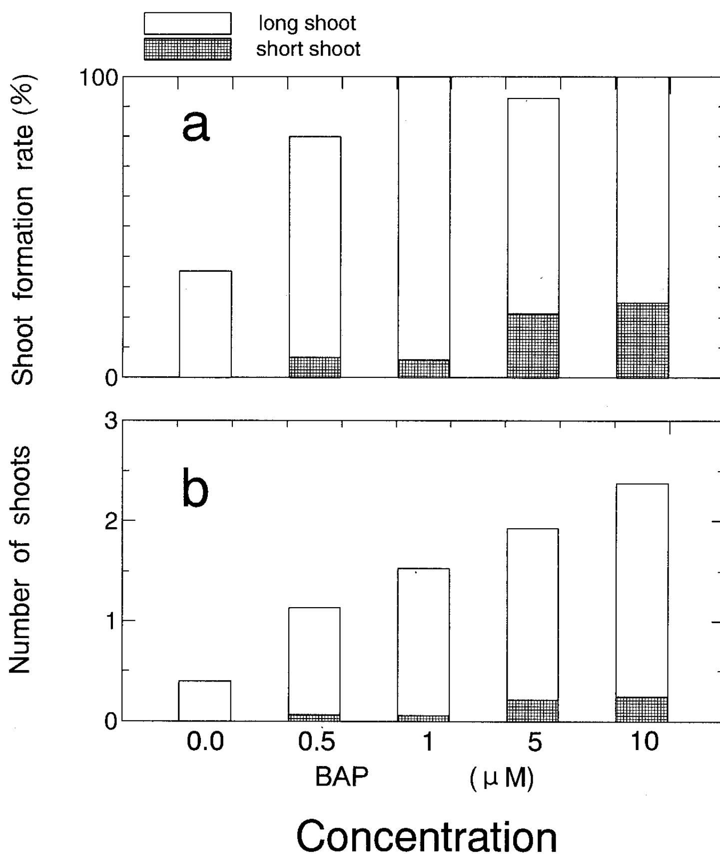

Fig. 2 Effect of BAP concentration on shoot formation.
Shoot formations rate (a) and number of shoots per explant (b) were counted after 35 days of culture. Developed shoots were classified into long shoots (the length of stem was more than 1. 5 cm) and short shoots.

shoots were directly dipped in 100 μM indole-3-acetic acid (IAA) solution for a few seconds. Then they were inoculated on the rooting medium with no growth regulators. Method 2 ; the shoots were transferred onto the rooting medium containing BAP (0, 1, 5 μM) and IAA (0, 10, 20 μM). Each shoot was cultured in two types of culture vessels, test tubes (25 mm × 120 mm, closed by plastic caps) and Erlenmeyer flasks (100 ml). The Erlenmeyer flasks were capped with aluminum foil with vents covered by Milli-seals (Nihon Millipore Kogyo, Yonezawa, Japan).

After 20 days of culture, the highest percentage of rooted explants was 33% on the rooting medium containing 10 μM IAA (**Table 1, Fig. 1-b**). Only one explant dipped in 100 μM IAA solution developed roots.

Rooting was observed only in the aerated Erlenmeyer flasks suggesting that aeration has an effect on root formation. Further investigations are needed to assess the effect of aeration on root differentiation. In this experiment, root formation was found to be optimum with 10 μM IAA in aerated Erlenmeyer flasks. By microcutting of *A. mangium*, high rooting percentage (70%) was achieved on the half-strength MS medium containing 0. 05 mg/l IBA[8]. However, in the present experiment, the shoots developed on MS medium containing BAP did not differentiate the roots on half-strength MS medium containing IBA (data not shown). These two results suggest that BAP in the medium of shoot differentiation influences root formation.

Table 1. Effect of IAA and BAP concentrations on root formation.

Method*[1]	Growth regulators in rooting medium		Number of tested explants	Number of rooted explants*[2]
	IAA (μM)	BAP (μM)		
1	0	0	10	1
2	0	0	9	0
2	0	1	9	0
2	0	5	9	0
2	10	0	9	3
2	10	1	9	0
2	10	5	9	0
2	20	0	9	0
2	20	1	9	0
2	20	5	9	0

*[1] Two methods of rooting induction were tested. See text.
*[2] Rooted explants were counted twenty days after shoots were transplanted to rooting medium.

Subculture of plantlets

The rest of shoots, those less than 1. 5 cm in length, differentiated from the nodal segments, were transferred to the subculturing MS medium in order to develop a multi-shoot induction system. The medium contained 3% sucrose and 0. 8% agar with various combinations of 5 μM BAP, 5 μM indole -3-butyric acid (IBA) and 0. 5 μM gibberellic acid (GA_3). Five explants were cultured in a 200 ml conical flask.

After 45 days of the first subculture, multiple shoots developed as showh in **Fig. 1-c**. All media tested were effective for shoot formation (**Table 2**). The percentage of explants producing long shoots was high on the media containing BAP. The number of shoots was higher on the medium containing BAP than on the medium without BAP. Optimal conditions of subculture were obtained with the media containing 5 μM BAP alone, those with 5 μM BAP and 5 μM IBA or those with 5 μM BAP, 5 μM IBA and 0. 5 μM GA_3.

Table 2. Effect of BAP, IBA and GA_3 in the subculturing medium on shoot and root formation.

Growth regulators in subculturing medium			Long shoot (>1. 5 cm)*[1]		Short shoot (<1. 5 cm)*[1]		Root formation rate (%)*[2]
BAP	IBA	GA_3 (μM)	Shoot formation rate (%)	Number of shoots per explant	Shoot formation rate (%)	Number of shoots per explant	
5. 0	-	-	47	1. 6	100	6. 1	14. 3
5. 0	5. 0	-	50	1. 5	95	5. 9	20. 0
5. 0	-	0. 5	55	1. 2	95	2. 2	-
5. 0	5. 0	0. 5	65	1. 8	95	4. 2	0. 0
-	5. 0	0. 5	0	0. 0	100	1. 3	-
-	5. 0	-	16	1. 0	84	1. 1	-
-	-	0. 5	17	1. 0	94	1. 2	-

*[1] Explants which developed shoots were counted thirty five days after shoots were transplanted to subculturing medium.
*[2] Rooted explants were counted twenty days after long shoots (>1. 5 cm) were transplanted to rooting medium.
The rooting medium was half-strength MS medium containing 10 μM IAA.

The long shoots developed in subculture were transferred onto the rooting medium, which was half-strength of MS medium containing 10 μM IAA with 1% sucrose and 0.8% agar. The shoots were cultured in the aerated Erlenmeyer flasks. After 20 days of culture on the rooting medium, explants produced roots as shown in **Table 2**. This experiment shows that subcultures of shoots from axillary buds can be continued with the media used. The shoots developed on the subculturing medium containing BAP, IBA and GA_3 did not develop roots. The BAP added to the previous shoot formation medium may have a prolonged effect on root formation of *A. mangium*[8]. The combination of growth regulators added to the previous medium may also influence root formation. Therefore the medium containing BAP, IBA and GA_3 was not suitable as a subculture medium for plantlets.

After 45 days of culture of the first subculture, short shoots were inoculated on fresh medium. The medium composition was the same as the first subculturing medium. After 45 days of culture, over 50% of the explants produced multiple shoots. The average being 3 shoots per explant.

Acclimatization

The plantlets developed on the rooting medium were rinsed with tap water and transplanted on to potting mixture composed of vermiculite and peat moss (1 : 1). Pots were set in an airtight container to maintain high humidity (90%). One week later, the container was opened for several minutes a day and the time for opening was gradually lengthened day by day. Three weeks later, the number of surviving plantlets was counted.

In this acclimatization processes, 56% of plantlets survived after they were transferred to potting mixture. This indicates that the plantlets produced using these procedures can be acclimatized without difficulty.

Plant regeneration from axillary buds of 6-month-old seedlings and subculture of multiple shoots was achieved in this experiment. This technique facilitates the clonal propagation of selected trees of *A. mangium*. The technique can be used for various purposes such as stock supply for afforestation and breeding for tolerances, and studies of physiological processes. The establishment of a subculture system will be of great use in the mass propagation of clonal stocks.

Acknowledgement

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《和文要約》

熱帯産マメ科樹木 *Acacia mangium* の腋芽からの試験管内増殖

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熱帯のマメ科早生樹である *Acacia mangium* の 6 ケ月生稚樹を用いて試験管内増殖をおこなった．稚樹の腋芽からマルチプルシュートを発生させ，このシュートを材料としてさらに継代的にシュートを得ることができた．また，試験管内で得たシュートを発根させて幼植物体を再生し，順化することに成功した．