NATURE IN SINGAPORE 2011 **4**: 383–392
Date of Publication: 14 December 2011
© National University of Singapore

AIR-LAYERING: A SUITABLE METHOD FOR MASS-PROPAGATING THE NATIONALLY CRITICALLY ENDANGERED *FAGRAEA AURICULATUM* JACK (GENTIANACEAE)

C. K. Yeo, B. Y. Q. Ng, P. X. Ng, K. Y. Chong, A. F. S. L. Lok, W. F. Ang, S. Y. Tan and **H. T. W. Tan**[*]
Department of Biological Sciences, National University of Singapore
14 Science Drive 4, Singapore 117543, Republic of Singapore
([*]Corresponding author: dbsttw@nus.edu.sg)

INTRODUCTION

Pelir musang, *Fagraea auriculata* Jack (Gentianaceae) is a lithophytic species that grows on coastal cliffs in Singapore. It can be found on several offshore islands south of the main island, namely on Pulau Sakijang Pelepah (Lazarus Island) (Fig. 1), Pulau Tekukor (Fig. 2), Pulau Biola (Fig. 3), and Pulau Pawai (largest specimens seen in the Republic ≈ 25 m tall, photos not available as the locality is militarily-protected). Presently, it is listed as being "Critically Endangered" (Chong et al., 2009), and therefore it is a species that deserves more conservation attention. *Fagraea auriculata* has a wide ecological amplitude, and is found in primary and secondary forests, in lalang savanna, scrubland, rocky coasts, and mangrove forests near estuaries, from sea-level up to an altitude of 1,900 m. It is distributed from south Indo-China, Thailand, Peninsular Malaysia, Borneo, Java, Sumatra, Bali, Moluccas, and the Philippines (Leenhouts, 1962).

In Singapore, the loss of habitat is probably the main threat to the continued existence of this species, where it is found predominantly in natural coastal habitats such as rocky shorelines. In Singapore, mangrove forest and other coastal habitats were exploited for charcoal in the late 19th century, while significant land reclamation started from the 1960s to add significant land area to the land-scarce nation, followed by the barraging of estuaries for freshwater reservoirs in the 1970s to satisfy its drinking water needs (Corlett, 1992). This loss of coastal habitats is not only restricted to just Singapore, but rather throughout much of its natural range, which includes the wider Indo-Malay-Philippines Archipelago, identified by Polidoro et al. (2010). This loss of coastal habitats in the region is likely to further exacerbate the decline of coastal *Fagraea auriculata* populations worldwide.

Fig. 1. *Fagraea auriculata* plant scrambling over a cliff face at Lazarus Island. (Photograph by: Hugh Tan Tiang Wah).

Fig. 2. Young plants and seedlings established on a rock face at Pulau Terkukor. (Photograph by: Hugh Tan Tiang Wah).

Fagraea auriculata is noted for its large yellow flowers up to 15 cm long and 30 cm across, and recommended as a native species with ornamental potential (Tan, 1995), thus its conservation under cultivation may be a viable option. Therefore, mass propagation of this species may have more than a narrow regional interest, and may have relevance to the ex situ conservation of the species. Through the representative collection of genotypes from wild populations, both the long term conservation of the species and the maintenance of a sustainable stock for horticulture are possible.

No conservation measures were previously suggested by Tan et al. (1995), probably owing to the slow growth of the species and the difficulty of rooting it from cuttings, though a viable vegetative propagation method from root cuttings was developed by HTWT (unpublished data) (Fig. 4). The main drawbacks to this simple method are the limited availability of thick exposed roots from wild and cultivated plants, as well as the long generation time (six months to a year) it takes for plantlets to grow to a size able to survive separation from the roots (WFA, unpublished data).

In the present study, we examine the effectiveness of air-layering, a method of vegetative propagation invented by the Chinese over 4,000 years ago (Huxley et al., 1992), as a quick way of mass-propagating the threatened species so that sizable propagules suitable for planting can be produced with regularity from plants of native stock brought under cultivation. This method has shown its potential for the propagation of a range of mangrove species from various families, such as *Exoecaria agallocha* (Euphorbiaceae), *Heritiera fomes* (Malvaceae), *Intsia bijuga* (Leguminosae), *Sonneratia apetala* (Lythraceae), *Xylocarpus granatum* (Meliaceae), *Rhizophora mangle* (Rhizophoraceae), *Avicennia germinans* (Acanthaceae), and *Laguncularia racemosa* (Combretaceae) (Carlton & Moffler, 1978; Kathiresan & Ravikumar, 1995; Eganathan et al., 2000).

Our optimism that this is also likely to work for *Fagraea auriculata* is based on the success obtained with another slow-growing cogeneric, *Fagraea berteroana* A.Gray ex Benth., which Whistler and Elevitch (2006) reported to root in about eight weeks from air-layering and be ready for planting out by about six months. Should this method work for our species, conservation of this native genotype can be further aided and supplemented by the growing of plants from seeds, easily obtainable from cultivated plants when they fruit.

Fig. 3. A large tree growing at the high tide mark on Pulau Biola. (Photograph by: Hugh Tan Tiang Wah).

Fig. 4. *Fagraea auriculata* plantlets growing from root cuttings. (Photograph by: Yeo Chow Khoon).

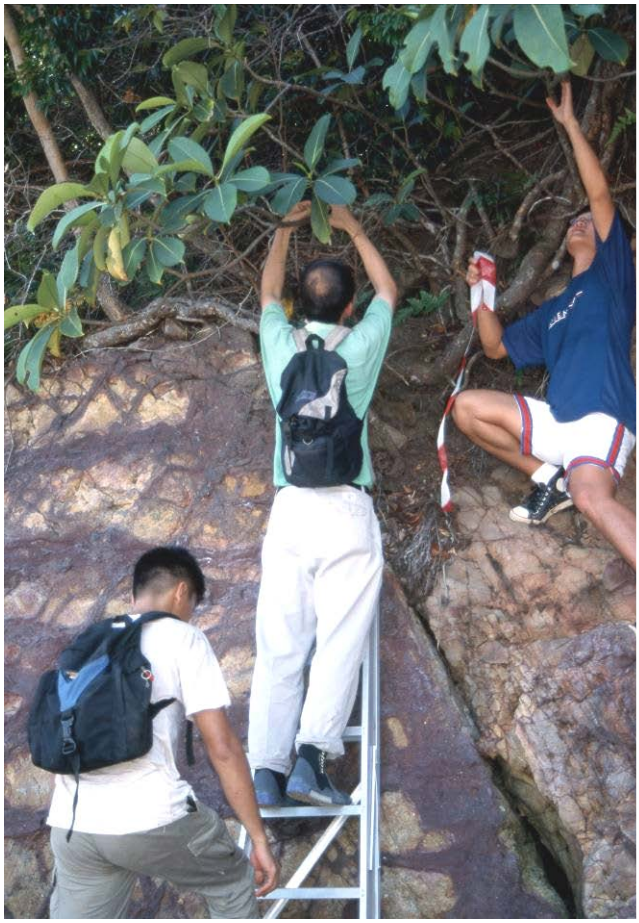
MATERIAL AND METHODS

Air-layering. **—** The species was brought under cultivation in the Native Plant Demonstration Garden at the National University of Singapore (NUS), located in the Department of Biological Sciences and along Research Link outside the Native Plant Nursery, largely through the extensive collection and propagation efforts of Tan Kai-xin and Morgany Thangavelu, as a continuation of the initial efforts of Adrian Loo, Lim Cheng Puay, and Soong Bee Ching (Fig. 5). Plants outside the NUS Native Plant Nursery were inspected for branches suitable for air-layering. Branches at least 20 cm long were identified as potential candidates. The number of leaves, shoot tips, and degree of branching were also recorded. The branch choice was constrained by the fact that no air-layering can be done along any branch distal to an air-layered one. The assignment of treatment was randomized such that the number of branches in each category was evenly split between the rooting gel versus control treatments. In all, 71 first-order branches and 68 second-order branches from 16 individuals were selected for the experiment.

The ringing method was used, in which a ring of outer stem tissue together with vascular cambium and phloem was completely removed (Fig. 6). The distal cut was then either coated with Clonex Red Gel, which contains 8 g L^{-1} indole-butyric acid (IBA) (Growth Technology, Australia), or left untreated as a control. The girdled stem was then wrapped in a moist mixture of three parts potting mix (Plantaflor Humus Verkaufs-GmbH, Germany) mixed with two parts of perlite in polyethylene sheets (Fig. 7). Aluminium foil was used to wrap over the entire air-layering to exclude light (Fig. 8). Of the 71 first-order branches, 35 were treated with Clonex Red Gel and 36 were not. Of the 68 second-order branches, 33 were treated with Red Gel and 35 were not.

The branches were checked weekly for signs of rooting seen through the polyethylene (Fig. 9). The date of the first visible rooting and the failure to root were recorded. The study was concluded after 20 weeks from the date the air-layering was conducted. Once the roots fill up the full length of the polyethylene-wrapped potting mix, the branches were severed below the ringed stem and planted in individual planting bags with potting mix. They were then hardened and grown until they are required for planting in the ground.

Statistical analysis. **—** Survival analysis was used to examine the success rate of rooting over time, where signs of rooting were treated as a "death" event. The Cox Proportional Hazards Regression Model was used to estimate the effects of (1) IBA treatment, (2) branch order, and (3) number of leaves at the commencement of the experiment on the



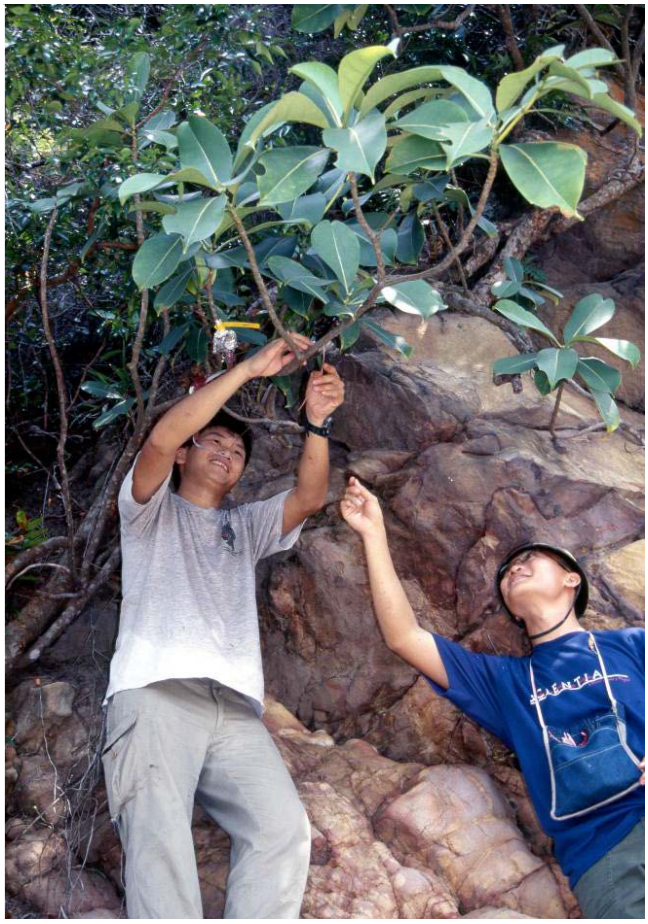


Fig. 5. Adrian Loo, Lim Cheng Puay and Soong Beng Ching carrying out air-layering in-situ on Lazarus Island. (Photograph by: Hugh Tan Tiang Wah).

Fig. 6. Air-layering with a ring of outer stem being removed (Photograph by: Yeo Chow Khoon).

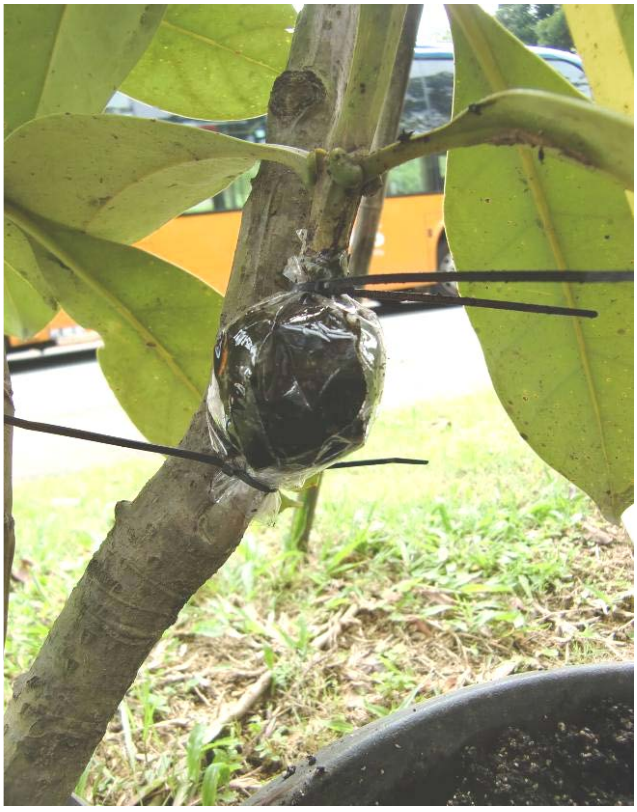


Fig. 7. Air-layering with the ringed portion wrapped in potting soil and perlite mixture under polyethylene sheet (Photograph by: Yeo Chow Khoon).

Fig. 8. Air-layering with the ringed portion wrapped in aluminium foil (Photograph by: Yeo Chow Khoon).

Fig. 9. Rooting seen through the polyethylene sheet (Photograph by: Yeo Chow Khoon).

hazard rate of rooting. We began with a full model with the three covariates listed as well as second-order interaction effects between the covariates, and used backward stepwise model selection to drop variables with the least significant p-values from the model while not violating model hierarchy (i.e., interaction terms were dropped before its first-order terms). When interaction terms with the number of leaves were included, we centered the number of leaves about its median for ease of interpretation of regression coefficients. The analyses were carried out in the statistical programming environment R version 2.13.0 (R Developmental Core Team, 2011) using the package "survival".

RESULTS

The success rate of rooting was extremely high, with 131 out of 139 branches (94.2%) showing signs of rooting by the end of 20 weeks. The median time of rooting (i.e., when 50% of branches showed signs of rooting) was five weeks (Fig. 10a). The mean time of rooting was 5.84 ± 2.25 weeks.

The final survival model included treatment, number of leaves, and an interaction between treatment and number of leaves as significant in explaining the hazard rate of rooting (Table 1). A branch with more leaves and treatment with auxin resulted in higher hazard rates of rooting, and the significant interaction term indicated that the effects of these two factors reinforced each other (Fig. 10b). This model, however, only explained 18.2% of variance.

An alternative model with treatment and branching order as significant covariates would have had an even lower explanatory value of 10.4%. Furthermore, second-order branches had more leaves than first-order branches (t-test, p-value < 0.001), and branching order did not have significant effects after controlling for the number of leaves. Thus, the alternative model was not considered.

Table 1. Final survival model retaining significant covariates using backward stepwise model selection. The number of leaves on each branch was centred about the median number of leaves of all branches.

Covariate	Coefficient (95% Confidence Interval)	p-value
Treatment	1.897 (1.336–2.694)	<0.001
No. of leaves	1.018 (1.006–1.030)	0.003
Treatment*No. of leaves	1.023 (1.000–1.047)	0.050

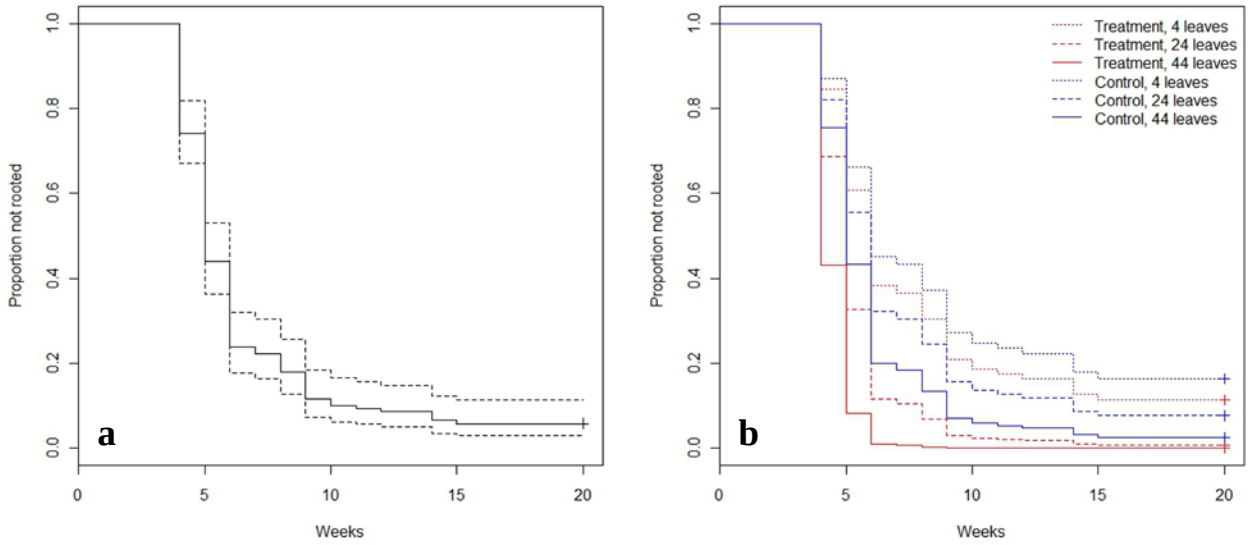


Fig. 10. a) Survival curve showing overall rates of rooting over the 20-week period. The 95% confidence intervals for the curve are given in broken lines. b) Expected survival curves generated from the final regression model to illustrate the main and interaction effects of increasing number of leaves and treatment on the hazard rate of rooting.

DISCUSSION

From the results, treatment with IBA clearly promotes root formation on the air-layerings. Endogenous and exogenous auxins play an important physiological role in adventitious root formation (Blakesley et al., 1991; Blakesley & Chaldecott, 1993). Yet interestingly, the number of leaves also had a significant effect, as did the interaction between the two factors. This is not unexpected as aside from shoot apices, leaves are also sources of endogenous auxin and indole-3-acetic acid (IAA) (Ljung et al., 2001).

The results of the present study clearly show the dependence of rooting on the number of leaves on the air-layered branches, but less so on the order of branching and thus the number of shoot apices. This suggests that leaves are more significant than the shoot apices in promoting root formation by virtue of being endogenous auxin sources. The interaction effect between the two factors further suggests that the number of leaves present on an air-layered branch raised the endogenous level of auxin closer to the rooting threshold, thus facilitating rooting promoted by the application of exogenous IBA. However, ultimate rooting success did not appear to be dependent on the application of IBA, which just sped up the rooting. Rooting appears to be promoted by the leafiness of the branches. Thus, the most economical method of air-layering would be just to select the leafiest branches for air-layering and omit the IBA.

The present method is much more effective than air-layering using the slit method, which is made from a deep cut on one side of the stem kept open with a tooth pick, and with auxin application, following the method of Adrian Loo, Lim Cheng Puay, and Soong Bee Ching (AFSL, unpublished data). The lower rooting rate suggests that the accumulation of auxin is less efficient when the cambial connection is not completely severed. Morris and Thomas (1978) have demonstrated the basipetal transportation of auxin by the cambium and the partially differentiated secondary vascular tissue, but not in mature xylem or phloem in the common pea, *Pisum sativum* (Fabaceae). Thus it is postulated that remnant cambial connection could abolish the threshold auxin accumulation needed for root differentiation in *Fagraea auriculata*. This also seems to be corroborated by our observation that intact branches which failed to root invariably showed some degree of formation of bark, which is assumed to have underlying cambial connections.

A simplified air-layering method not requiring the packing of the ringed branch in potting mix was also attempted on a small number of branches on plants not used in the experiment in the Native Plant Demonstration Garden. It involved only ringing the branches and wrapping the cuts in plastic film, with IBA applied or withheld (Fig. 11a). Rooting was observably slower, taking at least about three months from the start of treatment, while IBA application appeared to make no difference. The roots appeared to be tolerant of desiccation, and were able to grow normally exposed in the shade (Fig. 11b). This is probably an adaptation to the physiologically dry, lithophytic conditions that this species grows under. Though the sample size was too small to draw any statistically robust conclusion, it seems that this simplified method could be used to obtain propagules where time, location, or other resources do not allow proper air-layering to be performed. This is often the constraint faced in Singapore as the wild plants are often found on sea cliffs or rocky islands with low accessibility.

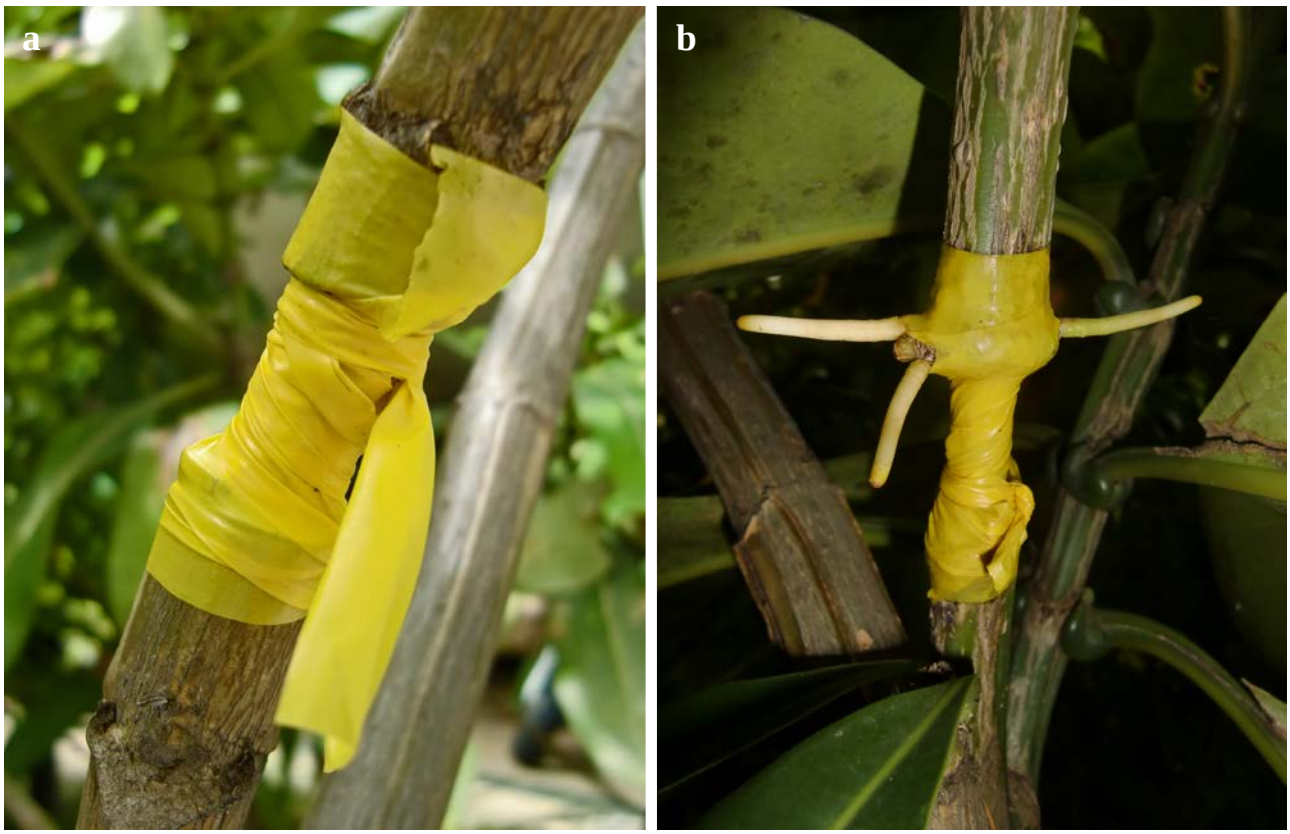


Fig. 11. a) A simplified air-layering method done without potting soil, with cut wrapped under plastic film. b) Root growing normally exposed on air-layered branch (Photograph by: Yeo Chow Khoon).

The rooted branches produced in the experiment were planted out at various sites within the Kent Ridge Campus of the National University of Singapore as a part of the ongoing programme to replace exotic plant species with native species to green the grounds and increase faunal biodiversity (Fig. 12). Though the time until planting out was not tracked systematically, being dependent on demands of the above-mentioned programme, most plantings were carried out well before the 20-week duration of the air-layering experiment, with the remaining rooted branches not planted out only because of the lack of suitable planting sites. This compares quite well against the eight weeks to rooting and six months needed for hardening needed for *Fagraea berteroana* (Whistler & Elevitch, 2006).

Havens et al. (2006) noted that botanical gardens and arboreta have expanded their roles from maintaining horticultural collections to including plant conservation and providing a framework for assessing their success. We agree with their emphasis, but would further extend the role of conserving native plant species to urban plantings as well, thus making whole towns, cities, or even nations participate in the ex situ conservation effort centred around these traditional institutions. Ang et al. (2010) articulated such a goal for Singapore. We believe that it could be encouraged by ensuring that native species, preferably from representative and genetically viable native stocks, are available for horticultural or landscaping applications. In a small way, our work has helped to make the future of an endangered species more secure.

CONCLUSIONS

A viable method has been found to mass-propagate *Fagraea auriculata*, an endangered slow-growing species. Rooting occurs on average about six weeks after air-layering, and rooting success rate was found to be high. Leafy branches were the best candidates for the vegetative propagation method, while IBA application was not strictly necessary for rooting. A small trial also showed that air-layering could be simplified to just ringing the stems and wrapping the cut in plastic film, vastly increasing the applicability in the field when resources are limiting.

From 16 plants, 131 air-layerings were obtained, averaging about eight air-layerings per plant, rooting in about six weeks and ready for planting within 20 weeks. The cultivated source plants were about 1.5–2 m tall, small relative to wild individuals, which are often 5 m or taller (pers. obs.), and up to 20 m tall (Leenhouts, 1962). The potential number of plants that can be produced from these larger wild individuals is likely to be considerably more. Even with a regeneration window period of two years between sets of air-layering, a significantly steady supply of propagules could be produced using this method. Besides satisfying the demand for the ornamental species, it will aid in ex situ conservation by ensuring this species' long-term survival.

Fig. 12. Hardened plants planted out on a slope in front of the National University of Singapore Kent Ridge Campus University Hall building as a part of the ongoing programme to replace exotic plant species with native ones. (Photograph by: Yeo Chow Khoon).

ACKNOWLEDGEMENTS

We would like to thank Chung Yi Fei who provided invaluable assistance in editing the figures. We would like to express our gratitude to Tan Kai-xin for her effort in establishing the native stock plants of *Fagrea auriculata* from propagules collected from off-shore islands of the Republic of Singapore in the NUS Native Plant Nursery, at the National University of Singapore, with considerable assistance from Morgany d/o Thangavelu. This project can succeed because of the pioneering efforts of Adrian Loo Hock Beng, Lim Cheng Puay, and Soong Beng Ching in trying to propagate the species.

LITERATURE CITED

Ang, W. F., R. T. Corlett, A. F. S. L. Lok, B. Y. Q. Ng, S. M. Suen, H. T. W. Tan, P. Y. Tan, & T. M. Tertilt, 2010. *A Field Guide to the Native Garden @ HortPark: An Urban Oasis of the Native Flora and fauna of Singapore*. Centre for Urban Greenery and Ecology (National Parks Board) and National University of Singapore, Singapore. 124 pp.

Blakesley, D. & M. A. Chaldecott, 1993. The role of endogenous auxin in root initiation. Part II: Sensitivity and evidence from studies on transgenic plants. *Plant Growth Regulation*, **13**(1): 77–84.

Blakesley, D., G. D. Weston, G. D. & J. F. Hall, 1991. The role of endogenous auxin in root initiation Part I: Evidence from studies on auxin application, and analysis of endogenous levels. *Plant Growth Regulation*, **10**(4): 341–353.

Carlton, J. M. & M. D. Moffler, 1978. Propagation of mangroves by air-layering. *Environmental Conservation*, **5**(2): 147–150.

Chong, K. Y., H. T. W. Tan, & R. T. Corlett, 2009. *A Checklist of the Total Vascular Flora of Singapiore: Native, Naturalised and Cultivated Species.* Raffles Museum of Biodiversity Research, National University of Singapore, Republic of Singapore. 273 pp.

Corlett, R. T., 1992. The ecological transformation of Singapore, 1819–1990. *Journal of Biogeography*, **19**(4): 411–420.

Eganathan, P., C. S. Rao & A. Anand, 2000. Vegetative propagation of three mangrove tree species by cuttings and air layering. *Wetlands Ecology and Management*, **8**(4): 281–286.

Havens, K., P. Vitt, M. Maunder, E. O. Guerrant Jr. & K. Dixon, 2006. Ex situ plant conservation and beyond. *BioScience*, **56**(6): 525–531.

Huxley, A., M. Griffiths & M. Levy (eds.), 1992. *The New Royal Horticultural Society Dictionary of Gardening*, *Volume 4*. The Macmillian Press Limited, London. 854 pp.

Kathiresan, K. & S. Ravikumar, 1995. Vegetative propagation through air-layering in two species of mangroves. *Aquatic Botany*, **50**(1): 107–110.

Leenhouts, P. W., 1962. Loganiaceae. *Flora Malesiana*. Series I, **6**: 293–387.

Ljung, K., R. P. Bhalerao & G. Sandberg, 2001. Sites and homeostatic control of auxin biosynthesis in *Arabidopsis* during vegetative growth. *The Plant Journal*, **28**(4): 465–474.

Morris, D. A. & A. G. Thomas, 1978. A microautographic study of auxin transport in the stem of pea seedlings (*Pisum sativum* L.). *Journal of Experimental Botany*, **29**(1): 147–157.

Polidoro, B. A., K. W. Carpenter, L. Collins, N. C. Duke, A. M. Ellison, J. C. Ellison, E. J. Farnsworth, E. S. Fernando, K. Kathiresan, N. E. Koedam, J. H. Primavera, S. G. Salmo III, J. C. Sanciangco, S. Sukardjo, Y. Wang & J. W. H. Yong, 2010. The loss of species: Mangrove extinction risk and geographic areas of global concern. *PLoS ONE*, **5**(4), 1–10.

R Developmental Core Team, 2011. *R: A Language and Environment for Statistical Computing*. The R Foundation for Statistical Computing, Vienna, Austria.

Tan, H. T. W., Ali bin Ibrahim & R. T. Corlett, 1995. Plant portraits and data sheets: Seed plants. In: Tan, H. T. W. (ed.), *A Guide to the Threatened Plants of Singapore*. Singapore Science Centre, Singapore. Pp. 33–114.

Whistler, W. A. & C. R. Elevitch, 2006. Fagraea berteroana *(pua kenikeni).* Species Profiles for Pacific Island Agroforestry, Version 3.2, Apr.2006. Pp. 1–11. http://www.traditionaltree.org. (Accessed 22 Sep.2011).