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Improved Clonal Propagation through Rejuvenation of Mature Branch Cutting of Four Important Acacia Species

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Abstract: Asexual propagation techniques for producing good quality germplasm for breeding and dissemination purposes have proven difficult for acacia species comprised of mature planting material. The study was conducted to study the effect of rejuvenation on the rooting ability of mature cuttings. Shoots were induced from the lower branch by cutting a part of the mature branch of the crown and leaving it horizontally on the propagation bench under the misting system. Shoots were harvested and further used as stem cuttings to evaluate their rooting ability through the application of rooting hormone. The rooting ability of the cuttings is highly variable among species. The percentage of stem sections producing juvenile shoots was similar for *Acacia mangium* Willd. (88%) and *Acacia auriculiformis* A.Cunn. ex Benth. (90%). Only 52% of stem sections were able to produce shoots for *Acacia crassicarpa* A.Cunn. ex Benth., followed by *Acacia aulococarpa* A.Cunn. ex Benth. with only 31%. Overall, *A. auriculiformis* rooted better and recorded the highest mean value for all traits tested. Hormone treatment significantly enhances the rooting ability of *A. auriculiformis* and *A. mangium*. However, *A. aulococarpa* and *A. crassicarpa* did not respond well to the treatment. Rejuvenated stem cuttings were rooted better than mature cuttings, producing the highest mean value for all traits tested in all species, with or without hormone treatment. Results indicated that it is possible to rejuvenate mature cuttings through bud break in a controlled environment.

Keywords: *Acacia* sp.; force flushing; rejuvenation; stem cutting; vegetative propagation

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1. Introduction

Vegetative propagation has been proposed as an alternative method of sexual breeding, to capture and preserve some unique characteristics of economically or aesthetically important tree species, in order to avoid undesirable variation among trees grown on the plantation. Long flowering and fruiting interval, along with the poor correlation between juvenile and mature sources, make field sources comprised of different ages and juvenility difficult to propagate. The maturation-related loss of adventitious rooting competence in trees becomes a major limiting factor in clonal forestry, since many desirable traits (wood quality, tree form, or seed production) are only expressed when the tree matures [1].

Adventitious root formation declines with the chronological age of the tree. Phase changes from juvenile and adolescent to mature (reproductive) trees result from the expression of certain genes at specific times in the life cycle of a plant, or in response to environmental or internal signals [2,3]. In terms of chronological age, the area near the base of the tree is considered the oldest, and the stem and branches are considered to be the youngest. However, stems and branches are oldest in maturity in terms of ontogenetic age, and are capable of reproducing [2,3]. Therefore, rooting potential decreases with increasing distance from the ground, and has been a limiting factor in the propagation of economically important tree species in a tree improvement program [4,5]. Although propagation of elite

mature sources is desirable to achieve and maintain genetic gains, outcome results using mature sources were still not as good as those produced when utilizing juvenile sources. Cuttings from some parts of young seedlings in most woody species are easily rooted compared to those produced from mature sources, which are rooted very slowly, or in some cases failed to root at all [6–8].

The phenotype, organizational structure, biochemical, and physiology of a juvenile tree are different from a mature tree [7,9]. Thus, maintaining the juvenility of the explants' sources is an important aspect of the upkeep of the active physiological state to promote active cell division. Rejuvenation of mature sources will restore the juvenile characteristics of a tree by increasing the activities of esterases, tyrosine phosphorylated proteins, and peroxidases, producing a higher degree of methylation, and improving the photosynthetic and respiratory rates. Juvenile tissues were also found to show higher hormone sensitivity with higher levels of endogenous hormones such as cytokinins, auxins, and gibberellins, which maintain the trees in a juvenile state [8,10–12]. A better understanding of rejuvenating these explants with pre-treatment is therefore useful and necessary to ensure success for induction and optimization in both macro-and micropropagation effects [13]. Thus, to facilitate the development of forest plantation programs utilizing mature sources with relevant economic traits, necessary workable protocols that could assist in rejuvenating them should be studied well and established before embarking on a mass propagation program.

Rejuvenation can be achieved through the application and modification of propagation technologies such as cryopreservation and coppicing [14]. Cryopreservation can be used as a technique to maintain the juvenility of some industrial species during simultaneous clonal testing in the field. However, this technology is applicable or practical mainly in extensive clonal forestry and where rejuvenation is difficult to achieve, especially for certain species such as conifers [15]. Stumping, pruning sprouts, force flushing, and serial grafting of mature branches and utilization of newly developed sprouts as planting material are some of the frequently attempted rejuvenation techniques in the propagation of elite sources [16–20]. In some cases, various ways of rejuvenation including serial propagation, repeated in vitro subculturing, successive pruning, mound layering, micrografting, coppicing, and etiolation, have also been practiced to improve adventitious root formation [7,8,21]. In some cases, where there is a risk of loss of some selected genotypes through destructive methods such as stumping, induction of shoots from the epicormic branch and branch cutting are more applicable. Shoots were induced from the lower branch by cutting a part of the mature branch of the crown (60–70 cm) and leaving it horizontally on the propagation bench/bed under the misting system [6]. The forced flushing process can be accelerated through the application of some chemicals. This forcing solution did not rejuvenate the explants directly, but acted as a stimulator to enhance the other plant growth-regulating chemicals such as gibberellin, cytokinin, and auxin [22,23].

Acacias, particularly the Australian species, are one of the most important forestry trees and multipurpose tree species in the tropics. They are distributed in warm and drier regions of the world, that is, mainly in the tropics and subtropics, and are more prevalent in Australia and Africa. There are more than 1000 Acacia species in Australia alone, where their taxonomic identification was refined for plantation purposes and only three were taken up for tropical plantations and grown for timber and pulp production. They are *Acacia mangium* Willd., *Acacia auriculiformis* A.Cunn. ex Benth., and *Acacia crassicarpa* A.Cunn. ex Benth. [24]. *A. mangium* mainly consists of juvenile materials that have been successfully propagated from traditional vegetative propagation methods such as cuttings. It was also noted that age is one of the factors that could affect the rooting ability of cuttings; for instance, maturation effects in superior mother trees have been shown to reduce clonal vigor in *A. mangium* and *A. crassicarpa*, making clonal forestry with matured species nonviable [24–26]. Propagation using mature trees is still a problem and most *Acacia* spp. are categorized as difficult to root [27]. To overcome the maturation effect on the rooting ability, cuttings were collected from coppice shoots of *A. manguim* × *A. auriculiformis*, whereas sprouting buds from stem cuttings were used in *A. auriculiformis* [28,29]. Rejuvenation

of mature Acacia species has been attempted before through stumping and rooting of sprouted young coppice material of 2- to 10-year-old *Acacia mearnsii* [30]. In this study, we propose the use of newly developed shoots through force flushing of 12-year-old branch cuttings of Acacia species to evaluate the rooting ability of rejuvenated stem cuttings. The study was conducted to study the effect of rejuvenation on the rooting ability of the cuttings in comparison to mature cutting. The study was also intended to study the effects of rooting hormone application on rejuvenated and mature cuttings originated from various superior clones of Acacia species.

2. Materials and Methods

This study was conducted for 6 months in the nursery of the Faculty of Forestry, Universiti Putra Malaysia (2°59′ N, 101°42′ E), Serdang, Selangor, Malaysia. The study area experiences a typically hot humid climate with temperatures ranging from 25–32 °C and a 12 h day and night cycle.

2.1. Selection of Mother Tree and Branch-Cutting Collection

For mass propagation of elite materials of Acacia species, branch cuttings were collected from selected mother trees of mature Acacia species. Branch cuttings were collected from *A. mangium*, *A. auriculiformis*, *A. crassicarpa*, and *A. aulococarpa* originated from the provenance–progeny trial established at How Swee Sdn. Bhd. Estate, Kampung Aur Gading, Kuala Lipis, Pahang, Malaysia, using seed sources supplied by ACIAR through CSIRO. Mother trees were selected based on their superior phenotypic characteristic. Trees were evaluated based on their quantitative and qualitative traits such as diameter at breast height (DBH), total height, crown emergence, crown diameter, crown form, stem straightness, branching and forking system, branching quality and quantity, the axis of branches, and pruning ability. The mature branches of each selected mother tree of Acacia species was collected during the rainy season around middle of October and the nursery setup, rejuvenation, and rooting experiment was extended for a period of one year. This was done to avoid moisture lost from the mature branch cutting during transportation to the nursery. Detailed information on the selection criteria and genotypes selection can be gathered from Kumar et al. [31]. Those individuals were identified as mother trees of each species and the three most superior individual trees were selected for further propagation (Table 1).

Table 1. Selected clones of Acacia species for clonal propagation.

Species	Region	Provenance	Selected Clone	Tree No	Code
Acacia mangium Willd.	PNG	SW of Boset WP	CG 1853	B1L11T6	M 1
			CG 1854	B4L67T1	M 2
	QLD	Russel & Gap CK	7	B1L28T8	M 3
Acacia auriculiformis A.Cunn. ex Benth.	PNG	Mibini	BVG 2724	B1L42T5	A1
		Bansbach	BVG 2657	B1L47T6	A2
	QLD	Wenlock River	JSL 363	B1L22T12	A3
Acacia crassicarpa A.Cunn. ex Benth.	PNG	Bensbach WP	BVG 2609	B1L27T7	C1
		Bimadebum WP	BVG 2748	B1L15T11	C2
	QLD	Chilli Beach	GJM 1135	B1L46T10	C3
Acacia aulococarpa A.Cunn. ex Benth.	PNG	Arufi E M WP	AR 000010	B4L61T13	U1
			MM 001016	B4L74T2	U2
		W Morehead	BVG 00834	B1L30T1	U3

Note: PNG = Papua New Guinea, QLD = Queensland.

2.2. Lateral Bud Induction from Cuttings and Forced Flushing of Mature Trees

Branches of selected *Acacia* genotypes from four species were collected from the lower part of the crown (from the first branch of the trunk). Branches were collected from three selected clones of each species growing in the same environmental conditions and of similar size in terms of height and diameter. Collected tree branches (<1 m) were dipped in water

and covered with a plastic cover to maintain their moisture content during transportation to the nursery of the Faculty of Forestry, UPM. Following transportation, the branches were further cut into smaller segments of 50 cm long and between 5.0 and 8.0 cm in diameter. Cuttings were kept at 4 °C for 4 days in black plastic bags as a cold pre-treatment before use [32]. The cuttings were then removed from the chiller and directly placed horizontally on the coarse wet sand. One half of the diameter was pressed into the sand to allow direct contact with the growth medium. A 4 × 3 factorial experiment in a completely randomized design with 4 species and 3 clones was used in this study. A total of 360 cuttings were planted, which included 10 cuttings per replicate with three replicates per treatment, and 12 possible treatment combinations were used for this experiment. The propagation trays were irrigated regularly and placed under sunlight with normal photoperiod. Data on the number of shoots, shoot length (cm), and survival percentage (%) was subjected to analysis of variance using SPSS statistical package. This was followed by a post hoc test using Duncan's multiple range test (DMRT). Shoots flushed in the experiment were collected for further rooting procedures as stem cuttings.

2.3. Preparation of Stem Cuttings and Hormone Treatment

Shoots above 4.0 cm in length were harvested and further used as stem cuttings which were subjected to subsequent rooting experiments. Additional stem cuttings were also obtained directly from the crown of the mature donor tree to evaluate the effect of rejuvenated and mature explants on root production of stem cuttings. Stem cuttings were cut into smaller segments with each segment containing two nodes with a pair of half-trimmed leaves from their original size. Cuttings were soaked and disinfected with 0.1% of fungicide Benlate for 30 min. Cuttings were separated into three groups representing the number of assessments for each treatment. There were three replications with 10 stem cuttings per replication and a total of 30 cuttings were used for each treatment. The basal end of the segment was dipped into distilled water (0) as control, 1000 ppm indole- 3-butyric acid (IBA) solutions for a minute, and commercial rooting hormone Seradix, also known as Agradix No. 3 (active constituent indole-3-butyric acid) (Agrimart Sdn Bdn, Seri Kembangan, Malaysia). IBA formulation was prepared by dissolving the IBA powder (Merck, Darmstadt, Germany) in absolute ethanol and diluting the dissolved solution in distilled water to obtain the required IBA concentration. The same amount of ethanol used to dissolve the IBA powder was also added to the control solution of distilled water. The ends of the cuttings were dipped lightly in Seradix 3 powder before they were planted vertically in propagation trays consisting of coarse wet sand. Propagation trays were irrigated regularly and covered with transparent plastic enclosures supported by steel frames to maintain high humidity of more than 80%. The enclosures were also shaded with black plastic netting (20% light intensity). The temperature around the cuttings ranged from 22 °C (night) to 35 °C (midday). The completely randomized design was adopted with 72 treatments with four species, three clones, two types of explants, and three groups (one control group and two experimental groups of IBA concentrations). Cuttings were dipped in distilled water (without any growth regulators) and they were served as control. A total of 720 cuttings were placed in a non-mist propagator with pure, sterilized river sand, and the experiment was repeated three times. Flow chart of research activities during collection and rooting experiment is presented in Figure 1. Data on root number, root length (cm), and rooting percentage were collected after 40 days and subjected to analysis of variance using SPSS statistical package. To test for significant differences, data were log-transformed and subjected to ANOVA. Duncan's Multiple Range Test (DMRT) was used to compare the treatment means. The results were considered significant when $p \leq 0.05$. The values are expressed in mean ± standard error (SE) or standard deviation (SD) when appropriate.

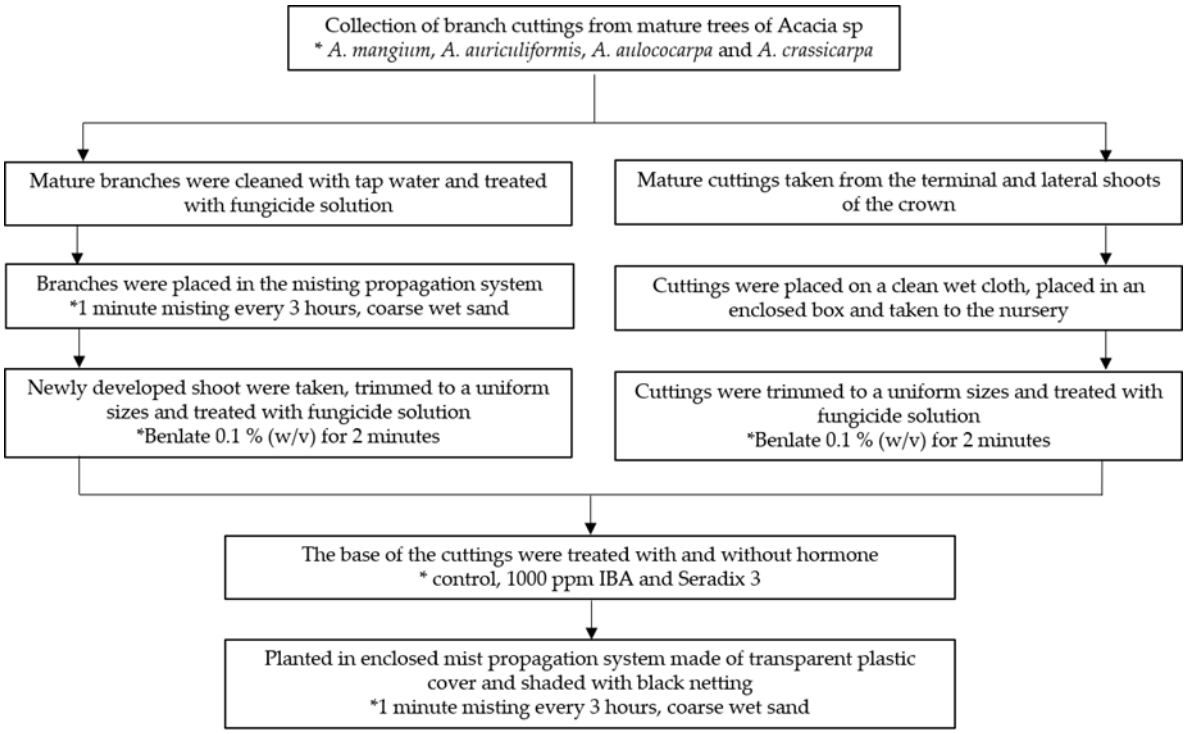

Figure 1. Flow chart of research activities during collection and rooting experiment in the nursery for the production of quality planting material of Acacia species. (* Specific concentration and time).

3. Results

Healthy vegetative shoots were successfully forced flushed in the greenhouse from excised branch cuttings of 12-year-old *Acacia* clones. The percentage of shooting and number of shoots produced per cutting as well as their shoot length indicated that they are highly variable among species but not within the clone of the species. The percentage of stem sections producing juvenile shoots was similar for *A. mangium* and *A. auriculiformis* with 88% and 90%, respectively. Only 52% of stem sections were able to produce shoots for *A. crassicarpa* followed by *A. aulococarpa* with only 31% (Table 2). A significant difference was obtained in terms number of shoots produced and shoot length between Acacia species. The average shoot number ranged from 0.60 in *A. crassicarpa* clones to 5.0 in *A. auriculiformis* clones. *A. auriculiformis* produced the highest number of shoots (4.7) followed by *A. mangium*, *A. aulococarpa*, and *A. crassicarpa*. Similarly, *A.auriculiformis* also produced the longest shoots (4.2 cm) among the other Acacia species however with no significant difference with the shoot length of *A. mangium* (4.0 cm). Even though *A. aulococarpa* recorded the lowest shooting percentage, it produced a relatively high number of shoots (1.36) and longer shoots (1.43 cm) compared to *A. crassicarpa*.

Table 2. Mean values of force flushing (bud break) ability of cuttings of four Acacia species clones.

Species	SP (%)	SN	SL (cm)
Acacia mangium	87.78 ± 3.64^{a}	3.44 ± 0.19^{b}	4.02 ± 0.20^{a}
Acacia auriculiformis	90.00 ± 3.33^{a}	4.70 ± 0.19^{a}	4.19 ± 0.32^{a}
Acacia crassicarpa	52.22 ± 3.64^{b}	0.72 ± 1.34^{d}	0.87 ± 0.14^{b}
Acacia aulococarpa	31.11 ± 3.51^{c}	1.36 ± 0.15^{c}	1.43 ± 0.15^{b}

Note: SP = shooting percentage, SN = shoot number, SL = shoot length. Values are expressed in mean ± standard error. Significant differences among species are indicated by different lower-case letters ($p \leq 0.05$). Similar letters are not significantly different at $p \leq 0.05$ based on Duncan Multiple Range Test.

The percentage of stem segments producing shoots was not significantly different for clones within the species. *A. mangium* clones and *A. auriculiformis* clones produced

the highest shooting percentage of 83% to 90% and 83% to 100%, respectively (Table 3). This was followed by the other two species, which were significantly different at $p \leq 0.05$ between clones, namely *A. crassicarpa* clones (50% to 53%), and *A. aulococarpa* clones (23% to 40%). *Acacia* clones were significantly different at $p \leq 0.05$ in terms of shoot number and shoot length. *A. crassicarpa* clones produced the least number of shoots with short shoots which eventually turned brown and died after a month in control growth condition. To obtain enough cuttings for the rooting experiment, more branches had to be force flushed for *A.aulococarpa* and *A. crassicarpa* so that only longer shoots with uniform size were selected for the rooting ability of the cutting experiment.

Table 3. Mean values for force-flushing (bud break) ability of cuttings of twelve clones of four *Acacia* species clones.

Species	Clone	BB (%)	SN	SL (cm)
Acacia mangium	M1	90.00 ± 10.00 ^{a}	3.50 ± 0.28 ^{cd}	4.08 ± 0.32 ^{ab}
	M2	90.00 ± 5.77 ^{a}	3.13 ± 0.31 ^{d}	4.21 ± 0.33 ^{ab}
	M3	83.33 ± 3.33 ^{a}	3.70 ± 0.36 ^{bcd}	3.78 ± 0.39 ^{ab}
Total mean		**87.78 ± 3.64**	**3.44 ± 0.19**	**4.02 ± 0.20**
Acacia auriculiformis	A1	86.67 ± 6.67 ^{a}	4.27 ± 0.34 ^{bc}	4.75 ± 0.62 ^{a}
	A2	83.33 ± 3.33 ^{a}	4.53 ± 0.40 ^{ab}	4.48 ± 0.42 ^{a}
	A3	100.0 ± 0.00 ^{a}	5.30 ± 0.22 ^{a}	3.32 ± 0.58 ^{b}
Total mean		**90.00 ± 3.33**	**4.70 ± 0.19**	**4.19 ± 0.32**
Acacia crassicarpa	C1	53.33 ± 6.67 ^{b}	0.60 ± 0.21 ^{f}	0.76 ± 0.24 ^{c}
	C2	53.33 ± 3.33 ^{b}	0.83 ± 0.19 ^{ef}	1.05 ± 0.24 ^{c}
	C3	50.00 ± 10.00 ^{b}	0.73 ± 0.24 ^{ef}	0.78 ± 0.23 ^{c}
Total mean		**52.22 ± 3.64**	**0.72 ± 1.34**	**0.87 ± 0.14**
Acacia aulococarpa	U1	23.33 ± 3.33 ^{c}	1.60 ± 0.30 ^{e}	1.52 ± 0.28 ^{c}
	U2	40.00 ± 5.77 ^{bc}	1.24 ± 0.24 ^{ef}	1.47 ± 0.26 ^{c}
	U3	30.00 ± 5.77 ^{c}	1.23 ± 0.26 ^{ef}	1.30 ± 0.25 ^{c}
Total mean		**31.11 ± 3.51**	**1.36 ± 0.15**	**1.43 ± 0.15**

Note: BB = Bud break, SN = shoot number, SL = shoot length. Values are expressed in mean ± SE. Significant differences among species are indicated by different lower-case letters ($p \leq 0.05$). Similar letters are not significantly different at $p \leq 0.05$, based on Duncan's Multiple Range Test.

Analysis of variance conducted on some rooting ability traits indicated there was less significant two-way interaction. Most variation clearly can be seen from source and treatment. Variation due to the interaction between main sources of variation was not significant for most of the rooting traits studied in this study. Overall, *A. auriculiformis* was rooted better than the other species and recorded the highest mean value for all traits tested (Table 4). This was followed by *A. mangium* and *A. aulococarpa*, which obtained similar mean shooting percentages of 58% and 61%, respectively. However, the latter species failed to produce a sufficient number of roots, obtaining a mean rooting percentage of only 35%, similar to *A. crassicarpa*'s 33%. Figure 2 describes the shooting percentage of the mature branch cuttings and rooting percentage of newly developed shoots of *Acacia* species for comparison.

Table 4. Mean values for rooting ability of four Acacia species cuttings.

Species	SP (%)	RP (%)	SN	SL (cm)	RN	RL (cm)
Acacia mangium	57.96 ± 3.12 ^{b}	67.22 ± 3.11 ^{a}	2.09 ± 0.11 ^{b}	3.94 ± 0.18 ^{b}	2.99 ± 0.12 ^{c}	3.47 ± 0.13 ^{c}
Acacia auriculiformis	74.26 ± 1.86 ^{a}	61.67 ± 3.12 ^{b}	3.88 ± 0.16 ^{a}	6.24 ± 0.22 ^{a}	5.70 ± 0.23 ^{a}	6.30 ± 0.21 ^{a}
Acacia crassicarpa	21.85 ± 2.37 ^{c}	33.15 ± 2.29 ^{c}	1.08 ± 0.10 ^{c}	1.64 ± 0.48 ^{d}	1.74 ± 0.12 ^{d}	1.95 ± 0.14 ^{d}
Acacia aulococarpa	60.56 ± 1.95 ^{b}	35.56 ± 2.31 ^{c}	2.16 ± 0.13 ^{b}	2.50 ± 0.14 ^{c}	4.54 ± 0.21 ^{b}	4.20 ± 0.19 ^{b}

Note: SP = shooting percentage, RP = rooting percentage, SN = shoot number, SL = shoot length, RN = root number, RL = root length. Values are expressed in Mean ± Standard Error. Significant differences among species are indicated by different lower-case letters ($p \leq 0.05$). Similar letters are not significantly different at $p \leq 0.05$ based on Duncan Multiple Range Test.

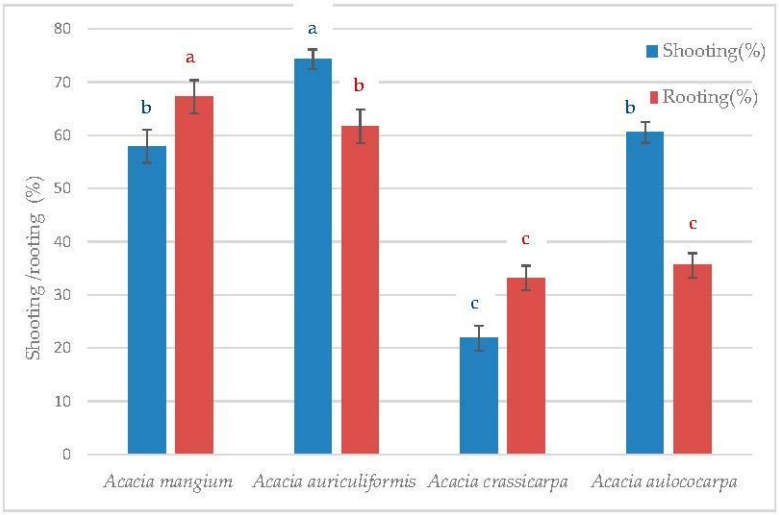

Figure 2. Shooting and rooting percentage of rejuvenated stem cuttings of *Acacia* species. Note: bar = ± standard error of the mean, Means of each variable with the same letters are not significantly different at $p \leq 0.05$.

Mean values are expressed as ± standard error of the mean, the means of each variable with the same letters are not significantly different at $p \leq 0.05$.

This indicates that the same number of cuttings failed to produce roots and shoots at the same time. Cuttings producing shoots without any development of a well-developed root system failed to survive and died after a month in the nursery bed. However, some rooted cuttings produced shoots only after 2 months. There was significant variation between clones within species for most of the traits studied (Table 5). *A. auriculiformis* clones produced the highest mean shooting percentage of 73% to 76%, followed by no significant variation among *A. mangium* clones (54% to 63%), *A. aulococarpa* clones (59% to 62%), and *A. crassicarpa* clones (16% to 28%). Young stem cuttings were rooted better than the mature cuttings, producing a high mean value for all traits tested in all species. Generally, young cuttings were also recorded to have similar shooting and rooting percentages (64% and 65%, respectively) compared to those obtained from mature cuttings, which produced 43% and 33%, respectively (Table 6).

Table 5. Mean values of some rooting ability traits of cuttings of four *Acacia* species clones.

Species	Clone	SN	SL (cm)	RN	RL (cm)
Acacia mangium	M1	2.27 ± 0.17^{b}	2.97 ± 0.20^{c}	3.23 ± 0.19^{de}	3.50 ± 0.23^{d}
	M2	2.10 ± 0.18^{bc}	3.26 ± 0.25^{c}	2.92 ± 0.20^{e}	3.21 ± 0.20^{d}
	M3	1.90 ± 0.20^{bc}	5.57 ± 0.39^{b}	2.81 ± 0.21^{e}	3.71 ± 0.23^{cd}
Acacia auriculiformis	A1	3.78 ± 0.25^{a}	5.87 ± 0.36^{ab}	5.08 ± 0.35^{bc}	5.98 ± 0.36^{b}
	A2	3.72 ± 0.28^{a}	5.81 ± 0.35^{ab}	5.59 ± 0.40^{b}	6.81 ± 0.40^{a}
	A3	4.13 ± 0.30^{a}	7.04 ± 0.45^{a}	6.43 ± 0.45^{a}	6.11 ± 0.32^{ab}
Acacia crassicarpa	C1	0.99 ± 0.17^{d}	0.93 ± 0.16^{d}	1.24 ± 0.17^{g}	1.64 ± 0.22^{e}
	C2	1.64 ± 0.22^{c}	3.09 ± 1.42^{c}	1.91 ± 0.23^{f}	2.00 ± 0.27^{e}
	C3	0.61 ± 0.12^{d}	0.92 ± 0.18^{d}	2.09 ± 0.23^{f}	2.22 ± 0.25^{e}
Acacia aulococarpa	U1	2.12 ± 0.19^{bc}	2.63 ± 0.21^{c}	3.64 ± 0.29^{d}	3.87 ± 0.29^{cd}
	U2	2.46 ± 0.25^{b}	2.70 ± 0.26^{c}	5.16 ± 0.39^{bc}	4.37 ± 0.35^{c}
	U3	1.90 ± 0.23^{bc}	2.16 ± 0.25^{cd}	4.83 ± 0.38^{c}	4.37 ± 0.35^{c}

Note: SN = shoot number, SL = shoot length, RN = root number, RL = root length. Values are expressed in mean ± SE. Significant differences among species are indicated by different lower-case letters ($p \leq 0.05$). Similar letters are not significantly different at $p \leq 0.05$, based on Duncan's Multiple Range Test.

Table 6. Mean values of some rooting ability traits of cutting sources (young and mature) of four Acacia species.

Clones	Source	SP (%)	RP (%)	SN	SL (cm)	RN	RL (cm)
Acacia mangium							
M1	Young	76.67 ± 12.25	86.67 ± 8.66	2.89 ± 2.33	3.56 ± 2.53	4.53 ± 2.49	5.04 ± 3.31
M2		74.44 ± 18.10	85.56 ± 10.14	3.28 ± 2.54	5.06 ± 3.35	4.17 ± 2.75	4.44 ± 2.36
M3		68.88 ± 22.61	88.88 ± 6.01	3.13 ± 3.33	7.16 ± 5.12	3.91 ± 3.01	5.03 ± 2.73
Total		**73.33 ± 17.76**	**87.04 ± 8.23**	**3.10 ± 2.76**	**5.26 ± 4.09**	**4.20 ± 2.76**	**4.84 ± 2.84**
M1	Mature	51.11 ± 12.69	46.67 ± 13.23	1.64 ± 1.93	2.38 ± 2.60	1.93 ± 2.01	1.97 ± 1.95
M2		34.44 ± 14.24	45.56 ± 16.67	1.63 ± 2.33	1.47 ± 2.47	1.65 ± 1.99	1.97 ± 2.30
M3		42.22 ± 18.56	50.00 ± 11.18	0.67 ± 2.74	4.03 ± 4.94	1.73 ± 2.10	2.37 ± 2.77
Total		**42.59 ± 16.31**	**47.41 ± 13.47**	**2.09 ± 2.47**	**2.63 ± 3.62**	**1.77 ± 2.03**	**2.10 ± 2.36**
Acacia auriculiformis							
A1	Young	76.67 ± 10.0	77.78 ± 12.02	4.59 ± 3.74	7.05 ± 5.56	6.37 ± 5.77	6.81 ± 5.50
A2		83.33 ± 8.66	74.44 ± 13.33	5.00 ± 4.24	8.09 ± 4.84	7.46 ± 6.21	8.13 ± 5.52
A3		83.33 ± 15.00	90.00 ± 7.07	5.33 ± 4.15	10.12 ± 6.03	9.07 ± 6.55	7.70 ± 4.27
Total		**81.11 ± 11.54**	**80.74 ± 12.69**	**4.97 ± 4.05**	**8.42 ± 5.62**	**7.64 ± 6.24**	**7.55 ± 5.14**
A1	Mature	75.56 ± 12.36	50.00 ± 11.18	2.97 ± 2.70	4.69 ± 3.62	3.78 ± 2.88	5.15 ± 4.02
A2		63.33 ± 13.23	40.00 ± 12.25	2.44 ± 2.50	3.52 ± 3.11	3.72 ± 3.52	5.48 ± 4.97
A3		63.33 ± 7.07	37.78 ± 12.02	2.92 ± 3.39	3.97 ± 4.15	3.77 ± 4.00	4.52 ± 3.83
Total		**67.41 ± 12.28**	**42.59 ± 12.59**	**2.78 ± 2.89**	**4.06 ± 3.67**	**3.76 ± 3.48**	**5.05 ± 4.31**
Acacia crassicarpa							
C1	Young	30.00 ± 11.18	34.44 ± 13.33	1.64 ± 2.82	1.21 ± 2.18	1.88 ± 2.88	2.43 ± 3.55
C2		48.88 ± 14.53	44.44 ± 8.82	3.03 ± 3.45	5.93 ± 26.60	3.05 ± 3.64	3.50 ± 4.37
C3		18.89 ± 9.28	56.67 ± 10.00	0.95 ± 2.11	1.50 ± 3.21	3.44 ± 3.56	3.64 ± 4.02
Total		**32.50 ± 17.01**	**45.19 ± 13.97**	**1.88 ± 2.96**	**2.88 ± 15.61**	**2.79 ± 3.43**	**3.19 ± 4.02**
C1	Mature	13.33 ± 10.00	23.33 ± 8.66	0.30 ± 0.98	0.66 ± 1.97	0.53 ± 1.21	0.81 ± 1.75
C2		7.78 ± 4.41	18.88 ± 7.82	0.25 ± 0.96	0.25 ± 0.99	0.74 ± 1.86	0.47 ± 1.88
C3		12.22 ± 12.02	21.11 ± 10.54	0.26 ± 0.79	0.34 ± 1.04	0.73 ± 1.50	0.79 ± 1.65
Total		**11.11 ± 9.34**	**21.11 ± 8.91**	**0.27 ± 0.91**	**0.42 ± 1.41**	**0.67 ± 1.54**	**0.69 ± 1.55**
Acacia aulococarpa							
U1	Young	68.89 ± 9.28	45.56 ± 8.82	2.80 ± 2.56	3.32 ± 2.52	4.58 ± 4.18	4.09 ± 3.51
U2		68.89 ± 14.53	52.22 ± 9.72	3.76 ± 3.73	3.99 ± 3.74	7.66 ± 5.68	6.40 ± 5.27
U3		71.11 ± 10.54	50.00 ± 7.07	2.73 ± 3.58	3.00 ± 3.74	7.09 ± 5.50	6.74 ± 5.26
Total		**69.63 ± 11.26**	**49.26 ± 8.74**	**3.10 ± 3.35**	**3.43 ± 3.40**	**6.44 ± 5.32**	**5.74 ± 4.88**
U1	Mature	52.22 ± 14.81	18.89 ± 7.82	1.44 ± 2.48	1.95 ± 2.91	2.70 ± 3.20	3.64 ± 4.32
U2		50.00 ± 7.07	22.22 ± 10.93	1.15 ± 2.36	1.41 ± 2.78	2.65 ± 3.22	2.33 ± 2.89
U3		52.22 ± 10.93	24.44 ± 14.24	1.07 ± 2.33	1.32 ± 2.55	2.57 ± 3.40	1.99 ± 2.39
Total		**51.48 ± 10.99**	**21.85 ± 11.11**	**2.16 ± 3.06**	**1.56 ± 2.75**	**2.64 ± 3.26**	**2.65 ± 3.36**

Note: SP = shooting percentage, RP = rooting percentage, SN = shoot number, SL = shoot length, RN = root number, RL = root length. Values are expressed in mean ± SD.

The rooting experiment was designed to create a quantitative description of the effects of the root-inducing hormone on the rooting ability of young and mature stem cuttings of *Acacia* species. Auxin-treated cuttings responded better than the non-treated cuttings for most of the rooting traits tested. Hormone treatment significantly increased the shooting percentage, rooting percentage, root number, and root length of *A. auriculiformis* and *A. mangium*. However, *A. aulococarpa* and *A. crassicarpa* did not respond well to the treatment, and the mean separation value did not reveal any significant differences between them. Overall, the highest rooting percentage of 72% and the greatest number of axillary roots of 4.4 occurred when *A. mangium* cuttings were treated with Seradix 3. Even though *A. auriculiformis* recorded the highest shooting percentage and rooting percentage (62% and 70%, respectively) in cuttings treated with 1000 ppm of IBA, the highest number of roots and the longest root length were achieved in those treated with Seradix 3 (Table 7).

Table 7. Mean values of some rooting ability traits of hormone-treated cutting sources of four Acacia species.

T	Source	SP (%)	RP (%)	SN	SL (cm)	RN	RL (cm)
Acacia mangium							
0 (control)		58.89 ± 16.91	81.11 ± 6.01	1.03 ± 1.30	2.65 ± 2.88	1.82 ± 1.62	3.76 ± 2.21
1000 ppm	Young	75.56 ± 14.24	88.89 ± 7.81	2.64 ± 1.98	5.50 ± 3.47	4.76 ± 2.43	4.18 ± 2.22
Seradix 3		85.56 ± 11.30	91.11 ± 7.82	5.62 ± 2.55	7.63 ± 4.20	6.03 ± 2.28	6.58 ± 3.13
0 (control)		31.11 ± 16.15	37.78 ± 13.01	0.43 ± 0.69	1.48 ± 2.82	0.67 ± 0.97	1.13 ± 1.48
1000 ppm	Mature	47.78 ± 13.94	51.11 ± 11.66	1.12 ± 1.23	2.80 ± 3.54	1.82 ± 1.91	2.15 ± 2.31
Seradix 3		48.89 ± 13.64	53.33 ± 11.18	1.69 ± 2.21	3.57 ± 4.09	2.82 ± 2.35	3.04 ± 2.75
Mean							
0 (control)		**45.00 ± 1.49**	**59.44 ± 24.37**	**0.73 ± 1.08**	**2.07 ± 2.91**	**1.25 ± 1.45**	**2.45 ± 2.29**
1000 ppm		**61.67 ± 19.78**	**70.00 ± 21.69**	**1.88 ± 1.81**	**4.15 ± 3.75**	**3.28 ± 2.63**	**3.16 ± 2.48**
Seradix 3		**67.22 ± 22.44**	**72.22 ± 21.57**	**3.66 ± 3.09**	**5.60 ± 4.61**	**4.43 ± 2.81**	**4.81 ± 3.43**
Acacia auriculiformis							
0 (control)		76.67 ± 10.00	76.67 ± 10.00	1.61 ± 1.47	5.54 ± 4.16	2.98 ± 2.39	4.73 ± 3.35
1000 ppm IBA	Young	86.67 ± 11.18	88.89 ± 14.53	5.06 ± 2.17	11.88 ± 5.87	6.81 ± 2.90	7.32 ± 3.53
Seradix 3		80.00 ± 12.25	76.67 ± 10.00	8.26 ± 4.51	7.84 ± 4.81	13.13 ± 7.16	10.59 ± 6.23
0 (control)		62.22 ± 9.72	46.67 ± 10.00	0.81 ± 0.81	3.16 ± 3.32	1.46 ± 1.45	3.49 ± 3.16
1000 ppm IBA	Mature	73.33 ± 12.25	48.89 ± 11.67	3.32 ± 2.46	5.09 ± 3.97	4.66 ± 3.26	6.69 ± 4.75
Seradix 3		66.67 ± 13.3	32.22 ± 9.72	4.20 ± 3.50	3.90 ± 3.47	5.18 ± 3.97	4.98 ± 4.29
Mean							
0 (control)		**69.44 ± 12.11**	**61.67 ± 18.23**	**1.21 ± 1.25**	**4.36 ± 3.93**	**2.21 ± 2.11**	**4.11 ± 3.30**
1000 ppm IBA		**80.00 ± 13.28**	**68.89 ± 24.23**	**4.19 ± 2.47**	**8.49 ± 6.05**	**5.73 ± 3.26**	**7.01 ± 4.19**
Seradix 3		**73.33 ± 14.14**	**54.44 ± 24.79**	**6.23 ± 4.51**	**5.87 ± 4.63**	**9.16 ± 7.02**	**7.78 ± 6.03**
Acacia crassicarpa							
0 (control)		32.22 ± 17.16	45.56 ± 11.30	1.91 ± 3.02	4.69 ± 6.67	2.30 ± 2.90	2.73 ± 3.47
1000 ppm IBA	Young	30.00 ± 15.81	44.44 ± 18.10	2.18 ± 3.47	2.23 ± 3.61	3.27 ± 3.93	3.94 ± 4.89
Seradix 3		35.56 ± 19.44	45.55 ± 13.33	1.54 ± 2.29	1.71 ± 2.69	2.81 ± 3.36	2.91 ± 3.44
0 (control)		10.00 ± 8.66	20.00 ± 7.07	0.17 ± 0.53	0.21 ± 0.72	0.44 ± 1.07	0.41 ± 1.00
1000 ppm IBA	Mature	13.33 ± 10.00	23.33 ± 11.18	0.44 ± 1.25	0.71 ± 1.99	0.94 ± 1.98	0.79 ± 1.59
Seradix 3		10.00 ± 10.00	20.00 ± 8.66	0.20 ± 0.78	0.34 ± 1.18	0.63 ± 1.43	0.86 ± 1.89
Mean							
0 (control)		**21.11 ± 17.45**	**32.78 ± 16.02**	**1.05 ± 2.33**	**2.46 ± 9.00**	**1.37 ± 2.38**	**1.58 ± 2.80**
1000 ppm IBA		**21.67 ± 15.43**	**33.89 ± 18.19**	**1.31 ± 2.75**	**1.47 ± 3.01**	**2.12 ± 3.32**	**2.38 ± 3.97**
Seradix 3		**22.78 ± 19.94**	**32.78 ± 17.08**	**0.87 ± 1.83**	**1.02 ± 2.18**	**1.72 ± 2.80**	**1.89 ± 2.95**
Acacia aulococarpa							
0 (control)		65.56 ± 12.36	46.67 ± 10.00	2.10 ± 2.55	2.48 ± 2.72	4.70 ± 4.44	4.15 ± 3.52
1000 ppm IBA	Young	74.44 ± 10.14	50.00 ± 5.00	2.91 ± 3.26	3.48 ± 3.22	5.94 ± 4.52	8.36 ± 5.96
Seradix 3		68.89 ± 10.54	51.11 ± 10.54	4.28 ± 3.80	4.34 ± 3.93	8.69 ± 6.08	4.72 ± 3.71
0 (control)		52.22 ± 13.01	21.11 ± 10.54	0.48 ± 1.42	0.76 ± 1.65	1.12 ± 1.36	1.34 ± 1.57
1000 ppm IBA	Mature	46.67 ± 10.00	24.44 ± 10.14	1.10 ± 1.99	1.73 ± 2.92	2.81 ± 3.15	3.69 ± 4.31
Seradix 3		55.56 ± 8.82	20.00 ± 13.23	2.09 ± 3.15	2.20 ± 3.36	3.99 ± 4.03	2.95 ± 3.23
Mean							
0 (control)		**58.89 ± 14.10**	**33.89 ± 16.50**	**1.29 ± 2.21**	**1.62 ± 2.40**	**2.91 ± 3.73**	**2.74 ± 3.6**
1000 ppm IBA		**60.56 ± 17.1**	**37.22 ± 15.26**	**2.00 ± 2.84**	**2.60 ± 3.14**	**4.38 ± 4.19**	**6.03 ± 5.69**
Seradix 3		**62.22 ± 11.66**	**35.56 ± 19.77**	**3.18 ± 3.65**	**3.27 ± 3.80**	**6.34 ± 5.66**	**3.83 ± 3.58**

Note: T = Treatment, SP = shooting percentage, RP = rooting percentage, SN = shoot number, SL = shoot length, RN = root number, RL = root length. Values are expressed in mean ± SD.

4. Discussion

Vegetative propagation can assist in maintaining superior genotypes, shorten the reproductive stage and encourage consistent flowering; overcome problems when using seeds, such as dormancy, germination, viability, and storage; and contribute to the genetic uniformity of trees in plantations [33]. However, many of these economically important

tree species have a low genetic or physiological capacity for adventitious root formation, especially when the tree has reached the maturity stage. This group of trees is considered recalcitrant to routine vegetative propagation via stem cuttings [3]. Thus, factors affecting the rooting of mature Acacia species cuttings, such as the juvenility of the donor plant and auxin treatment, were investigated. In the first part of the experiment, branch cuttings were force flushed for the formation of juvenile shoots to be used in the second rooting experiment and compared with the mature cuttings taken directly from the crown of the mother tree.

Results revealed that the bud break and shoot formation from the mature branches of Acacia species are highly variable among species, but not among clones within the same species. Bud break and shooting ability varied between species and clones, regardless of the age of the branch segment. Acacia species responded well to the force-flushing technique by producing healthy shoots with acceptable shoot length for the cutting experiment. However, shoot formation from *A. crassicarpa* was not very encouraging, and mature branches produced short shoots with a low survival rate in the nursery. To obtain enough cuttings for the rooting experiment, more branches had to be force flushed for *A. crassicarpa* so that more shoots were available, and only longer shoots with uniform size were selected for the rooting ability of the cutting experiment. However, in contrast to our study, shoots generated through the same technique, and the rooting ability of rejuvenated 35-year-old *Quercus* sp., were found to be highly variable and greatly depend on the type of clones [34]. In addition, it was shown that the shooting and rooting ability of forced-flush shoots depends on the tree age and height or position from which the branch segment was collected [35]. Some of the additional benefits of using this technique for rejuvenation include the fact that it can be accomplished under controlled growth conditions, and that optimization of the cultural condition is possible and easy for monitoring the shoot development [32].

Shoots from forced flushing of branch segments were reported to be ontogenetically juvenile and have enhanced the rooting ability of mature sources. This technique is one of the frequently attempted methods as an initial stage of pretreatment for micropropagation of mature tree species. For instance, epicormic shoots from the basal trunk of 40-year-old *Quercus rubra* were force flushed after cold treatment for 3 months in the growth cabinet. A 70- to 300-year-old *Q. robur* tree was also flushed for the same reason from the 30 cm branch segment. The newly developed flushed shoots were then used as explants in micropropagation. Rooting of these shoots under in vitro conditions was better compared to the adult materials [36]. The same technique was also found to be effective for micropropagation in *Q. robur* [37].

To further study the effects of in vivo rooting of flushed shoots, another experiment was also designed. Forced-flush shoots were further rooted under control conditions, and the rooting ability of these shoots was compared with stem cuttings harvested directly from the crown of the tree branches belonging to the same mother tree used in the force-flushing experiment. These stem cuttings were subjected to a rooting experiment without any initial rejuvenation and referred to as mature sources in the subsequent rooting study. To increase the productivity and effectiveness of rooted cuttings, materials used for cutting propagation usually were restricted to young materials because age reduces drastically the root ability of cuttings, regardless of species. Most of the mature cuttings in the study were not as responsive to rooting treatment as the young, rejuvenated cuttings. The unrooted cutting could not survive longer in the nursery condition in the absence of adventitious roots. The inability of acacia cuttings to survive longer with very limited potential for callus formation in this species has been addressed in few studies [38,39]. Different rooting abilities, both in juvenile and mature cuttings, and their response to hormone treatment might be due to the deficiency in endogenous promoters and increase in inhibitors of root inducers in the mature cuttings [38]. Cuttings are usually collected from coppice materials or forced flushing materials to be rooted under control conditions. A similar approach has been

adopted in this study. Young materials rooted better than the ones from mature cuttings for all *Acacia* species tested in this study.

A similar rejuvenation method has been tested for improving the rooting ability of 5- to 6-year-old *Acacia mearnsii* adult seed tree by using newly developed sprout from coppice materials [40]. They found that the young, rejuvenated cutting is rooted more easily with the use of rooting hormones. However, they noted that a better rooting was achieved in rooting powder containing NAA (1-Naphthylacetic acid) in the form of commercial rooting powder ABT, rather than IBA, which was used in this study. As indicated in the study, the incorporation of the hormone IBA had positive effects on enhancing the rooting ability of Acacia cuttings regardless of the source (maturity) of cuttings. However, the best root production of cuttings was obtained when Acacia cuttings were treated with Seradix 3. Untreated cuttings generally showed lower rooting and shooting percentages in the present study. These results are in agreement with the findings of other researchers, such as in *A. auriculiformis* [41], *Acacia catechu* [42], *Acacia cyanophylla* [43], *Acacia* hybrid (*A. mangium* × *A. auriculiformis*) [44], *A. mangium* [38,45], and *Acacia senegal* [46], where the IBA-treated cuttings show better root formation and growth development. In accordance with our study, Seradix 3 was also found to be effective in improving the rooting ability of *A. mangium* cuttings in a previous study [38]. Similarly, the 6-year-old *A. mangium* coppice treated with Seradix 3 showed a lower rooting percentage than the average rooting percentage recorded for that particular species in our study, but it exhibited a better rooting percentage compared to its adult material. In contrast, treating the base of cuttings with Seradix 3 had no overall effect on the rooting rates, nor on root length, in two sub-experiments conducted by Monteuuis et al. [39]; however, an increase was observed in root number. The variation between the studies might be due to the difference in the part of the stem cuttings used in the rooting experiment. Although this study did not address the effect of this factor, the previous study utilized cuttings from terminal shoot and nodal cuttings, whereas only nodal cuttings were used in this study. Nodal cuttings were found to be less responsive to adventitious rooting in *A. mangium* cuttings [38].

The fundamental mechanism that can trigger or regulate the formation of adventitious roots from stem cuttings, especially those derived from woody species, is a complex physiological, genetic, and environmental process and still unknown [47]. The successive phase in the development and division of parenchyma cells in adventitious root formation can be summarized as dedifferentiation, induction, outgrowth in the stem, and outgrowth from the stem [48]. Dedifferentiation occurs when the cells are activated by wound-related compounds and auxins. This is followed by the induction phase, where the auxin stimulates the formation of root meristemoids. Root primordia elongate and finally grow out of the stem during the adventitious rooting process [3]. The application of auxins at the wounded site of cuttings will enhance the formation of a layer of parenchyma cells into the callus and induce the formation of roots from the cuttings. Maturation of forest tree species delays the root induction from cuttings, decreases the quality and ability of adventitious root produced, and decreases the growth rate of the cuttings [49]. The degree of lignifications in the primary phloem of mature, woody cuttings affects the rooting ability of cuttings by hindering the root primordia tissue from developing root initials [50]. Rooting of cuttings from woody trees showed that the degree of lignifications in the primary phloem affects the rooting ability of cuttings from trees by hindering the root primordia tissue from developing root initials. An increase in the production of rooting inhibitors; a decrease in the phenolic content, which is an auxin cofactor in root initial of cuttings; and the presence of anatomical barriers, such as a sclerenchymatous sheath, are some of the other important factors related to the poor rooting response of mature cuttings [51].

Rooting hormone can exert influence on polysaccharides hydrolysis and increase the physiologically active sugar in the cuttings. More energy in the cuttings can stimulate the formation of adventitious roots from meristematic tissues and root primordia [52,53]. Thus, rooting hormone is commonly applied exogenously to cuttings to promote better hormonal balance if the endogenous auxin content is not readily available or insufficient

in the cuttings. The rooting response to the supplied hormone in the cutting depends on the endogenous auxins in the species and varies greatly with the age of the donor plant, physiological factors, the genetic constituent of the selected mother plant, and time of year of collection [54]. Some woody species will not induce adventitious roots even when the cuttings are treated with root-promoting hormone auxins such as in *Quercus rubra* [1] and *A. leprosa* [27]. Cuttings collected from 10-year-old *A. leprosa* failed to initiate any root even when treated with auxin IBA. Similarly, 10-year-old *A. cyanophylla* showed a very poor rooting response to auxin treatment [27]. Regeneration through coppicing and pollarding for *A. mangium* is poor and it has poor rooting ability [55]. The rooting percentage of *A. manguim* cuttings decreased significantly with the increasing age of the stock plant. It was noted that cuttings taken from 6- and 12-month-old seedlings rooted better and faster compared to the cuttings obtained from old stock plants [51]. The addition of one step of rejuvenation, through the initiation of sprout production through partial or complete felling at 30 cm above ground of adult *A.* hybrid (*A. mangium* × *A. auriculiformis*) tree enhanced the rooting ability of rejuvenated cuttings. 12-week-old sprouts treated with 50 or 100 ppm IBA rooted better within 2–3 weeks, with 70%–95% of them rooting, compared to 40%–55% in untreated cuttings [44]. Cuttings collected from 1- to 30-year-old *Quercus robur* trees showed wide variation in their rooting ability. The rooting percentage of the cuttings decreased from 89%, obtained in 1-year-old cuttings, to 11%, recorded in 30-year-old cuttings [56].

5. Conclusions

Our findings provide evidence that mature *Acacia* species are capable of resprouting under some modified environmental conditions, suggesting the possibility of rejuvenation through the force-flushing technique. Two-step rejuvenation could be applied to improve the effects of aging on the rooting ability of cuttings from the adult donor plant. Based on the findings, the following conclusions can be drawn: (1) Propagation of *Acacia* sp. cuttings was greatly influenced by the age of the donor plant and the root-inducing hormone. (2) Rejuvenation technique through force flushing has less effect in stimulating the bud break and rooting response of branch cuttings from *A. crassicarpa* and *A. aulococarpa*. (3) Successfully rejuvenated planting material showed vigorous growth both in terms of shooting and rooting performance. In addition, the application of the rooting hormone on rejuvenated cuttings enhanced the rooting ability of *Acacia* species. Overall, the study provides additional information on strategies for sustainable production of planting material of *Acacia* species selected from mature elite individuals. Further study is needed on the modification of techniques to determine the limiting factor of age on mature cuttings of *A. crassicarpa*, because they do not respond well to any treatment tested in this study.

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