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Full Length Article

Adventitious Rooting in Microcuttings of Selected Indigenous Landscape Trees of Malaysia

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ABSTRACT

Rooting performance in microcuttings of five indigenous and one exotic landscape tree species was evaluated in a 60 days period. Rate of rooting varied greatly among species as *Ilex cymosa, Tabebuia heterophylla* and *Agalaia korthalsii* reached 100% rooting at 24, 42 and 48 days after insertion into the rooting medium followed by *Lepisanthes rubiginosa* and *Hopea odorata* species (95 & 80% rooting at 60 days, respectively). The poorest rooting ability (30% rooting at 60 days) was shown by *Aporusa globifera* and was first noticed at 30 days. Results of *Ilex cymosa* species for adventitious root induction per microcutting was markedly different from other species and it produced the highest number of adventitious roots (14.07 roots/microcutting). *A. korthalsii* generated the fewer, but the heaviest roots compared to *I. cymosa*. Rooting ability of microcuttings was highly correlated with its number of leaves produced during rooting (r = 0.869-0.981) as well as, with regenerative shoot length of the mother plants (r = 0.690-0.954). Regenerative leaf growth of mother plants and the rooting of their microcuttings was species-specific suggesting that developing association between the two parameters is difficult.

Key Words: Microcutting; Rooting; Vegetative propagation; Landscape tree

INTRODUCTION

Rapid demand for the indigenous species for landscaping projects in Malaysia requires efficient and cost effective propagation techniques. Presently, production of planting materials of such trees relies mainly on the collection of seeds or seedlings from the wild plant sources. Irregular flowering and fruiting habits (Ashton *et al.*, 1988) and recalcitrant nature of seeds of many tropical tree species (Tompsett, 1987) make seed collection difficult. Seedlings collected from the wild, if not properly handled could cause high mortality (Palmiotto, 1993). A rapid and cost effective vegetative propagation suitable for these trees is therefore required.

Use of cuttings is one of the possible techniques for vegetatively propagation of trees. Earlier efforts conducted to evaluate rooting ability of cuttings of tropical trees were mainly focus on important timber species (Dick & Aminah, 1994). Huge diversity among species of interest makes generalization of results impossible. Study of a wide range of tropical rainforest tree species (100 species) revealed that rooting ability of members of *Dipterocarpaceae*, *Lauraceae*, *Euphorbaceae*, *Rubiaceae* and *Annonaceae* varied between 0 - 100% with a mean rooting percentage of 37.7% only (Itoh *et al.,* 2002).

Use of microcuttings for propagation of different tropical tree species emerged as a popular technique recently (Titon *et al*., 2006) and has been equally effective and reliable for mass propagation of landscape trees such as *Dillenia philippinensis*, *Lopanthera lactescens* and *Xanthestemon chryanthus* (Ab Kahar *et al*., 2009). Plant material used in the technique comprised of stem cutting (4 cm long) obtained from juvenile mother plants raised from seed or micropropagation grown in high planting density under partially protected condition and pruned regularly.

In this study, rooting performance of microcuttings of five selected indigenous forest species and one exotic landscape species was determined and associations were established between regenerative growth of mother plants and rooting ability of their microcuttings.

MATERIALS AND METHODS

Plant materials. In this study, five selected indigenous candidate species having potential for use as landscape trees along with one exotic species, *T. heterophylla* were used (Table I). The seedlings (as mother plants) were obtained from a commercial nursery (Syarikat Perniagaan Tunas Harapan, Tanjung Malim, Perak).

Seedlings of 10 - 20 cm in height and about 2 months old were selected and carefully extracted from sand beds in the nursery with intact roots. They were packed in moist plastic bags, labeled and immediately brought to Serdang, Selangor and planted at the Ornamental Research Complex

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of the Malaysian Agricultural Research and Development Institute (MARDI) on the same day. The seedlings were raised in plastic trays (25 cm x 30 cm x 10 cm) on a loamy top soil-cocopeat (3:1) medium (8 cm deep) with additional NPK fertilizer (15:15:15 g L^{-1}). The plants were raised under a 50% netted structure, which helps in reducing the incoming light radiation and moderates temperature at mid-day. The plants were watered twice daily to ensure their establishment.

Propagation technique. Microcuttings 4 ─ 6 cm in length having 2 - 3 nodes were harvested using a sharp knife from mother plants after four months of establishment. The microcuttings were propagated in propagation chamber on a soilless medium comprising of perlite (8 cm deep) underlying with light expanded clay aggregates (LECA). The propagation chamber was constructed using polystyrene boxes (55 cm x 43 cm x 30 cm) and covered with polyethylene sheet to maintain a humid environment. A water reservoir was created and maintained at the bottom of the box and water would then flow through capillary rise into perlite. A hole was thumbed on sidewall at 5 cm above the base of each box so that a good air-water relationship atmosphere can be created in the rooting zone. The propagation chambers were placed under 50% nettings. For rooting process, microcuttings were inserted into the perlite for one cm deep without application of rooting hormone. The root and shoot growth of the microcuttings were monitored to a maximum period of 60 days.

Data Collection

Root growth of microcutting. Root growth was monitored by counting the number of rooted cuttings up to 60 days after insertion of microcuttings into the rooting medium. The recording of rooted cuttings was stopped, when 100% rooting was achieved prior to 60 days. Rooting success was examined by carefully pulling out the microcuttings from the medium, afterwhich the cuttings were inserted back. This process did not adversely affect the rooting as perlite used was very loose and the whole process was completed in less than 10 seconds. Data obtained were used to calculate the percentage of rooted cuttings. At final harvest, all roots generated from 10 microcuttings for each plot were harvested and the length of the longest three roots for each cutting was measured and mean calculated. Root dry weight was obtained after drying in a forced drought oven at 70°C for 3 days. For each species, 10 samples were used for the above measurements for each plot.

Shoot growth of microcuttings. Leaf number (including the intact leaves retained before propagation) was counted at every six days after the insertion of microcuttings into medium, up to 60 days except for species that have achieved 100% rooting earlier. Ten samples per plot were used for determination of leaf number for each species. At the final harvest, all the leaves retained on the cuttings were separated and their areas were measured using a leaf area meter (Li-COR® MODEL LI-3000, Lincoln, NE, USA). Leaf dry weight was also recorded.

Regenerative growth of mother plants. The growth of mother plants after the harvesting of microcuttings was monitored by measuring the increment of shoot length and number of new leaves generated after the harvesting of microcuttings. Shoot length was measured from the point of harvest of the microcuttings to the tip of the shoots at every six days. Number of leaves generated by new shoots was counted up to the date as, when the microcuttings reached to 100% rooting. For both measurements, nine samples of the mother plants from each species were used per plot.

Experimental design and statistical analysis. The experiment was laid out in randomized complete block design (RCBD) with three replicates and each plot contained 10 microcuttings. Analysis of variance (ANOVA) of data was performed using Statistical Analytical System (SAS 9.1, SAS Institute, Inc. Cary, NC, USA). Least significant different (LSD) at a 0.05 significant level was used for mean comparison among treatments. Correlation analysis was performed to determine the association between rooting performance with some other parameters measured in the study.

RESULTS AND DISCUSSION

Root growth of microcuttings. The patterns of root production differed markedly between species as shown by a significant interaction of species and days interaction (p<0.0001, Fig. 1). Only three species *Ilex cymosa*, *Tabebuia heterophylla* and *Agalaia korthalsii* reached 100% rooting within 60 days period after 24, 42 and 48 days of insertion into the rooting medium, respectively. Microcuttings of *Hopea odorata*, one of the favorite landscape tree species for street planting in Malaysia produced 80% rooting at 60 days. The ability of *Aporusa globifera* to root was the poorest (30% microcuttings rooted at 60 days).

Differences in rooting ability of the indigenous species recorded here are inline with the result obtained by Itoh *et al.* (2002), who reported that the rooting of indigenous species ranged from 0 to 100%, with an average of 37.7%. In our case rooting percentage varied from 30 to 100% with an average of 52.9%. Such variation is expected as the rooting process is highly affected by many possible factors including concentration of inhibitors in the stem tissues (Kibbler *et al*., 2002) and food reserves (Druege *et al*., 2004), mineral nutrients (Schwambach *et al*., 2005) and genetics (Titon *et al*., 2006).

Propagation technique is considered successful, when adventitious roots are generated from the cuttings. Roots can be either initiated from the stem or at the based-stem (Hartmann *et al.,* 2002). Number of adventitious roots produced by micrcuttings was significantly different (p<0.0001) for different species (Table II). *I. cymosa* generated the highest number of adventitious roots (14.07 roots/microcutting) and this was followed by *T. heterophylla* (5.33 roots/microcutting). Rest of the species

Table I. Six selected tree species used in the study

Species	Common Name	Family
Agalaia korthalsii	Agalaia	*Meliaceae*
Aporusa globifera	Sebasa	*Euphorbiaceae*
Hopea odorata	Merawan Siput Jantan	*Dipterocarpaceae*
Ilex cymosa	Mensirah, Timah-timah	*Aquifoliaceae*
Lepisanthes rubiginosa	Mertajam	*Sapindaceae*
Tabebuia heterophylla	Tecoma	*Bignoniaceae*

Table II. Number of adventitious roots, root length and root dry weight of microcuttings of six tree species

Species	No. of adventitious roots	Root length (cm)	Root dry weight (g)
A. korthalsii	4.53	3.10	0.04
A. globifera	1.00	4.39	0.02
H. odorata	3.13	3.96	0.02
I. cymosa	14.07	2.41	0.02
L. rubiginosa	1.91	3.75	0.02
T. heterophylla	5.33	3.62	0.02
$LSD_{0.05}$	0.95	2.79	0.01

Table III. Number of leaves, leaf area and leaf dry weight of microcuttings of six tree species

Species	No. of leaves	Leaf area (cm^2)	Leaf dry weight (g)
A. korthalsii	3.6	52.18	0.29
A. globifera	4.1	46.98	0.19
H. odorata	4.0	24.25	0.13
I. cymosa	4.1	25.87	0.13
L. rubiginosa	9.3	34.34	0.14
T. heterophylla	4.9	10.63	0.06
$LSD_{0.05}$	0.1	10.82	0.06

Table IV. Regenerative leaf number of mother plant of six tree species

Species	Number of leaves
A. korthalsii	3.43
A. globifera	5.22
H. odorata	1.44
I. cymosa	4.89
L. rubiginosa	12.89
T. heterophylla	4.89
$LSD_{0.05}$	2.63

Table V. Correlation coefficients (r) between percentage of rooting on microcuttings with shoot length of mother plants, leaf number of mother plants and with the leaf of microcuttings

Species parameters	*A. korthalsii*	*A. globufera*	*H. odorata*	*I. cymosa*	*L. rubiginosa*	*T. heterophylla*
		Percentage of rooting of microcuttings and shoot length of mother plants				
r values	0.954	0.896	0.960	0.899	0.932	0.690
Significant levels	P<0.0001	P=0.0002	P<0.0001	P=0.0002	P<0.0001	P=0.0189
		Percentage of rooting of microcuttings and leaf number of mother plants				
r values	0.683	0.313	0.762	0.581	0.660	0.929
Significant levels	P=0.0205	P=0.3486	P=0.0064	P=0.0607	P=0.0269	P<0.0001
		Percentage of rooting of microcuttings and leaf number of microcuttings				
r values	0.955	0.898	0.981	0.971	0.869	0.860
Significant levels	P<0.0001	P=0.0002	P<0.0001	P<0.0001	P=0.0005	P=0.0007

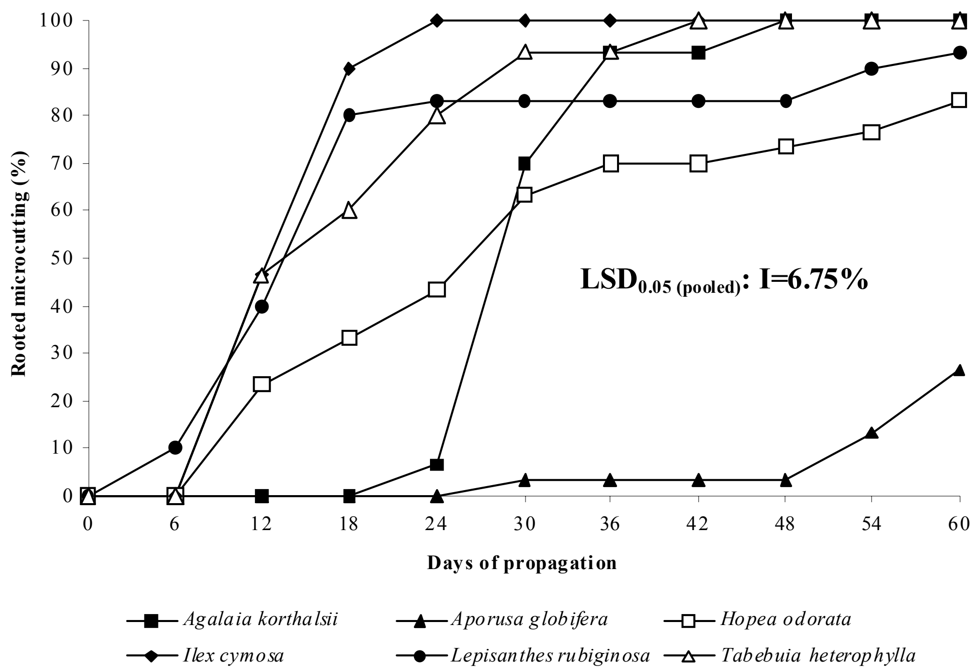
produced 1-5 roots per cutting. In contrast to the root number, root length for all species was similar as indicated by a non-significant difference among them.

A. korthalsii generated the heaviest roots (0.04 g/microcutting) and this was significantly different ($p<0.05$) from the weight of roots produced by other species (Table II). Heavier roots produced by *A. korthalsii*, despite of fewer roots and similar root length compared to the root produced by *I. cymosa*, indicated that roots of *A. korthalsii* were larger in diameter. This suggests that root growth of *I. cymosa* may involve a higher degree of cell differentiation, whereas root growth in *A. korthalsii* mainly relies on cell enlargement, which leads to larger roots in *A. korthalsii* than *I. cymosa*. Coarse roots observed in *A. korthalsii* give an indication that this species can penetrate hard soils and reach deep horizon (Gross, 1977), provide better anchorage and store more carbohydrate, nutrients and water (Boot & Mensink, 1990), thus could be advantageous under long drought stress. In contrast, plants with more fine roots as in the case of *Illex cymosa* are more active in the absorption of water and nutrients as well as, have higher growth rates and turnover (Wilcox *et al*., 2004).

Results of earlier studies indicated that seedlings with large root volume and/or high number first-order lateral roots (FOLR) have been shown directly correlated with reforestation success in both coniferous (Mc Millin & Wagner, 1995; Rose *et al*., 1997) and deciduous species (Thompson & Schultz, 1995; Schultz & Thompson, 1997; Dey & Parker, 1997). Strong linear association between root volume and/or FOLR would become important indicators for the performance and competitive ability of the seedlings once transplanted in the field. Jacobs *et al*. (2005) reported that seedling's root volume accounted for 91 and 77% of the variation in respective height and stem diameter of first year red oak seedlings. In lieu of these informations, *Illex cymosa*, *A. kothalsii* and *T. heterophylla* would have better field performance than the other species. The significance of having large root volume could be higher if the seedlings to be planted on low resource soils (Schreeg *et al*., 2005), which is a common scenario in urban areas of the tropics.

Shoot growth of microcuttings. Importance of leaves during rooting has been shown in a tropical tree species by Aminah (1996), who recommended that a single, whole leaf shall be retained on each cutting to obtain 85% rooting percentage for *H. odorata.* Among the species, *Lepisanthes rubiginosa* produced leaves at the highest rate (9.3 leaves/60 days, Table III). However, it is interesting to note that this species did not produce much roots (only 1.91 roots, Table II), suggesting that the roots and shoot growth might be competing for the same assimilates. Such phenomenon is

Fig. 1. Percentage of rooted cutting of six species of trees over a period of 60 days



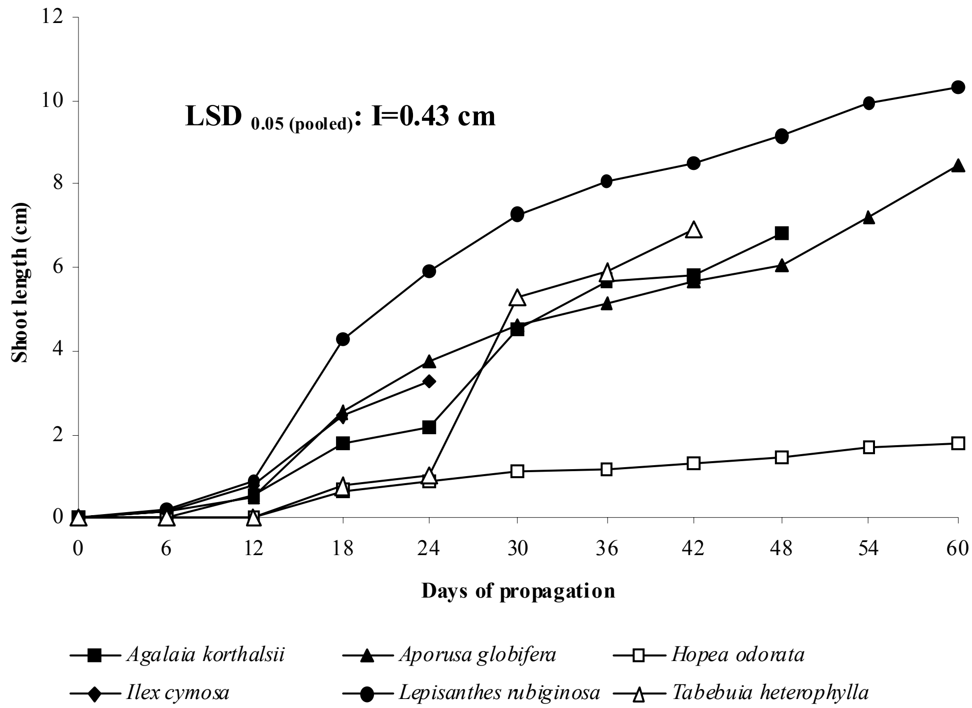
common for perennials (Bevington & Castle, 1985). Overall, leaf number was recorded as having a strong association with the rooting ability of species as shown by highly significant coefficients of correlation between the two parameters (r = 0.86-0.97, p<0.0001, Table V). Such a strong association is vital as leaves and roots are interdependent to ensure continuous production of assimilates and growth hormones and absorption of water and mineral nutrients that mainly occur in shoots (leaves) and roots, respectively. Leaf area produced by microcuttings differed significantly among species (p<0.0001, Table III). The greater the leaf area, the higher the leaf ability of plants to accept sunlight, hence increasing photosynthesis efficiency. *A. korthalsii* yielded the biggest leaf area with 52.18 cm^2/microcutting and this is followed by *A. globifera* (46.98 cm^2). However, root number of these two species was lower relative to *I. cymosa* (25.87 cm^2). Lower leaf area could be associated with its inherent characteristic. Leaf for *A. korthalsii* was the heaviest compared to other species and the difference was highly significant (p = 0.0004), which is parallel to the result for leaf area. Lower leaf dry weight of *L. rubiginosa* despite of higher number of leaves is indicating of its small size of leaves.

Shoot regeneration of mother plants. An interaction effect of species and days (p<0.0001) depicted the trend in shoot development overtime and varied among the six species (Fig. 2). *L. rubiginosa* continuously generated shoots faster than the other species to a maximum length of 10.33 cm.

Apparently, the growth of shoots play an important role in influencing the capability of the microcuttings to initiate new roots as shown by strong positive correlations between shoot length of mother plants and rooting percentage of all species (Table V). The coefficients of correlation (r) for most species (except *T. heterophylla*) ranged from 0.89 to 0.96 (p<0.0001). Such relationships may not exist at the earlier stage of propagation, as ability of root initiation at this stage may be more associated with conditions of mother plants. This might have contributed to variations in rooting performance of the microcuttings over period of the study.

Number of leaves produced by mother plants differed significantly (p<0.0001) for different species (Table IV). Plants experiencing a fast growth after harvesting of cuttings would yield more planting materials within given time and this is applicable for *L. rubiginosa.* Number of leaves produced by mother plants after the harvesting of microcuttings correlated positively with the rooting percentage of *A. korthalsii*, *H. odorata*, *L. rubiginosa* and *Tabebuia heterophylla* with their respective co-efficients of correlation (r) of 0.683 (p = 0.0205), 0.762 (p = 0.0064), 0.660 (p = 0269) and 0.929 (p<0.0001). Results recorded here are consistent with the finding of Itoh *et al.* (2002), who reported that species with a faster growth rate, measured as diameter growth had a better rooting ability. Low significant level of co-efficient of correlation (r = 0.581, p = 0.0607) between leaf number and rooting percentage observed for *I. cymosa* may be associated to a short duration of data collected as 100% rooting was achieved in 24 days. Different results could have been obtained if the data collection was extended as for other species. It is worth to note that both microcuttings and mother plants of *A. globifera* had produced substantial leaf area (Table III) and leaf number (Table IV), respectively, but this species had the lowest percentage of rooting (Fig. 1). This is confirmed by a non-significant correlation co-efficient between rooting percentage of microcuttings and leaf number

Fig. 2. Regenerative shoot length of mother plants of six species of trees over a period of 60 days after the harvesting of microcuttings



produced for this species (r = 0.313, $p = 0.3486$). Rapid initial generation of new leaves by the cuttings could be detrimental in some species since immature leaves are net importer and may compete for resources with developing roots (Bovington & Castle, 1985).

As the rooting ability of cuttings for most species is generally positively related to the regenerative growth, such relationship indicated that easy-rooted species would have the ability to recover from growth disturbance and physical damage (Guerrero-Campo, 2006). This characteristic is considered as an important trait in sustaining tree population in tropical forest as well as, in maintaining the aesthetic and functional roles of trees in urban landscape (Paciorek *et al*., 2000; Bond & Midgley, 2001; Guerrero-Campo *et al*., 2006). The regenerative leaf growth of mother plants and the rooting of their microcuttings was found to be highly species-specific suggesting that developing association between the two parameters as a principle is difficult and need further exploration.

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