VEGETATIVE PROPAGATION OF ENDOSPERMUM MALACCENSE BY LEAFY STEM CUTTINGS: EFFECTS OF INDOLE BUTYRIC ACID (IBA) CONCENTRATIONS AND PROPAGATION SYSTEMS (MIST AND NON-MIST)

H. Aminah

Forest Research Institute Malaysia, Kepong, 52109 Kuala Lumpur, Malaysia

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H. AMINAH. 2003. Vegetative propagation of Endospermum malaccense by leafy stem cuttings: effects of indole butyric acid (IBA) concentrations and propagation systems (mist and non-mist). Two experiments were carried out separately. In experiment 1, IBA dissolved in 50% ethyl alcohol (0, 30, 60 and 90 μ g per cutting) was applied to the base of single node leafy stem cuttings of Endospermum malaccense taken from six-monthold potted seedlings. It was found that the application of IBA significantly increased the speed of rooting of the cuttings and increased the number of roots per cutting. At week three of the propagation period, 70% of cuttings treated with 30 μ g IBA rooted compared with only 32% of the untreated cuttings. Eight weeks after treatment, the mean number of roots on cuttings treated with IBA was significantly greater than cuttings not treated with IBA. Mean number of roots per cutting was 5.8, 5.9 and 5.9 respectively for 30, 60 and 90 µg IBA compared with 4.2 for cuttings without the IBA treatment. In experiment 2, leafy stem cuttings of E. malaccense were propagated in two propagation systems (mist and non-mist). Results indicate that cuttings of this species can be propagated in both systems. The mean accumulated number of roots per cutting was significantly higher in the non-mist (6.3) compared with those in the mist system (4.0).

Key words: E. malaccense - rooting - number of roots - speed of rooting

H. AMINAH. 2003. Pembiakan tampang Endospermum malaccense melalui keratan batang berdaun: kesan kepekatan asid indola butirik (IBA) dan sistem pembiakan (renjisan berkabus dan tanpa renjisan berkabus). Dua percubaan yang berbeza telah dijalankan. Percubaan 1: Beberapa kepekatan IBA yang dilarut dengan 50% etil alkohol $(0, 30, 60 \text{ and } 90 \mu \text{g satu keratan})$ dibubuh pada bahagian bawah keratan batang berdaun Endospermum malaccense yang diambil daripada anak benih berumur enam bulan. Rawatan IBA memberi kesan bererti dalam mempercepatkan pertumbuhan akar dan penambahan bilangan akar pada keratan. Dalam tempoh tiga minggu, 70%keratan yang dirawat dengan 30 μ g IBA telah berakar berbanding dengan hanya 32% keratan yang tidak menerima rawatan IBA. Purata bilangan akar bagi keratan yang dirawat dengan IBA pada minggu kelapan menunjukkan perbezaan yang bererti berbanding dengan keratan yang tidak menerima rawatan IBA. Purata bilangan akar satu keratan masing-masing ialah 5.8, 5.9 dan 5.9 untuk 30, 60 dan 90 µg IBA berbanding dengan 4.2 untuk keratan tanpa rawatan IBA. Percubaan 2: Keratan batang berdaun E. malaccense ditanam dalam dua sistem pembiakan (renjisan berkabus dan tanpa renjisan berkabus). Keputusan percubaan menunjukkan bahawa keratan spesies ini boleh dibiak dalam kedua-dua sistem. Purata kumulatif bilangan akar keratan yang ditanam dalam sistem tanpa renjisan berkabus adalah bererti dan lebih banyak (6.3) berbanding dengan akar keratan yang ditanam dalam sistem yang mempunyai renjisan berkabus (4.0).

Introduction

Endospermum malaccense belongs to the family Euphorbiaceae and the preferred vernacular name for it is sesenduk. The tree occurs in all states of Malaysia except Perlis. The species is also found in the neighbouring countries of Thailand, Sumatra and Kalimantan. It is a light-demanding species and is widely distributed in the lowland secondary forest especially in logged-over areas up to 1000 m altitude (Whitmore 1972).

The tree of *E. malaccense* can reach 40 m in height and 3 m in girth. The trunk is stout and columnar with smooth grey bark. Mature tree has dome-shaped crown with massive spreading branches. The leaves with long stalks, about 13 cm, are usually clustered at the tip of the twig. The leaf blade is ovate, measuring 13×7.5 to 17×17 cm (Whitmore 1972).

The growth rate of this species is fast, with mean annual increment of 1.9 m in height and 2.5 cm in trunk diameter, as indicated by measurements taken from 13-year-old trees planted at Sungei Tekam Forest Reserve in the state of Pahang, Peninsular Malaysia (Darus *et al.* 1990). It is anticipated that the species would attain a harvestable size of 40 to 50 cm diameter in less than 30 years after planting (Darus *et al.* 1990).

The timber of *E. malaccense* is classified as light hardwood. The wood is bright yellow and is well-suited to the current market demand for light-coloured wood (Vermeer 1991). The timber is not durable but it is extremely easy to treat with preservative. The wood is commonly used for drawing boards, furniture parts, pattern making, plywood and other small articles such as chopsticks, match boxes, clogs and toys (Mohd Shukari 1982). *Endospermum malaccense* produces fruit regularly since the tree flowers twice a year, i. e. in February/March and again in August/September. The seeds can be stored up to six months. Seed viability is reduced by severe infestation of the fruit borer *Dichocrosis puntiferalis* (Lepidoptera: Pyrilidae) (Yap & Razali 1980), which limits mass production of planting stock from seeds. Vegetative propagation by stem cuttings is therefore sought as an alternative for the production of planting stock, especially since *E. malaccense* is one of the species recommended for plantation (Darus *et al.* 1990, Ab. Rasip 1994).

Several factors can affect the rooting of cuttings. Benefit of auxin application in promoting adventitious root development of stem cuttings have been reported (Leakey *et al.* 1982, Hartmann *et al.* 1990, Aminah *et al.* 1995, Tchoundjeu & Leakey 1996). In addition, it is important to test the necessity of using mist for propagation, as this affects the cost of the operation (Leakey *et al.* 1990). Two separate experiments were therefore carried out to determine the auxin concentration and type of propagation system most suitable for rooting of the stem cuttings of *E. malaccense*.

Materials and methods

Two separate experiments were carried out in the nursery of the Forest Research Institute Malaysia (FRIM). The stock plants for experiment 1 were raised from seedlings (approximately 5 to 6 cm tall) collected from three mother trees in the Sungai Menyala Forest Reserve, Negeri Sembilan, Peninsular Malaysia. For experiment 2, the stock plants were raised from rooted cuttings. There were 21 clones used for the experiment with one clone per stock plant. These plants were potted in black perforated polythene bags (9 cm diameter \times 17 cm height). The potting medium used was forest topsoil and sand (3:1 v/v). To every cubic meter of the medium,1.2 kg triple superphosphate (46% P₂O₅) and 1.6 kg ground magnesium limestone (33% CaO) were added. The potted seedlings were kept on transplanting beds shaded with plastic netting. Measurements for three consecutive sunny days gave an average midday irradiance of 770 µmol m⁻² s⁻¹ (measured using SKP 215/200 light sensor, Skye Instruments, UK). Granular compound commercial fertiliser, NPK Blue (12N:12P₂O₅:17K₂O:2MgO + trace element), was applied at 1 g plant⁻¹month⁻¹. Watering was carried out manually in the mornings and late afternoons. Weeding and insecticide and fungicide applications were carried out as needed.

A weekly assessment of the cuttings was carried out from the first week after planting. For each assessment, the number of rooted, unrooted, dead cuttings and the number of roots produced were recorded. The unrooted cuttings were replanted into rooting bed and reassessed until the eighth and ninth week for experiments 1 and 2 respectively. By this time leaves of unrooted cuttings would have turned yellow and dropped off from the cuttings. These cuttings eventually died when carbohydrate reserves from the stem were depleted. In the assessment, a cutting was scored as rooted when it produced at least one root that was at least 1 cm long. The cuttings were considered dead when the whole stem turned brown. The mean accumulated root number was calculated by dividing the total number of roots produced by the total number of rooted cuttings at each assessment week.

A stepwise regression procedure (Genstat 5, 1987) was used to determine which treatment was significantly associated with percentage of rooted, unrooted and dead cuttings. Analysis of variance was used to test for significant differences in the treatments for initial cutting diameter, cutting length and number of roots. The results were considered significant when p < 0.05.

Other details of each experimental procedure are described separately.

Experiment 1: effect of indole butyric acid (IBA) concentrations

A total of 60 seedlings was selected for the experiment when the stock plants reached the age of six months. The average height of the selected seedlings was 60 cm. Single node stem cuttings were taken from these stock plants from the third through sixth nodes from the top of the stem. The apical undeveloped shoots were discarded as they were not suitable for cuttings. Four cuttings were taken from each stock plant and each node was randomly allocated to one of the four indole butyric acid (IBA) treatments. The length of the cuttings was 4 cm and the leaf area retained on each cutting was 30 cm². The leaf area was cut using a 30-cm² paper template measured with a leaf area meter (Delta-T series, Taiwan). The initial diameter of each cutting was recorded. Initial stem diameter of cuttings

used was not significantly affected by the treatments. Mean diameters were 3.7, 3.8, 3.7 and 3.8 mm for 0, 30, 60 and 90 µg IBA respectively. The base of the cutting was cut at a right angle and treated with one of the four IBA concentrations. The IBA formulation was prepared in liquid form using absolute ethyl alcohol which was diluted to 50% with distilled water. IBA was applied to each cutting using a micropipette (model F10, Gilson Medical Electronic France), after which the alcohol was immediately evaporated by a stream of air from a fan. The treated cuttings were inserted into rooting media of cleaned river sand containing sand particles $\leq 2 \text{ mm} (60\%)$ and 2 to 5 mm (40%). Each treatment consisted of 60 cuttings were covered with translucent plastic enclosures supported by aluminium frames to maintain high humidity. The plastic enclosures were then shaded with black plastic netting. The rooting beds were kept moist by an automatic mist system set at hourly intervals of one-minute misting.

The average midday irradiance value under this shade on a sunny day was 275 μ mol m⁻² s⁻¹. The red/far red ratio was quantified using a SKR 110 with a 660/730 light sensor (Skye Instruments, UK) and the average midday value of this ratio, taken from readings of three consecutive days, was 1.10.

Experiment 2: effect of mist and non-mist propagation systems

Stem cuttings were taken from six-month-old stock plants. The average height of these plants was 40 cm. Single node stem cuttings were taken and prepared as in experiment 1. The length and diameter of each cutting were recorded. There was no significant difference in stem diameter and length of cuttings used. Mean diameter was 6.0 mm for both treatments while lengths of cuttings were 3.5 and 3.3 cm for mist and non-mist propagation systems respectively. The base of the cuttings was immediately treated with 30 μ g IBA dissolved in 50% ethyl alcohol. After the alcohol evaporated, cuttings were planted in cleaned river sand (as in experiment 1) in mist and non-mist propagation systems. The cuttings for each stock plant were randomly assigned to each system and were replicated in two blocks, each comprising 30 cuttings. The non-mist system was constructed based on the principal used by Leakey et al. (1990) while the mist propagation system used was as described in experiment 1. Both propagation systems were located in the cutting shed and were shaded with one layer of black plastic netting. Environmental data, namely, temperature, relative humidity and irradiance level, were recorded by a data logger (Model: 21X, Campbell Scientific, UK) using a thermocouple (model: 1XA-K-15, Campbell Scientific, UK), a rotronic relative humidity probe (model MP100, Campbell Scientific, UK), and a quantum sensor (model SKP100, Skye Instrument, UK) respectively. The sensors were placed in all the blocks used for the experiment. Data from each type of sensor was recorded every minute and a five minute average was stored. Data measurement was carried out for 15 days. The red/far red ratio was measured at 10 spots in each block of each treatment as described for experiment 1. The average values of red to far red ratio in the mist and non-mist propagators were 1.10 and 1.08 respectively.

Results

Experiment 1

IBA treatments significantly accelerated the rooting of single node leafy stem cuttings of *E. malaccense* taken from six-month-old seedlings compared with cuttings without IBA (Figure 1). At week three, 62 to 70% of the IBA-treated cuttings had rooted in contrast with only 32% for the untreated cuttings. However, no significant difference among treatments was observed in rooting percentages of cuttings at week eight. The accumulated rooting percentages at week eight for cuttings with IBA treatments of 30, 60 and 90 μ g were 86.7, 81.7 and 71.7% respectively while that for untreated cuttings was 71.7%.



Figure 1 Effect of various concentrations of indole butyric acid on the mean accumulated rooting percentage of *Endospermum malaccense* stem cuttings

The mean accumulated number of roots was increased by IBA treatments throughout the assessment period (Figure 2), and, in the eighth week, it was significantly different compared with untreated cuttings. The means for accumulated number of roots per rooted cutting at week eight for 30, 60 and 90 μ g IBA were 5.8, 5.9 and 5.9 respectively compared with 4.2 for cuttings without IBA treatment. No significant difference in the mean accumulated number of roots per cutting was observed between the different IBA concentrations used.



Figure 2 Effect of various concentrations of indole butyric acid on the mean accumulated number of roots of *Endospermum malaccense* stem cuttings

Experiment 2

There was no significant difference in the percentage of rooting for stem cuttings of *E. malaccense* planted in mist and non-mist propagation systems. The accumulated rooting percentages at week nine were 63.4 and 51.6% for mist and non-mist systems respectively.

Cuttings from the non-mist propagation system produced significantly more roots compared with cuttings from mist propagation system (Figure 3). The means for accumulated number of roots for cuttings in the mist and non-mist propagation systems were 4.0 and 6.3 respectively.

The number of dead cuttings was significantly higher in the non-mist (46.7%) than in the mist system (28.3%). There were significantly more unrooted cuttings in the mist (8.3%) than in the non-mist (1.7%) propagation system.

Environmental data showed that the non-mist environment had higher air and leaf temperatures (> 40 °C), higher irradiance (> 500 μ mol m⁻² s⁻¹) and lower humidity (< 90%) (Figures 4 (a)-(d)).



Figure 3 Effect of propagation systems on the mean accumulated number of roots of *Endospermum malaccense* stem cuttings

Discussion and conclusions

In this experiment, the use of IBA induced earlier rooting of *E. malaccense* cuttings as indicated by the significantly higher percentage of rooting in treated than in untreated cuttings at week three. The application of IBA increase the speed of transformation and movement of sugar to the base of cuttings and thus indirectly stimulate rooting (Haissig 1974, 1982). Similar results were obtained in *Shorea macrophylla* (Lo 1985) and *S. leprosula* stem cuttings (Aminah *et al.* 1995). Early rooting is desirable because the faster the rooting, the lower the chances of mortality of cuttings due to water stress. Rooted cuttings can be potted earlier, allowing the rooting beds to be used for a new batch of cuttings. Results obtained at the end of this experiment did not reflect the effect of auxin in stimulating early rooting since no significant differences were obtained between treatments. Similar results were reported by Lo (1985) and Aminah *et al.* (1995).

The number of roots produced per rooted cutting in *E. malaccense* was significantly greater with auxin application. More roots on cuttings are beneficial as it enhances anchorage in the field. The effect of auxin in increasing the number of roots has been observed in other species such as *S. leprosula* (Aminah *et al.* 1995), *Milicia excelsa* (Ofori *et al.* 1996), *Ricinodendron heudelotii* (Shiembo *et al.* 1997), *Khaya ivorensis* (Tchoundjeu & Leakey 1996), and *Calliandra calothyrsus* (Wolf & Jaenicke 2000). However, in *E. malaccense*, there was no advantage in applying higher IBA concentrations since even after eight weeks treatment, no significant difference in the number of roots was obtained between the IBA concentrations used.

There was no advantage of the mist over the non-mist propagation system since differences in rooting percentage between the two systems were not significant. This showed that a non-mist propagation system can be used as an alternative system for rooting cuttings as previously reported for *S. leprosula* (Aminah *et al.* 1996). However, mortality of cuttings was higher (46.7%) in the non-mist than in the mist propagation system (28.3%). This result could be due to higher air and leaf temperatures, higher irradiance, and lower humidity in the non-mist system (Figures 4(a)-(d)). Even though similar shade cloth was used, higher irradiance



Figure 4 Daily maximum air temperature, leaf temperature, irradiance and relative humidity in the mist and non-mist propagation systems measured from day 1 to day 15 of the experiment. Each variable was calculated as a five-minute average.

levels were recorded in the non-mist propagation system compared with the mist system. This higher irradiance could be due to the more exposed location of the non-mist system. High temperature (> 40 °C) and low humidity (< 90%) have been reported to be detrimental to rooting of many tropical species because cuttings suffer from water stress (Yasman & Smits 1988). In *Shorea macrophylla* cuttings, 75% mortality occurred as a result of water stress (Lo 1985). High temperature induced water stress in leafy woody cuttings (Gay & Loach 1977) . With reduced humidity, the leaves of cuttings wilted and lost their turgor. They are not likely able to recover to their turgid condition and so, the cuttings either died or did not root. Grange and Loach (1983) recommended shading to keep a maximum irradiance of 100 Wm⁻² (45 μ mol m⁻² s⁻¹), which could reduce the temperature and yet still be adequate for photosynthesis. It is anticipated that if the air and leaf temperatures in our systems can be reduced to less than 40 °C through heavier shading, and high humidity is maintained, more cuttings would survive and increased rooting of *E. malaccense* would be obtained.

Cuttings that rooted in the non-mist propagation system had more roots than those rooted in the mist propagation system. The higher number of roots obtained in the former could perhaps be due to optimum use of auxin at the rooting zone. In the mist propagation system, leaching of applied auxin may occur due to water from misting. Similar results were observed with *S. leprosula* cuttings (Aminah *et al.* 1996).

In conclusion, a treatment of $30 \,\mu g$ IBA dissolved in 50% ethyl alcohol is suitable for good rooting of juvenile stem cuttings of *E. malaccense*. Cuttings of this species can be rooted either in mist or non-mist system. The non-mist system offers advantages of being simple and inexpensive to construct and does not require facilities like piped water or electricity. Rooting percentages of more than 50%, with an average of six roots per cutting, can be obtained within eight to nine weeks of using this system.

References

- AB. RASIP, A. G. 1994. Endospermum malaccense: sesenduk. Pp. 30–34 in Zakaria, I. & Wickneswari, R. (Eds.) Selected Tree Species for Forest Plantation in Peninsular Malaysia: A Preliminary Consideration. Research Pamphlet No. 116. Forest Research Institute Malaysia, Kepong.
- AMINAH, H., DICK, J. MCP., LEAKEY, R. R. B., GRACE, J. & SMITH, R. I. 1995. Effect of indole butyric acid (IBA) on stem cuttings of Shorea leprosula. Forest Ecology and Management 72(2&3): 199–206.
- AMINAH, H., DICK., J. MCP. & GRACE, J. 1996. Vegetative propagation of Shorea leprosula Miq. by stem cuttings in the non-mist and mist propagation systems. Commonwealth Forestry Review 75(3): 221-226.
- DARUS, A., HASHIM, M. N., AB. RASIP, A. G. & LOK, E. H. 1990. Khaya ivorensis and Endospermum malaccense as potential species for future reforestation programme. Pp. 60–65 in Appanah, S., Ng, F. S. P. & Roslan, I. (Eds.) Proceedings of the Conference on Malaysian Forestry and Forest Products Research. Forest Research Institute Malaysia, Kepong.
- GAY, A. P. & LOACH, K. 1977. Leaf conductance changes on leafy cuttings of Cornus and Rhododendron during propagation. Journal of Horticultural Science 52: 509-516.
- GRANGE, R. I. & LOACH, K. 1983. Environmental factors affecting water loss from leafy cuttings in different propagation systems. *Journal of Horticultural Science* 58: 1–7.
- HAISSIC, B. E. 1974. Influences of auxin synergists on adventitious root primordium initiation and development. *New Zealand Journal of Forest Science* 4: 311-323.

- HAISSIG, B. E. 1982. Carbohydrate and amino acid concentrations during adventitious root primordium development in *Pinus banksiana* Lamb. cuttings. *Forest Science* 28: 813–821.
- HARTMANN, H. T., KESTER, D E. & DAVIS, JR. F. T. 1990. Plant Propagation: Principals and Practices. 5th edition. Prentice-Hall International Editions, New Jersey. 647 pp.
- LEAKEY, R. R. B., CHAPMAN, V. R. & LONGMAN, K. R. 1982. Physiological studies for tropical tree improvement and conservation. Factors affecting root initiation in cuttings of *Triplochiton* scleroxylon K. Schum. Forest Ecology and Management 4: 53-66.
- LEAKEY, R. R. B., MESEN, J. F., TCHOUNDJEU, Z., LONGMAN, K. A., DICK, J. MCP., NEWTON, A. C., MATIN, A., GRACE, J., MUNRO, R. C. & MUTHOKA, P. K. 1990. Low technology techniques for the vegetative propagation of tropical trees. *Commonwealth Forestry Review* 69: 247–257.
- Lo, Y. N. 1985. Root initiation of *Shorea macrophylla* cuttings: effect of node position, growth regulators and misting regime. *Forest Ecology and Management* 12: 42–52.
- MOHD SHUKARI, M. 1982. *Malaysia Timber: Sesenduk*. Malayan Forest Service Trade Leaflets No. 66. The Malaysian Timber Industry Board, Kuala Lumpur. 6 pp.
- OFORI, D., NEWTON, A. C., LEAKEY, R. R. B. & GRACE, J. 1996. Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentration, leaf area and rooting medium. *Forest Ecology and Management* 84: 39–48.
- SHIEMBO, P. N., NEWTON, A. C. & LEAKEY, R. R. B. 1997. Vegetative propagation of Ricinodendron heudelotii, a West African fruit tree. Journal of Tropical Forest Science 9(4): 514–525.
- TCHOUNDJEU, Z. & LEAKEY, R. R. B. 1996. Vegetative propagation of African mahogany: effects of auxin, node position, leaf area and cuttings length. *New Forests* 11: 125–136.
- VERMEER, M. 1991. European Tropical Timber Market Update. MTIB Marketing Seminar, Market Opportunity: Post Gulf War. MTIB and Ministry of Primary Industry Malaysia. 27 pp.
- WHITMORE, T. C. 1972. Tree Flora of Malaya. Volume 2. Longman, Malaysia. 444 pp.
- WOLF, J. DE. & JAENICKE, H. 2000. Propagation of *Calliandra calothyrsus* through cuttings: effect of stock plant shading. *Journal of Tropical Forest Science* 12(3): 571-580.
- YAP, S. K. & RAZALI, H. 1980. The reproductive behaviour of sesenduk (Endospermum malaccense). Malaysian Forester 43(1): 37-43.
- YASMAN, I. & SMITS, W. T. M. 1988. Methoda Pembuatan Stek Dipterocarpaceae. Balai Penelitian Kehutanan Samarinda. Edisi Khusus No. 3. 36 pp.