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INDOLE-3-BUTYRIC ACID (IBA) AND LEAF TRIMMING REGULATE THE ADVENTITIOUS ROOT FORMATION OF STEM CUTTINGS DERIVED FROM MATURE *AQUILARIA CUMINGIANA*

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Aquilaria is known to produce agarwood, but its natural population is declining. Apart from being recalcitrant, not all *Aquilaria* trees are agarwood producers, hence yield is not guaranteed in plantations established from seedlings originated from seeds. Since cloning can produce progenies with similar genetic quality as parent trees, this study evaluated the effect of various leaf trimmings, indole-3-butyric acid (IBA) doses, and their interaction in rooting induction of cuttings from mature *Aquilaria cumingiana*. Analysis of variance revealed a significant ($p < 0.001$) increase in most of the rooting parameters with increasing leaf surface areas (except on shoot length) and IBA concentrations (aside from shoot length, percentage of survival and percentage of callus). Interaction between IBA (1000 ppm) and cuttings, with half of the leaf surface trimmed, detected the highest rooting percentage (46.67%, $p < 0.001$), number of roots (1.16, $p < 0.05$), average root length (14.05 mm, $p < 0.001$), number of secondary roots (3.49, $p < 0.001$) and length of the longest roots (18.56 mm, $p < 0.001$). Correlations among root traits ($r^2 = 0.11–0.95$), between rooting trait and cutting size ($r^2 = -0.09–0.09$), and among cutting sizes ($r^2 = 0.69–0.96$) were found to be highly significant ($p < 0.001$). The results showed potential to produce high agarwood yielding *A. cumingiana* using low-cost cloning technique.

Keywords: Agarwood, true-to-type cloning, maturation, low-cost, leaf lamina

INTRODUCTION

Aquilaria cumingiana of the Thymelaeaceae family is among the three identified agarwood-producing tree species in the Philippines together with *A. malaccensis* and *A. filaria* (CITES 2004, Lee & Mohamed 2016, Piñon & Reyes 2021). Several studies have been made on this genus owing to its special byproduct, which is known internationally as 'gaharu' or 'agarwood' (Tan et al. 2019, Naziz et al. 2019). These include phenology, vegetative and reproductive biology, ecology, distribution, uses, origin, initial growth and survival in the natural forest, and seedling production (Soehartono et al. 2002, CITES 2004, Lee & Mohamed 2016, PROSEA 2019).

Agarwood is a resinous material made from pathological processes caused either by biotic or abiotic stressors (Tan et al. 2019, Naziz et al. 2019). Despite being ugly and diseased, agarwood infected tree is highly sought in the wild and considered more expensive than gold (Xu et al. 2013). The rising global demand for agarwood is further observed with increasing variations in its utility due to the advancement in science and industrial technology (Suharti et. al. 2011). Other economic potentials of the agarwood include medicines, incense for religious ceremony and sculpture for interior decorations (Saikia et al. 2013, Xu et. al. 2013). However, not all *Aquilaria* trees are agarwood producers. Previous studies show that only 10 percent of them are capable to produce agarwood, yet more than 90 percent of *Aquilaria* trees, particularly in the wild, are suspectedly felled for agarwood collection (Gibson 1977, Soehartono et al. 2002).

Parallel to the increasing global demand is the rising need to increase the supply of agarwood. Previous researchers have tried artificial induction, but this technique is oftentimes tedious with various hit and miss issues (Blanchette & Heuveling 2009, Tan et al. 2019). To address this problem, planting materials from identified agarwood-producing mother trees should be used for plantation establishment. Several countries are into *Aquilaria* plantations such as Indonesia, Thailand, Malaysia, Cambodia and Vietnam (Barden et al. 2000, Lata 2007). In the Philippines, there is no large-scale tree plantation despite having nine (9) naturally growing *Aquilarias*, with the majority identified as endemics (Barden et al. 2000, CITES 2004, The Plant List 2013, Lee & Mohamed 2016). Apart from rampant harvesting, felling and hacking of even the non-infected trees, over-collection of naturally grown seedlings has resulted in the rapid decline of the wild population (Soehartono et al. 2002, Lee & Mohamed 2016, Esyanti et al. 2019). This problem is further exacerbated by the recalcitrant characteristics of the seeds with low viability and poor germination (Kundu & Kachari 2000).

Alternative propagation of vegetative means should be considered for *Aquilaria*. Unfortunately, true-to-type cloning is both hard and challenging as many forest trees are considered hard to root (Lata 2007, Pijut et al. 2011, Zobel & Talbert 2003). This is because maturation affects the rooting ability as it declines with the increasing age of the tree (Trueman 2006, Wendling et al. 2014). As such, many of the planting materials produced for timber production and the greening program in the Philippines, that used this technique, were collected from shoots of young and juvenile seedlings (Patricio et al. 2006, ERDB 2017).

Fortunately, initial findings suggest that rooting induction of *Aquilaria* is feasible, particularly of cuttings derived from young seedlings (Borpuzari & Kachari 2018, Piñon & Reyes 2021, Piñon et al. 2022). This emphasises the ability to mass-produce the species using young and juvenile cuttings from seedlings derived from high agarwood yielding mother trees. Although such technique will increase the available seedlings, true-to-type cloning should be carried out to ensure that the seedlings planted, just like the mother trees, are susceptible to infection and produce agarwood.

While rooting induction of mature stem cutting is not new, it has been tried only in few high valued forest tree species such as *Paulownia fortunei*, *Eucalyptus benthamii* and *Pterocarpus indicus* (Stuepp et al. 2014, Baccarin et al. 2015, Piñon et al. 2021). Since wild population is known to produce agarwood with higher quality than those from plantations, true-to-type cloning will not only conserve and protect them, but will ensure that the seedlings planted are high-agarwood yielding (Chakrabarty et al. 1994, Barden et al. 2000). Study to optimise the production of *Aquilaria* trees using cuttings from mature trees could revolutionise the agarwood industry in the country. As such, this study was carried out to determine the ability to induce roots in mature cuttings derived from the wild population of *A. cumingiana* using a simple cloning protocol and low-cost rooting chamber.

MATERIALS AND METHODS

Planting material

Stem cuttings were collected from 50 mother trees of *A. cumingiana* within Quezon Protected Landscape (QPL) in Quezon Province, Philippines in September 2021. All mother trees identified at the site were collected with 1–3 branches from the base of each tree. During collection, several mother trees were found to be flowering and fruiting. This phenology coincides with the study reported in East Kalimantan, Indonesia where most of the *Aquilaria* species produced flowers and fruits between September and December (Soehartono & Newton 2001). To ensure the natural proliferation of the species, only twigs and branches without fruits and flowers were collected with cuttings. The global positioning system (GPS) readings were recorded but were not disclosed here for the purpose of protecting the wild population from illegal collection. Total height (height from the ground up to the topmost portion, measured using meter tape) and root collar diameter (10 cm from the ground, measured using diameter tape) were also gathered and used to compute the volume (Table 1).

Each twig was separated into nodal cuttings with one leaf per cutting of about 2.15 cm long. Four (4) leaf trimmings were used such as leafless [(LL – ± 0 cm^2) had 100% of the leaf removed], one-fourth [(OF – ± 5.97 cm^2) had 75% of the leaf surface trimmed], one-half [(OH – ± 6.79 cm^2) had 50% of the leaf surface trimmed], and three-fourth [(TF – ± 8.59 cm^2) had 25% of the leaf surface removed]. Processed nodal cuttings were sterilised by soaking in Dithane M-45 fungicide solution with six tablespoonfuls per 16 L of water for 10 min. Cuttings were bundled (45 pieces per treatment) using a rubber band before immersing the basal portion in 0, 500 and 1000 ppm of indole-3-butyric acid (IBA) for 30 min.

Table 1. Tree measurements of 50 mother trees of *A. cumingiana* in Quezon Protected Landscape (QPL)

Tree no.	Root collar diam (cm)	Total height (m)	Volume (m^3)
1	1.3	0.7	0.0001
2	3.4	1.1	0.0010
3	4.7	3.0	0.0052
4	6.5	4.1	0.0136
5	2.2	1.7	0.0006
6	3.5	3.5	0.0034
7	2.0	1.1	0.0003
8	1.6	1.1	0.0002
9	3.0	0.8	0.0006
10	1.9	1.0	0.0003
11	3.7	2.4	0.0026
12	4.7	2.2	0.0038
13	1.5	0.8	0.0001
14	1.5	0.6	0.0001
15	2.5	2.7	0.0013
16	3.6	2.7	0.0027
17	1.5	1.1	0.0002
18	3.5	3.2	0.0031
19	3.0	3.0	0.0021
20	2.6	2.5	0.0013
21	2.1	1.4	0.0005
22	2.4	2.1	0.0010
23	2.7	2.1	0.0012
24	4.5	1.4	0.0022
25	2.5	1.9	0.0009
26	1.9	1.2	0.0003
27	1.2	1.2	0.0001
28	6.0	3.8	0.0107
29	2.8	1.4	0.0009
30	2.6	1.2	0.0006
31	3.0	1.8	0.0013
32	1.7	1.8	0.0004
33	2.0	1.3	0.0004
34	2.7	2.5	0.0014
35	3.4	2.6	0.0024
36	4.5	3.2	0.0051
37	5.1	3.7	0.0076
38	5.7	2.4	0.0061
39	1.4	0.8	0.0001
40	2.1	1.4	0.0005
41	2.0	3.4	0.0011
42	2.6	1.8	0.0010
43	1.4	2.1	0.0003
44	3.3	1.8	0.0015
45	5.7	4.4	0.0112
46	2.0	1.7	0.0005
47	2.9	1.7	0.0011
48	2.0	0.8	0.0003
49	1.5	0.9	0.0002
50	7.4	5.6	0.0241
50	**149.3**	**102.7**	**0.128**

Planted cuttings were profusely watered thrice a week using a 16-L stainless knapsack sprayer. Both rooted and unrooted cuttings were harvested after 72 d.

Rooting chamber

The study used the raised concrete bed in the Forests and Agroforests Nursery Learning Laboratory (FANLL) at the Institute of Renewable Natural Resources, College of Forestry and Natural Resources, University of the Philippines, Los Baños, Laguna. Using the concept of the improvised rooting chamber used by others, the concrete bed was made into a makeshift rooting chamber (Leakey et al. 1990, Piñon & Reyes 2021, Piñon et al. 2022).

The rooting chamber had a dimension of 0.8 m × 1 m × 2 m. One layer of stones (2.5–6 cm) was laid at the bottom part of the concrete bed. On top of it, a layer of black plastic net was laid before adding the gravels (0.5–1.0 cm) to a total depth of about 5 cm. Another layer of black net was placed on top of the gravel before adding the washed river sand (about 20 cm depth). Then, a galvanised welded wire mesh (1 m × 2 m) with 2 in × 2 in square opening and 0.12 in diameter was painted (quick-drying enamel) and installed on top of the rooting bed. Lastly, a single layer of polyethylene plastic film Anti-UV (gauge number 8), which measured 2 m × 3 m was installed on top of the wire mesh to cover the rooting chamber. Daily temperatures ranging from 26–30 °C during the rooting period were recorded using a thermometer. (0–110 °C).

Data analysis

A 3 x 3 factorial with two treatments in a complete randomised design was used. Treatments were IBA of various concentrations [0 ppm (distilled water), 500 ppm and 1000 ppm] and different leaf trimmings [leafless (0%), one-fourth (25% leaf surface area), one-half (50% leaf surface area) and three-fourth (75% leaf surface area)] with a total of nine (9) possible treatment combinations replicated three times. A total of 540 nodal cuttings were planted with 15 cuttings per replication and 45 cuttings per treatment.

All planted leafless cuttings did not survive, hence they were excluded prior to the statistical analysis. A total of eight (8) rooting parameters were used such as percentage of rooting [PR — total number of rooted cuttings over the total number of cuttings planted multiplied by 100), percentage of survival [PS — total number of survived (rooted and callused) cuttings over the total number of cuttings planted multiply by 100), percentage of callus (PC — total number of callused cuttings over the total number of cuttings planted multiply by 100), number of roots (NR — total number of roots produced per cutting), average root length (ARL — average length of produced roots per cutting, measured using a ruler), number of secondary roots (NSR — number of secondary roots produced per cutting), length of the longest root (LLR — longest root produced per cutting, measured using a ruler), and shoot length (SL — length of shoots produced per cutting). Cuttings with at least one adventitious root ($\geqslant$ 1 mm) produced were measured (Amri et al. 2010). Collected data were arranged, organised and transformed (square root) for normality distribution using Microsoft Excel Office 365 before statistical analysis (Osborne 2002). Correlation coefficients were calculated using the 'metan' packages in R. All statistical computations including analysis of variance (ANOVA) followed by post-hoc test using the Duncan Multiple Range Test (DMRT) were performed using R-Statistics software version 4.0.5. of 2021.

RESULTS AND DISCUSSION

Leaf effects on rooting

Analysis of variance revealed highly significant ($p < 0.001$) variations in all rooting parameters tested except on shoot length (0.18 mm, $p > 0.05$) due to varying leaf surface area. Rooting responses with the overall mean values in percentage of rooting (21.48%), percentage of survival (54.07%), number of roots (0.45), average root length (5.44 mm), number of secondary roots (1.21), length of longest root (6.96 mm) and percentage of callus (22.47%) were all found to be highly significant ($p < 0.001$) (Figure 1). Both cuttings with wider leaf laminas (OH and TF) recorded the highest percentage of rooting. Similarly, they also achieved the highest overall mean values in terms of the number of roots. On the other hand, cuttings with the smallest leaf surface area (OF) achieved the lowest value both in terms of percentage of rooting and the number of roots (Figure 4). Meanwhile, rising overall mean values were detected with increasing leaf surface area in the case of percentage of survival, shoot length and percentage of callus. The mean comparison of all treatments showed that all leaf surface treatments were significantly different from each other, except for shoot length. Cuttings with the lowest leaf lamina (OF) differed significantly from the others with wider leaf surface areas (OF and OH) in all root parameters assessed, aside from shoot length

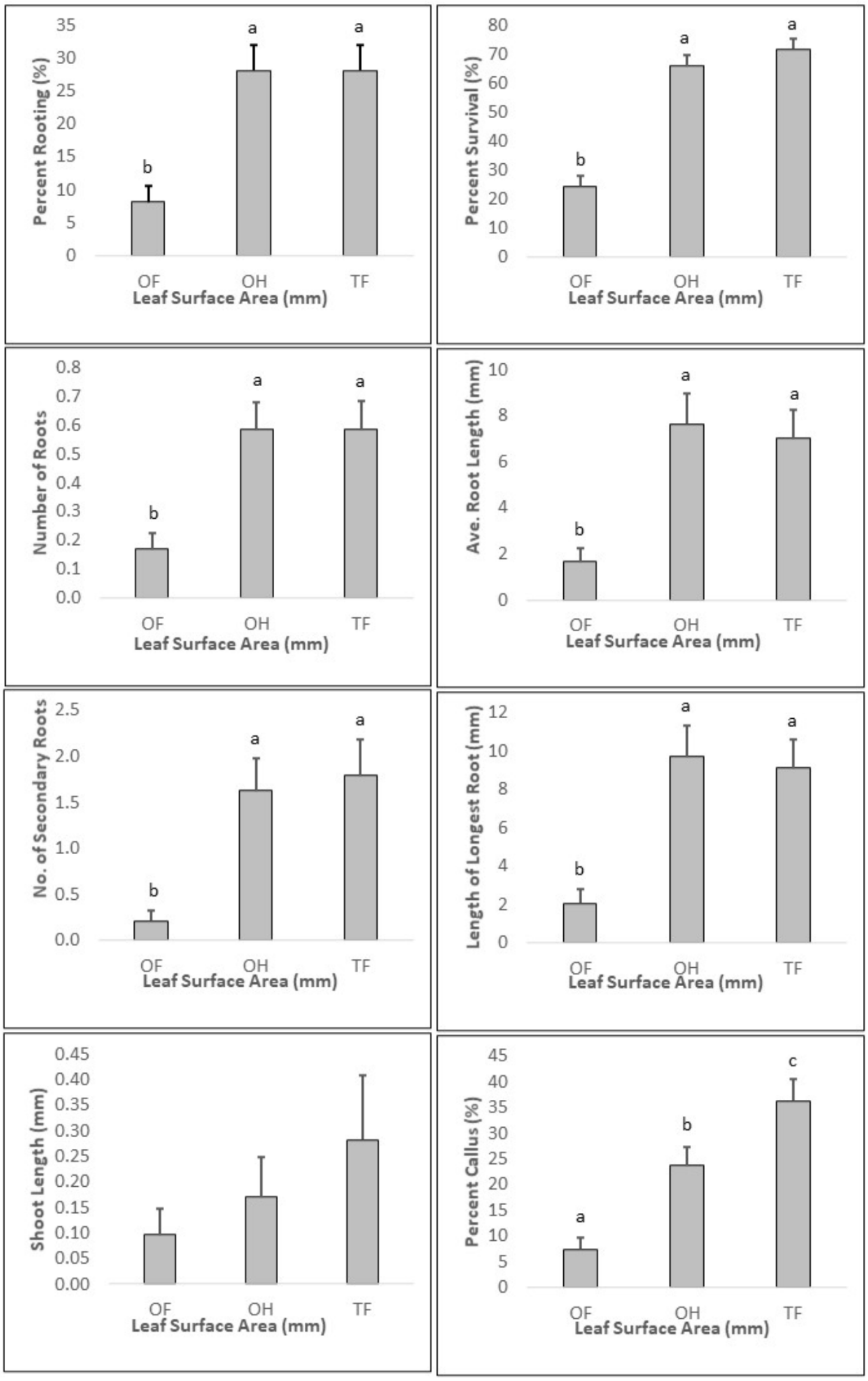

Figure 1 Effect of varying leaf surface area on vegetative propagation of cuttings from mature *A. cumingiana;* means followed by the same letter(s) are not significantly different at 5% level according to Duncan's Multiple Range Test

and percentage of callus. Finally, a significant increase in percentage of callus with increasing leaf laminas was obtained.

A significant variation in percentage of rooting as influenced by varying leaf areas was detected in the present study. This result conforms with previous vegetative propagation studies using various high-valued tree species (Ky-Dembele et al. 2011, Leakey 2014). However, the effect on rooting may vary using cuttings taken from the same species of different ages. In the present study, the variations in seven (7) out of eight (8) parameters assessed were found to be statistically significant after 72 days. Interestingly, previous studies with cuttings derived from young seedlings of *A. cumingiana* that tested the effect of varying leaf surface areas achieved different results. For instance, among five (5) root parameters assessed, only the number of secondary roots had generated a significant effect with leaf trimming application after 50 days (Piñon & Reyes 2021). Meanwhile, with intact leaf laminas, the varying leaf surface areas, due to differences in leaf numbers, generated significant effects in all root parameters evaluated after 63 days (Piñon et al. 2022). These observations may suggest two possible implications — the presence of photosynthetically active leaves with optimal leaf area, and the time the cuttings stayed in the propagation chamber seem to affect the rooting due to variable leaf laminas. The leaf is affected by various factors (stomatal density, leaf morphology, leaf thickness and age of the leaf), hence the balance between photosynthesis and transpiration is crucial at this stage (Leakey 2004). In addition, cuttings with actively photosynthesising leaves affect the supply of carbohydrates to the intensive metabolic processes related to adventitious root formation (Leakey 2004, Tombesi et al. 2015). As such, severed cuttings with optimal leaf areas that are capable to photosynthesise actively while planted in a propagation chamber are crucial during root initiation. On the other hand, the duration that severed cuttings are planted in the propagation chamber may also influence the variation in rooting as the time for root initials to develop, after cuttings are planted in propagation chambers, varied within and between species (Hartmann et al. 2011). Hence, rooted cuttings that stayed in the propagation chamber for a much longer period obtained more significant variations, particularly in the case of rooting of juvenile cuttings from young seedlings.

This study recorded the highest percentage of rooting (28.15%) due to varying leaf laminas using cuttings from mature trees. In contrast, a relatively higher percentage of rooting was reported using cuttings from young seedlings. With trimmed leaf lamina, a previous study achieved higher percentage of rooting (67.19%) (Piñon & Reyes 2021), yet much higher using untrimmed leaves (83.62%) (Piñon et al. 2022). Perhaps, the presence of other endogenous plant factors, aside from phytohormone could possibly affect the rooting responses of cutting, particularly when the cells' age increases. After comparing the shoot morphology between mature and juvenile shoot of *Ficus pumila*, Hartmann et al. (2011) postulated that the presence of continuous 'sclerenchyma ring' or 'sclereids', as it gets older, could possibly block the root emergence, hence may affect the formation of adventitious roots. However, although the species studies had 'sclereids' (Hartmann et al. 2011), the presence of such lignified cells in the subject tree could not be reported as plant anatomy was not undertaken in this study.

Phase change or maturation, which can be controlled through rejuvenation may also play a vital role to achieve better success in rooting (Wendling et al. 2014). For instance, across averages, the present study had recorded a percentage of rooting of 21.48%. The value is higher than those recorded in other species that used cuttings from older trees like *Khaya senegalensis* (5%), but lower than *Pterocarpus indicus* (45.83%) and *Paulownia fortunei* (58.12%) (Ky-Dembele et al. 2011, Stuepp et al. 2014, Piñon et al. 2021). However, it is important to note that cuttings used in the study were unrejuvenated. Since the response to auxin is known to decline in rooting competence as sources of cuttings gets older, the present study detected lower rooting success compared to other studies that also used mature trees but underwent rejuvenation prior to rooting induction (Wendling et al. 2014, Bannoud & Bellini 2021). As a result, lesser rooting success was achieved in the study, unlike with *P. fortunei* and *P. indicus* (Stuepp et al. 2014, Piñon et al. 2021).

Effect of varying indole-3-butyric acid (IBA) concentrations in rooting

Highly significant differences ($p < 0.001$) were recorded in most of the rooting parameters evaluated due to varying IBA concentrations, except for percentage of survival, shoot length and percentage of callus (Figure 2). A significant increase in overall mean values with rising IBA concentrations was recorded in percentage of rooting, number of roots, number of secondary roots and length of the longest root. Cuttings

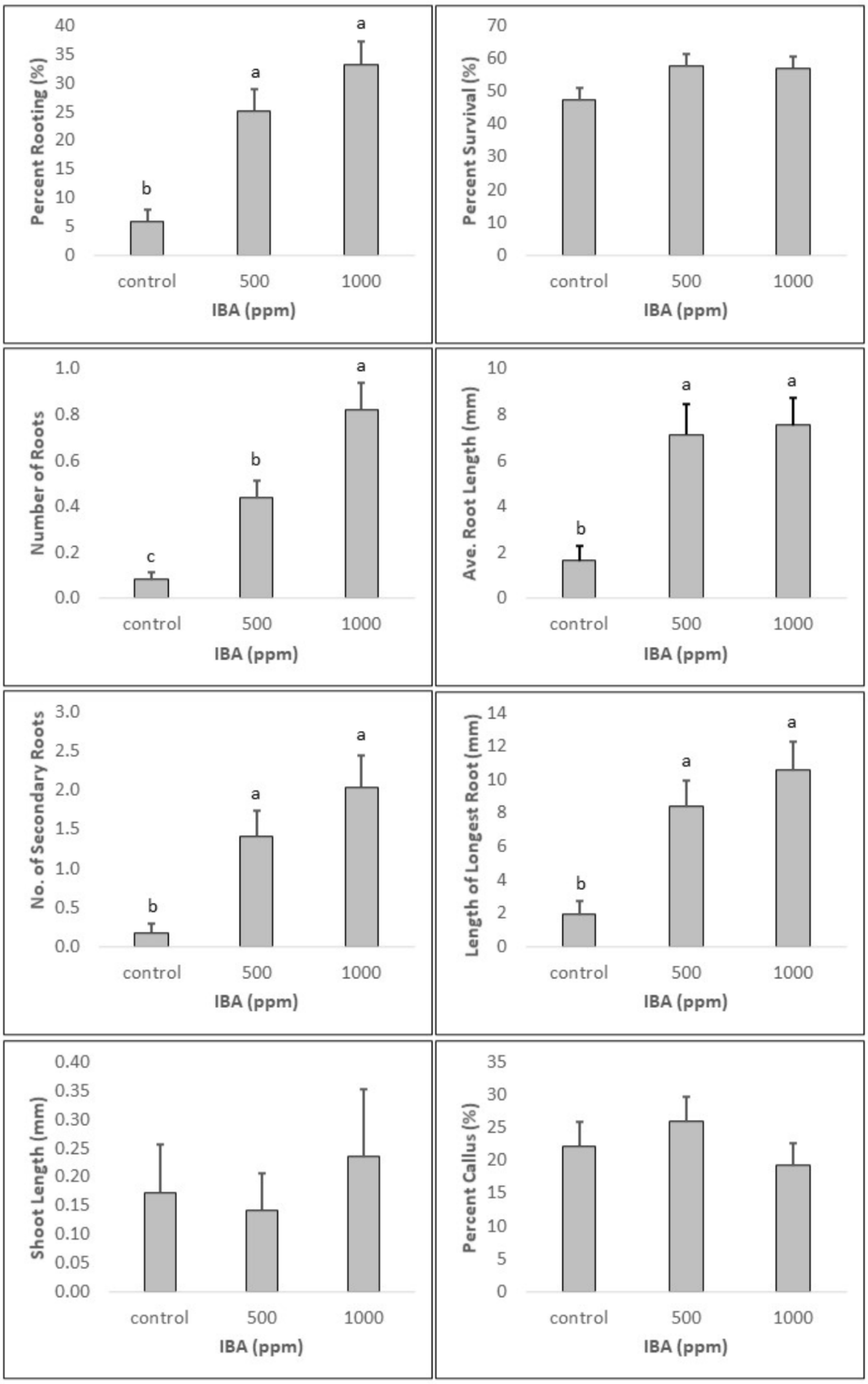

Figure 2 Effect of IBA treatment on vegetative propagation of cuttings from mature *A. cumingiana*; means followed by the same letter(s) are not significantly different at 5% level according to Duncan's Multiple Range Test

soaked in 500 ppm showed a consistent increase with the highest percentage of survival, although not that far from cuttings subjected to 1000 ppm. Meanwhile, cuttings treated with the highest IBA concentration have recorded the greatest average root length. Similarly, it is quite close to cuttings soaked in 500 ppm. Untreated cuttings, however, registered the lowest values in terms of percentage of survival and average root length. A comparison of the treatment means revealed that the untreated cuttings were found significantly different ($p < 0.001$) from IBA treated cuttings in terms of percentage of rooting, average root length, number of secondary roots and length of the longest root. This suggests the need for IBA to increase the rooting ability with the use of cuttings derived from mature *A. cumingiana*.

The IBA is known to induce roots of cuttings collected from seedlings and mature trees of various tree species, where 500 ppm seems the most effective concentration in most tree species (Patricio et al. 2006, Leakey 2014, Piñon et al. 2021). In this study, IBA applications of 500 ppm also achieved the most effective dose. In contrast, similar 500 ppm of IBA gave poor rooting and survival rates in semi to hardwood cuttings of *A. malaccensis* (Borpuzari & Kachari 2018). This would imply that rooting responses tend to vary with cuttings of various maturation and ontogenetic ageing, even with cuttings derived from the same species (Wendling et al. 2014, Leakey 2014). In fact, the present study generated significant variations in five (5) out of eight (8) rooting parameters used. In contrast, only one (1) from five (5) root parameters tested differed significantly due to varying IBA doses in cuttings derived from seedlings of the subject *Aquilaria* (Piñon & Reyes 2021). These observations imply the different levels of suspected naturally occurring endogenous auxin (NOA) in the subject species at different ages with a relatively higher amount at the seedling stage, hence high rooting success was achieved even in the non-IBA treated cuttings (Piñon et al. 2022). This is consistent with the studies that explored the role of NOA in the root initiation of cuttings. Accordingly, variation in rooting at different ages within the same species was strongly correlated with variation in auxin content (Blakesley et al. 1991). Apparently, NOA in plants is highest in the youngest leaves of the seedling but rapidly declines as it gets older (Ludwig-Muller 2000, Overvoorde et al. 2010). Meanwhile, indole-3-acetic acid (IAA) is considered the most common NOA in plants, which was previously suspected to affect the rooting of the subject species (Ludwig-Muller 2000, Piñon & Reyes 2021). However, a recent study discovered that endogenous IAA does not have a direct influence but rather a supportive role, which when applied exogenously could enhance the rooting induction of cuttings taken from trees of the genus Dalbergia (Kumar et al. 2022). This may imply that apart from IBA, other rooting hormones, like IAA should also be tested, particularly in trees with high amount of NOA. Endogenous stimuli, however, do not necessarily correspond to auxin as there are other factors (carbohydrates, mineral nutrition and other chemical compounds) that are also affecting the rooting performance of cuttings (Bannoud & Bellini 2021). Unfortunately, all these observations remain assumptions only, unless proven empirically.

Leaf trimming and IBA interaction effect

Aside from a significant increase in the number of roots ($p = 0.002$), highly significant interaction effects ($p < 0.001$) between leaf surface area and IBA were detected in most of the rooting parameters assessed, except for percentage of survival, shoot length and percentage of callus ($p > 0.05$) (Table 2). Among the interactions, the greatest values were observed between OH (50% of the leaf removed) and IBA1000 ppm. These were detected in rooting parameters such as percentage of rooting, number of roots, average root length, number of secondary roots and length of the longest root. A comparison of the means using DMRT revealed a significant increase in root parameters with increasing leaf laminas in cuttings treated with IBA500 ppm in terms of percentage of rooting, number of roots, average root length, number of secondary roots and length of the longest root. On the other hand, it appears that interaction between IBA500 ppm with the smallest leaf lamina was non-beneficial for rooting. In fact, not a single cutting of such treatment, soaked in 500 ppm of IBA, produced root (Table 2).

Most of the rooting traits examined (5 out of 8) revealed a significant interaction between IBA and leaf trimming. In contrast, with cuttings from young seedlings, the same

Table 2 Interaction effect of different indole-3-butyric acid (IBA) doses and various leaf trimmings of cuttings derived from mature *A. cumingiana*

LSA/IBA	PR (%)	PS (%)	NR	ARL (mm)	NSR	LLR (mm)	SL (mm)	PC (%)
OF								
0 ppm	6.67ab	22.22	0.09ab	2.76a	0.36a	2.89a	0.18	8.89
500 ppm	0.00b	15.56	0.00b	0.00a	0.00a	0.00a	0.00	4.44
1000 ppm	17.78a	35.56	0.42a	2.22a	0.27a	3.29a	0.11	8.89
OH								
0 ppm	4.44b	53.33	0.07c	0.44c	0.00c	0.73c	0.16	22.22
500 ppm	33.33a	77.78	0.53b	8.49b	1.40b	9.84b	0.18	37.78
1000 ppm	46.67a	66.67	1.16a	14.05a	3.49a	18.56a	0.18	11.11
TF								
0 ppm	6.67b	66.67	0.09a	1.72b	0.18b	2.18b	0.18	35.56
500 ppm	42.22a	80.00	0.78a	12.93a	2.84a	15.29a	0.25	35.56
1000 ppm	35.56a	68.89	0.89b	6.38a	2.36a	9.87a	0.42	37.78
SE	0.02^{**}	0.02^{ns}	0.05^{*}	0.65^{**}	0.18^{**}	0.83^{**}	0.05^{ns}	0.02^{ns}

Note: * significant, ** highly significant, SE = standard error, means followed by the same letter(s) are not significantly different at 5% level according to Duncan's Multiple Range Test, OF = one-fourth leaf trimming, OH = one-half leaf trimming, TF = three-fourth leaf trimming, PR = percentage of rooting, PS = percentage of survival, NR = number of roots, ARL = average root length, NSR = number of secondary roots, LLR = length of longest root, SL = shoot length, PC = percentage of callus

interaction effect did not vary significantly in all parameters tested (Piñon & Reyes 2021). These observations may suggest the influence of age-related auxin sensitivity of cuttings due to maturation or ontogenetical ageing. Such phenomenon is known to occur in trees that affect their physiological response to exogenous auxin application (Leakey et al. 2004, Hartmann et al. 2011, Wendling et al. 2014). The response of juvenile and mature tissues to auxin application differs even in the same plant (Pijut et al. 2011). The wider the age gap, the greater the degree of such differences observed. That is because, physiological characteristics of cuttings vary at different ages, and since stages of *de novo* adventitious root formation are correlated with changes in auxin concentration, rooting responses to IBA application vary at different ages of cuttings (Hartmann et al. 2011, Wendling et al. 2014). However, while these findings may seem probable, the reason for the decline or loss of competence to rooting in physiologically mature-phase cuttings compared with physiologically juvenile-phase cuttings remains unclear, unless an in-depth understanding is conducted, like the use of molecular analysis (Hartman et al. 2011, Wendling et al. 2014).

Association among root traits and cutting sizes

Significant ($p < 0.05$) and highly significant ($p < 0.001$) positive with weak to very strong correlations that ranged from $r^2 = 0.11–0.95$ were calculated among eight (8) rooting traits, but not between root traits and cutting sizes (Figure 3). On the other hand, moderately high to highly positive correlations ($r^2 = 0.69–0.96$) among all cutting sizes demonstrated highly significant ($p < 0.001$). The strongest positive relationship among rooting traits was detected between percentage of rooting and the number of roots, followed by between percentage of rooting and average root length, and then between average root length and the number of secondary roots. Meanwhile, the weakest association, although positive and highly significant among rooting traits, was recorded between the number of secondary roots and shoot length. Among cutting sizes, the strongest association was detected between cutting length and cutting diameter, with the weakest value between leaf surface diameter and cutting diameter. On the other hand, low and negative, yet significant correlation was generated between percentage

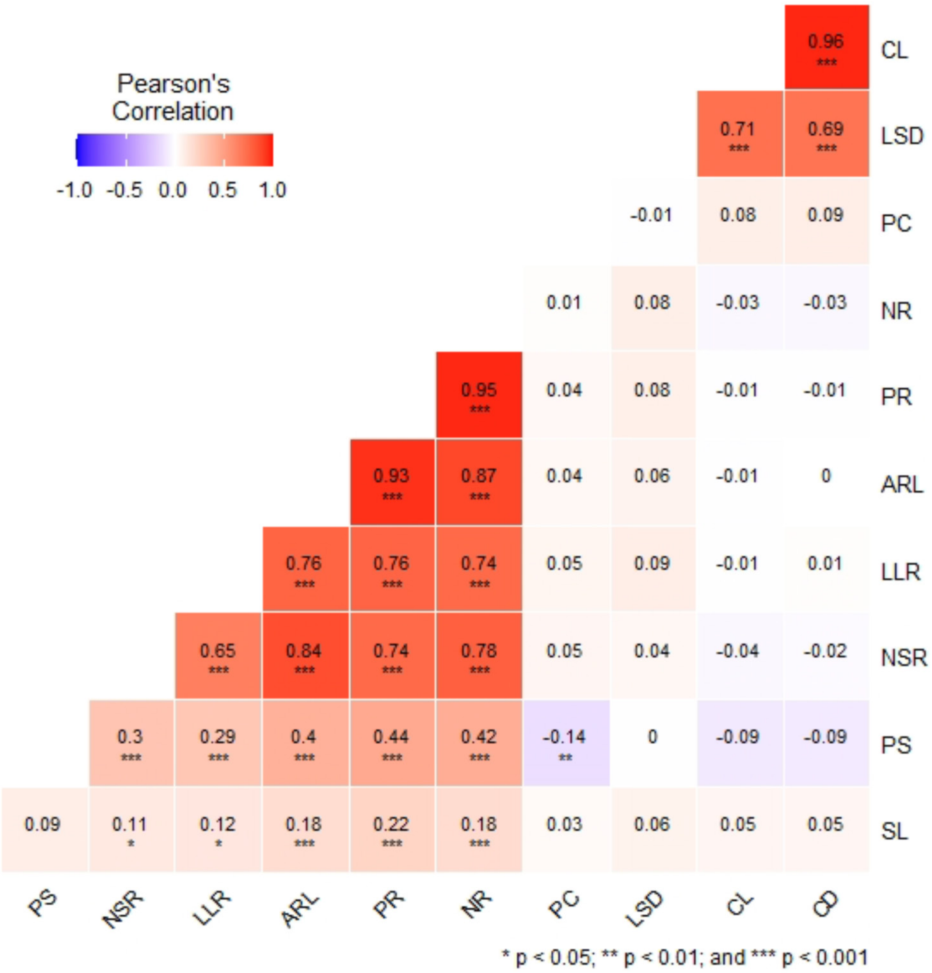

Figure 3 Correlation among rooting traits, cutting sizes and between rooting trait and cuttings size on vegetative propagation using stem cuttings from mature trees of *A. cumingiana*; root traits were denoted as PR (percentage of rooting), PS (percentage of survival), NR (number of roots), ARL (average root length), NSR (number of secondary roots), LLR (length of the longest root), SL (shoot length) and PC (percentage of callus); while cutting sizes were denoted as CL (cutting length), CD (cutting diameter) and LSD (leaf surface diameter); the colour represents the strength of correlations: strong and highly significant relationships among the majority of the rooting traits but less between root trait and cutting size, non-colored square represents zero to insignificant relationships

of callus and percentage of survival. Finally, very weak and insignificant associations ($p > 0.05$) were detected in all associations between rooting traits and cutting sizes, indicating the absence of any possible relationships as far as rooting traits and cutting sizes are concerned.

Correlations of more than 64% (among root traits), 0% (between rooting trait and cutting size), and 100% (among cutting sizes) were found highly significant in the subject species. While in cuttings derived from seedlings, highly significant associations were recorded among all rooting traits (100%), but not between rooting trait and cutting size (0%), and between cutting sizes (0%) despite the non-IBA application (Piñon et al. 2022). Statistically significant associations among root traits, both in mature and young cuttings, although less in the former, may imply the possible accumulation of a high level of NOA, especially when the subject species are still young. Depleting auxin, however, may probably occur as they grow old, hence the effect is less variable, despite the IBA application. Amount, distribution and transportation of auxin influence the formation of the adventitious roots (Ahkami et al. 2013). Differences in the rate of

Figure 4 Samples of rooted cuttings of *A. cumingiana* with various leaf surface areas [25% (a-e), 50% (f–j) and 75% (k–o)] showing pronounced newly induced roots, average root length, secondary roots, and length of the longest root in cuttings with half of the leaf trimmed (f–j)

such auxin factors may probably widen as the source of cutting gets older. However, although some scientists have proved the variation of auxin transport and distribution in plants, comparing the differences in the rate of auxin transport and distribution between young and mature plants of the same species remains uncertain. Most of the studies used seeds or young seedlings, but limited mature plants (Petrasek & Friml 2009, Ni et al. 2001, Ahkami et al. 2013).

All associations in the present study, due to cutting sizes, were highly significant, however, not conforming to the previous research on seedling-based rooting (Piñon et al. 2022). This is because, auxin application stimulates carbohydrate mobilisation which increases the translocation of assimilates to root primordia (Ahkami et al. 2013). However, as auxin biosynthesis was not undertaken and remains a presumption that needs verification.

CONCLUSIONS

Aquilaria trees are not all agarwood producers. As such, true-to-type cloning of high agarwood yielding trees could be used to ensure that the seedlings produced are capable to produce such resource. Generally, despite the low overall rooting success, the present study was able to macro-somatically produce this pricey species using a less complex facility and a simple rooting induction protocol. Results revealed that rooting ability tends to increase with wider leaf surface area and higher auxin concentration, applied exogenously. The presence of photosynthetically active leaves with optimal leaf area, and the duration the cuttings were allowed to stay in the propagation chamber, seems to affect the rooting as caused by variable leaf laminas. In the case of IBA application, the different levels of suspected NOA in *A. cumingiana* at different ages were probably causing significant variation to rooting, due to varying IBA doses. Meanwhile, significant interaction effects in most of the root parameters assessed suggest the influence of age-related auxin sensitivity of cuttings due to maturation. Finally, the significant associations among root traits may imply the possible accumulation of high levels of NOA. However, the amount of such auxin may probably reduce as they grow old, hence the effect is less variable, despite the application of IBA in the present study. These observations suggest that clonal optimisation is important, but precision and accuracy may have increased significantly, considering the phytohormone, auxin biosynthesis and plant anatomy of *Aquilaria*.

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REFERENCES

Ahkami AH, Melzer M, Ghaffari MR et al. 2013. Distribution of indole-3-acetic acid in *Petunia hybrida* shoot tip cuttings and relationship between auxin transport, carbohydrate metabolism and adventitious root formation. *Planta* 238: 499–517.

Amri E, Lyaruu HVM, Nyomora AS & Kanyeka ZL. 2010. Vegetative propagation of African Blackwood (*Dalbergia melanoxylon* Guill. & Perr.): effects of age of donor plant, IBA treatment and cutting position on rooting ability of stem cuttings. *New Forests* 39: 183–194.

Baccarin FJB, Brondani GE, De Almeida LV, Vieira IG, DE Oliveira LS & De Almeida M. 2015. Vegetative rescue and cloning of *Eucalyptus benthamii* selected adult trees. *New Forests*: 46: 465–483.

Bannoud F & Bellini C. 2021. Adventitious rooting in *Populus* species: update and perspectives. *Frontiers in Plant Science* 12: 1–22. https://doi.org/10.3389/fpls.2021.668837.

Barden A, Anak NA, Mulliken T & Song M. 2000. *Heart of The Matter: Agarwood Use and Trade and CITES Implementation for Aquilaria malaccensis*. TRAFFIC International, Cambridge.

Blakesley D, Weston GD & Hall JF. 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: 341–353.

Blanchette R & Heuveling VBH. 2009. *Cultivated Agarwood*. U.S. Patent No. 7638145. University of Minnesota, Minnesota.

Borpuzari PP & Kachari J. 2018. Roots stimulation of selected genotypes of *Aquilaria malaccensis* Lamk. through indole-butyric acid (IBA): a most economically important species of the northeastern region. *International Journal of Botany Studies* 3: 16–20.

Chakrabarty K, Kumar A & Menon V. 1994. *Trade in Agarwood.* WWF-India/TRAFFIC-India, New Delhi.

CITES [Convention on International Trade in Endangered Species of Wild Fauna and Flora]. 2004. *Amendments to Appendices I and II.* CITES: Thirteenth Meeting of the Conference of the Parties, Bangkok.

ERDB [Ecosystems Research and Development Bureau]. 2017. *Annual Report.* Department of Environment and Natural Resources, College, Laguna.

Esyanti RR, Fadholi M, Rizki RM & Faizal A. 2019. Shoot multiplication and growth rates of *Aquilaria malaccensis* Lamk. shoot cultures in temporary immersion system (IT IS)-RTA and bubble column bioreactors. *Pakistan Journal of Botany* 51: 1317–1321.

Gibson IAS. 1977. The role of fungi in the origin of oleoresin deposits of agaru in the wood of *Aquilaria agallocha* Roxb. *Bano Biggyan Patrika* 6: 16–26.

Hartmann H, Kester D, Davies F & Geneve R. 2011. *Plant Propagation. Principles and Practices.* 8th edition. Prentice Hall, New Jersey.

Kumar P, Chaturvedi A & Vaishnav V. 2022. Relationship between endogenous auxin (Indole-3-acetic acid) and adventitious rooting in *Dalbergia* species of different rooting ability. *Journal of Tropical Forest Science* 34: 149–158.

Kundu M & Kachari J. 2000. Desiccation sensitivity and recalcitrant behavior of seeds of *Aquilaria agallocha* Roxb. *Seed Science and Technology* 28: 755–760.

Ky-Dembele C, Tigabu M, Bayala J, Savadogo P, Boussim IJ & Oden PC. 2011. Clonal propagation of *Khaya senegalensis*: the effects of stem length, leaf area, auxins, smoke solution and stockplant age. *International Journal of Forestry Research* 2011: 10.

Lata A. 2007. Investigation of seed longevity and viability and cutting propagation for *Aquilaria crassna.* Literature Review, Postgraduate Diploma of Research Methods, James Cook University, Cairns.

Lee SY & Mohamed R. 2016. The origin and domestication of *Aquilaria*, an important agarwood-producing genus. Pp 1–20 in MOHAMED R. (ed) *Agarwood*: *Tropical Forestry.* Springer, Singapore.

Leakey RRB, Mesen JF, Tchoundjeu Z et al. 1990. Low-technology techniques for the vegetative propagation of tropical trees. *Commonwealth Forestry Review* 69: 247–257.

Leakey RRB. 2004. Physiology of vegetative reproduction. Pp 1655–1668 in Burley J et al (eds) *Encyclopedia of Forest Sciences.* Academic Press, London.

Leakey RRB. 2014. Plant Cloning: Macropropagation. Pp 349–359 in van Alfen N (ed) *Encyclopedia of Agriculture and Food Systems.* Volume 4. Elsevier Publishers, San Diego.

Ludwig-Muller J. 2000. Indole-3-butyric acid in plant growth and development. *Plant Growth Regulation* 32: 219–230.

Naziz PS, Das R & Sen S. 2019. The scent of stress: Evidence from the unique fragrance of Agarwood. *Frontiers in Plant Science* 10: 840. http://dx.doi.org/10.3389/fpls.2019.00840.

Ni DI, Wang LJ, Ding CH & Xu ZH. 2001. Auxin distribution and transport during embryognesis and seed germination of *Arabidopsis. Cell Research* 11: 273–278.

Osborne J. 2002. Notes on the use of data transformations. *Practical Assessment, Research and Evaluation* 8: 1–7.

Overvoorde P, Fukaki H & Beeckman T. 2010. Auxin control of root development. *Cold Spring Harbor Perspectives in Biology* 2: 1–16. . http://dx.doi.org/10.1101/cshperspect.a001537.

Patricio HP, Castañeto YT, Vallesteros AP & Castañeto ET. 2006. Macropropagation of *Shorea guiso* using stem cuttings. *Journal of Tropical Forest Science* 18: 198–201.

Petrasek J & Friml J. 2009. Auxin transport routes in plant development. *Development* 136: 2675–2688.

Pijut PM, Woeste KE & Michler CH. 2011. Promotion of adventitious root formation of difficult-to-root hardwood tree species. Pp 213–251 in Janick J (ed) *Horticultural Reviews Volume 38.* Wiley-Blackwell, New Jersey.

Piñon AA, Tolentino ELJR & Reyes TDJR. 2022. Influence of leaf number, rooting trait and cutting size in vegetative propagation of *Aquilaria cumingiana* [Decne] Ridl. *Philippine Journal of Science* 151: 487–495.

Piñon AA, Reyes TDJR, Carandang WM & Carandang VQ. 2021. Rooting induction of a mature *Pterocarpus indicu*s Willd. using stem cuttings derived from stump epicormic shoots. *Philippine Journal of Science* 150: 1089–1098.

Piñon AA & Reyes TDJR. 2021. Vegetative propagation of *Aquilaria cumingiana* (Decne) Ridl.: effects of IBA concentration and leaf trimming. *Mindanao Journal of Science and Technology* 19: 96–115.

PROSEA [Plant Resources of South-East Asia]. 2019. *Bibliographic Details for Aquilaria cumingiana.* PROSEA, Wageningen.

Saikia M, Shrivastava K & Singh SS. 2013. Effect of culture media and growth hormones on callus induction in *Aquilaria malaccensis* Lam., a medicinally and commercially important tree species of North East India. *Asian Journal of Biological Science* 6: 96–105.

Soehartono T & Newton AC. 2001. Conservation and sustainable use of tropical trees in the genus *Aquilaria.* The impact of gaharu harvesting in Indonesia. *Biological Conservation* 97: 29–71.

Soehartono T, Newton AC & Mardiastuti. 2002. Factors influencing the survival and growth of *Aquilaria malaccensis* seedlings in Indonesia. *Journal of Tropical Forest Science* 14: 364–378.

Stuepp CA, Zuffellato-Ribas KC, Wendling I, Koehler HS & Bona C. 2014. Vegetative propagation of mature dragon trees through epicormic shoots. *Bosque* 35: 337–345.

Suharti S, Pratiwi P, Santosa E & Turjaman M. 2011. Feasibility study of business in agarwood inoculation at different stem diameters and inoculation periods. *Journal of Forestry Research* 8: 114–129.

Tan CS, Isa NM, Ismail I & Zainal Z. 2019. Agarwood induction: current developments and future perspectives. *Frontiers in Plant Science* 10: 122.

The Plant List. 2013. Version 1.1. http://www.theplantlist.org/.

Trueman SJ. 2006. Clonal propagation and storage of subtropical pines in Queensland, Australia. *Southern African Forestry Journal* 208: 49–52.

Tombesi S, Palliotti A, Poni S & Farinelli D. 2015. Influence of light and shoot development stage on leaf photosynthesis and carbohydrate status during the adventitious root formation in cuttings of *Corylus avellana* L. *Frontiers in Plant Science* 6: 1–13.

Wendling I, Trueman SJ & Xavier A. 2014. Maturation and related aspects in clonal forestry – part II: reinvigoration, rejuvenation and juvenility maintenance. *New Forests* 45: 473–486.

Xu Y, Zhang Z, Wang J et al. 2013. Identification of genes related to agarwood formation: transcriptome analysis of healthy and wounded tissues of *Aquilaria sinensis. BMC Genomics* 14: 227.

Zobel B & Talbert JT. 2003. *Applied Forest Tree Improvement.* John Wiley & Sons, New York.