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Macropropagation of macadamia nut (*Macadamia integrifolia* Maiden & Betche) through shoot cuttings

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ABSTRACT

Macadamia integrifolia, locally known as Macadamia, is a tree species native to Queensland, Australia. The species is also recognized as an appropriate option for land rehabilitation in Indonesia, with one location being the Lake Toba catchment area in North Sumatra. This species is planted on a large scale because of its adaptability and relatively short harvesting time. The high demand for macadamia seeds cannot be supported by the species' ability to regenerate, as natural regeneration is slow due to the thick and hard seed coat. As a result, a short-term, quality propagation technique that the community can easily use, such as shoot cuttings, is required. This study aimed to determine the success of the shoot cutting technique and the adventitious roots development on macadamia cuttings. The cutting material was obtained from a healthy mother tree from the Sipiso-piso garden, North Sumatra. The research was designed by using the factorial complete block design with two factors randomized design with two factors namely cutting media (A) and auxin application (B). The media treatment consisted of sand and topsoil mixture (A1: 1/1 v/v), pure sand (A2), and pure topsoil (A3), while the auxin application consisted of 0 g (B1), 200 ppm (B2), and 600 ppm (B3). All treatments were carried out with 3 replications, each consisting of 5 individual cuttings. The results showed that macadamia shoot cuttings varied survival rate ranging from 33.33 % to 86.66% with the rooting percentage ranging from 6.66%-40%. The highest survival rate was found in the top soil mixture: sand and without auxin treatment (88.66%) while the lowest was found in sand and without auxin (33.33 %). The cutting media and auxin application did not significantly affect all the observed parameters. Based on histological root observations, the adventitious roots of Macadamia form from the wound-induced root with a process that begins with callus formation and ends with the advent of adventitious roots. This research found that the juvenile level of the cutting material used is considered a very important factor in the success of macadamia shoot cuttings.

Keywords: *Macadamia integrifolia*, Macropropagation, Cutting, Auxin

1. Introduction

Macadamia integrifolia Maiden & Betche (Proteaceae) is a broadleaf tree species native to the Australian rainforest mainland (Queensland) and is a valuable source of nuts with the local name Queensland nut, macadamia nut, Australian bush nut [1]. This species prefers areas with high humidity and rainfall, warm, tropical climates but cannot tolerate cold temperatures [2]. *M.integrifolia* is the more common commercial species and grows at latitudes of 25-28 [3]. This species is considered endangered in the wild due to habitat fragmentation [3,4]. This species was classified as vulnerable by the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species since 2020 [5]. In Thailand, Macadamia has been tested and cultivated in the highlands of northern Thailand for more than 40 years [6]. Meanwhile, Macadamia has not been widely cultivated as an agricultural commodity in Indonesia. In fact, the tree can be found in several places in Indonesia, such as in several research locations, the Cibodas Botanical Gardens, and the Sipiso-piso Forest, North Sumatra.

Macadamia nuts have become one of the foods humans consume, providing minerals and energy sources [7]. Macadamia nuts are high in nutrients such as K, P, Mg, Cu, Mn, and Zn [8], as well as niacin (B3), thiamine (B1), retinol (A1), and riboflavin (B2) [9]. Macadamia nuts are also rich in oil, therefore being one of the world's highest-ranked nuts and the best luxury grade [9]. Macadamia oil also includes significant antioxidants, which benefit the skin and cosmetics [6,10]. Because of its high economic and ecological values and its fast character, this species offers the potential to be developed for important land rehabilitation [11]. The high price of macadamia nuts, its adaptability, and relatively short harvesting time make this species popular for planting. Low and inconsistent yields remain an issue for this economically valuable plant [12], making it difficult to provide seeds for generative propagation. Macadamia fruit production is limited by several factors, including pollen compatibility [13,14], irrigation sufficient [15,16], dry weather and heat [17], cross-pollination in flowers [18], and the presence of pollinators [19], where previous research indicates that honey bees are the only major pollinators [20]. The macadamia seeds are characterized as semi-recalcitrant, are very erratic and low, and germinate asynchronously [21]. The seeds of this species are also semi-recalcitrant, characterized by a short storage period, sensitivity to removal of water and drying process, and usually have a larger shape and seed coat.

Macadamia vegetative propagation is a recommended alternative for overcoming various problems related to seed availability. This technique can use various plant parts, including stems, roots, shoots, and leaves, where the seeds produced have characters similar to the parent plant [22]. Several studies reported that Macadamia can be propagated by grafting [23], cuttings [24], and tissue culture [25,26]. The success of macadamia cuttings using multiple organs, such as seedling stems, roots [27,28], and propagule tips [24], has also been reported. Semi-hardwood cuttings under a mist system have been used for developing clonal macadamia trees, with varying success rates. Based on the successful report of vegetative propagation on Macadamia, this study aimed to determine the success of the shoot cutting technique and the adventitious roots development on Macadamia cuttings. The success of this macadamia macro propagation technique is expected to provide an alternative for the community by providing easy-to-use propagation methods and producing uniform and good-quality seedlings.

2. Method

2.1. Cutting material

Macadamia shoots were collected from individual 9-year-old mature trees growing at Sipiso-piso Experimental Garden, Toba Samosir district, North Sumatra Province, Indonesia. The orthotropic shoot was taken from the trees and stored in an ice box before planting. The cutting media consisted of a 1:1 v/v sand and topsoil combination. Commercial auxin was utilized to improve root capacity. A shading net, propagator box, tray, and cutting scissors were all employed as instruments for this study.

The shoot cutting material was prepared using the KOFFCO technique [29] with minor modifications in the Forestry Faculty of Universitas Sumatra Utara, Indonesia, nursery. Materials for cutting were obtained from orthotropic branches and cut to a length of 7-10 cm before being promptly stored in a container of water. The cuttings were then repeatedly cleansed with sterilized water before being planted in a pot tray with sterilized material and placed in propagation boxes as directed. The propagation boxes were stored in a greenhouse, and the light intensity decreased to around 50%. Watering was done twice a day, once before 10 a.m. and once in the late afternoon after 4 p.m., to ensure that the seedlings received adequate water during the first growing stage. A complete factorial block design with two factors was used in this cutting experiment. The first factor was the treatment of cuttings media composition (factor A), including (i) sand and topsoil mixture with a ratio of 1:1 (A1), (ii) sand (A2), and (iii) topsoil (A3). The second factor was the treatment of commercial auxin addition (as plant growth regulator) consisting of (i) without commercial auxin (0 g) as B1, (ii) commercial auxin 200 ppm (B2), and (iii) commercial auxin 600 ppm. All treatments were carried out with 3 replications, each consisting of 5 individual cuttings.

2.2. Data analysis

Cuttings were observed from when the cuttings were planted until 12 weeks later. The parameters studied were cutting survival %, rooted cutting percentage, primary and secondary root length, and primary and secondary root number. Cuttings were assessed monthly for three months until no new roots emerged. At the end of each month, the number of rooted cuttings, the number of roots, and the length of roots were recorded. Roots from the cuttings are also observed in root histology observations. The data obtained was then analyzed using Statistical Package for the Social Sciences (SPSS) software version 25.0 [30] to obtain the ANOVA (analysis of variance) value. If the results obtained significantly affected the observed parameters, further testing was carried out using DMRT (Duncan Multiple Range Test) with a rate of 5% [31].

3. Results and Discussion

Morphological observation of shoot cuttings is an important step in determining the success of vegetative propagation of woody plants. This observation involves observing physical changes and growth during the rooting process, such as root production, shoot growth, and leaf condition at 12 weeks after, all cutting showed good cutting growth, healthy shoots and leaves indicated by the emergence of new shoots, increased number of leaves, presence of the root and fresh cuttings condition (Figure 1).

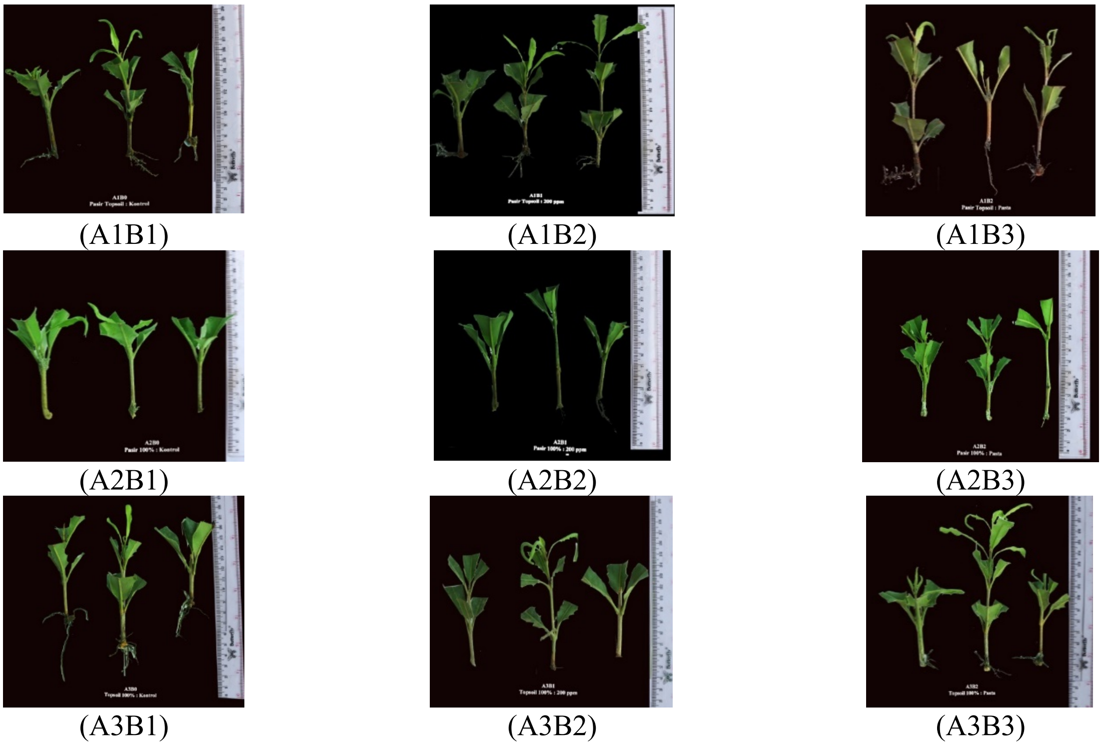

Figure 1. Growth of *Macadamia integrifolia* cuttings at 12 weeks after planting with various treatments: mixture of top soil: sand and without Rootone-F (A1B1), mixture of top soil: sand and Rootone-F 200 ppm (A1B2), mixture of top soil: sand and Rootone-F (A1B2), sand and without Rootone-F (A2B1), sand and Rootone-F 200 ppm (A2B2), sand and Rootone-F 600 ppm (A2B3), top soil and without Rootone-F (A3B1), top soil and Rootone-F 200 ppm (A3B1), top soil and Rootone-F 600 ppm (A3B2).

The survival percentage of macadamia cuttings varied from 33.33%-86.66% (Table 1). The survival percentage of this research was higher than that of the previous research by [32], which was only 10-37%. The juvenility of the donor plant might cause the variation in the survival percentage of macadamia cuttings. The level of juvenile cuttings material affects the success of cuttings [33], the high number of shoots and roots in species in the developmental stage is related to their physiological age or juvenile level. This juvenile level affects the phenol content of the material. Polyphenols are thought to be one of the components of complex compounds that are included in enzymatic factors in cellular tissue, where root initiation occurs when ortho-dihydroxy phenol reacts with the addition of auxin and enzymes, thereby accelerating the process of respiration and cell mitotic division, and subsequently leads to tissue and cell differentiation. The juvenility may be related to low levels of rooting inhibitors and high levels of photosynthates, but as the plant grows older, the inhibitor levels increase [32]. However, the overall survival rate of this study was higher compared to another research on Macadamia conducted by [32], which used materials from a 12-year-old tree, and only 10-37% of cuttings survived.

The percentage of rooted cuttings varied from 6.66% to 40% in various treatment combinations, with the highest percentage of rooted cuttings reaching 40% which was found in two different treatment combinations (Table 1), namely cuttings on topsoil: sand (1:1) media with auxin 200 ppm (A1B2) and cuttings on topsoil media without auxin (A3B1) suggested the difference in planting media used could be a factor in root formation on cuttings. This pattern was also supported by the result of ANOVA, which showed that cutting media affected the length of the primary root and the number of secondary roots (Table 2). According to [34], the rooting medium could affect the development of roots and shoots. The high porosity of the sand could form a suitable temperature and aeration for the growth of cutting roots. According to [35], the temperature in the root zone affects root initiation in stem cuttings. In addition, the mixture of topsoil and sand can also improve the suitability of the planting media because it has a more complex texture and structure and provides nutrients for emerging roots. This mixed topsoil and sand media was also reported by [36] as the best cutting medium for olive (*Olea europaea*).

Table 1. The average value of the parameters of *Macadamia integrifolia* shoot cuttings after 12 weeks of planting

Parameter	Treatment								
	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3	A3B1	A3B2	A3B3
Survival percentage (%)	86.66	53.33	40.00	33.33	46.66	46.66	66.66	46.66	60.00
Rooting percentage (%)	33.33	40.00	27.00	20.00	20.00	20.00	40.00	6.66	27.00
Number of leaves	1	3	2	2	1	1	2	1	2
Shoot height (cm)	1.36	4.9	2.23	2.81	2.27	1.67	2.17	1.11	2.20
Number of primary roots	2	3	4	2	2	1	4	1	2
Number of secondary roots	3	10	5	0	2	3	4	0	2
Primary root length (cm)	1.12	2.82	2.66	0.61	0.78	0.90	2.12	0.06	0.70
Secondary root length (cm)	0.09	0.50	0.33	0.00	0.04	0.20	0.22	0.00	0.70

Mixture of top soil: sand and without Rootone-F (A1B1), mixture of top soil: sand and Rootone-F 200 ppm (A1B2), mixture of top soil: sand and Rootone-F (A1B2), sand and without Rootone-F (A2B1), sand and Rootone-F 200 ppm (A2B2), sand and Rootone-F 600 ppm (A2B3), top soil and without Rootone-F (A3B1), top soil and Rootone-F 200 ppm (A3B1), top soil and Rootone-F 600 ppm (A3B2).

The plant growth regulators could also affect the rooting of the cuttings [37]-[39]. Auxins contained in growth regulators contribute to cuttings' root formation. The addition of exogenous auxin was reported to increase rooting and the number of roots [22]. Auxins play a role in mobilizing sugars and nutrients and promoting starch hydrolysis at the cutting base [40]. In addition, during auxin transport and cell division, auxin has a major role in selective proteolysis and loosening of the cell wall by inhibiting receptor-transporter protein 1 (RTP1) and auxin-binding protein 1 (ABP-1) [41]. However, the success of cuttings in forming roots without plant growth regulators in the present study indicated that the application of plant growth regulators was not an absolute determining factor in root formation on cuttings. This is supported by the results of ANOVA, which showed that the addition of plant growth regulators did not significantly affect the various parameters observed ($P > 0.05$) (Table 2). Similar results were also reported by [22], where treatment without adding growth regulators succeeded in forming roots on *Cotylelobium melanoxylon* cuttings.

The planting media and plant growth regulator factors, the level of juvenile of cuttings used, the nutritional content (carbohydrates, iron, manganese, and zinc) in the cuttings [22], and environmental conditions including temperature, light, and humidity [42] are also factors that influence the success of root formation, growth and development of cuttings. Cuttings derived from juvenile plants generally produce higher quality roots [43,44], while cuttings derived from mature trees fail to produce roots [45,46]. According to [32], the rooting ability of juvenile cuttings was thought to be associated with a high rate of photosynthesis and a low level of root inhibition. At the same time, the mature donor plants have a negative effect on the rooting performance of the cuttings, which may be due to the presence of sclerenchyma tissue in the phloem of mature trees that inhibits root emergence [47]. The low rooting ability of old donor plants was also reported by [48].

Table 2. The results of the analysis of the variance (ANOVA) of media treatment and plant growth regulators (Rootone-F) on the growth parameters of *Macadamia integrifolia* cuttings

Parameter	Treatment		
	Media	Plant Growth Regulator	Media x Plant Growth Regulator
Survival percentage (%)	0.103^{ns}	0.222^{ns}	0.072^{ns}
Rooting percentage (%)	0.348^{ns}	0.603^{ns}	0.405^{ns}
Number of leaves	0.616^{ns}	0.992^{ns}	0.133^{ns}
Shoot height (cm)	0.534^{ns}	0.669^{ns}	0.194^{ns}
Number of primary roots	0.200^{ns}	0.493^{ns}	0.215^{ns}
Number of secondary roots	0.054^{*}	0.709^{ns}	0.231^{ns}
Primary root length (cm)	0.049^{*}	0.942^{ns}	0.148^{ns}
Secondary root length (cm)	0.287^{ns}	0.606^{ns}	0.495^{ns}

Note: *: significant at 5% level probability; ns = not significant at 5% level probability

Based on the histology observations, it is known that the root formation of macadamia cuttings begins with the formation of meristem cells that develop in the cambium and then form callus that emerges from the surface of the base of the stem (Figure 2). Furthermore, primordial roots will be formed as the callus develops. The emergence of primordial roots is followed by adventitious roots, which are the last form of growth in root formation on macadamia cuttings. A similar root formation process was also found in some shoot cuttings, including *Cotylelobium melanoxylon* [22] and *Dryobalanops aromatica* [21].

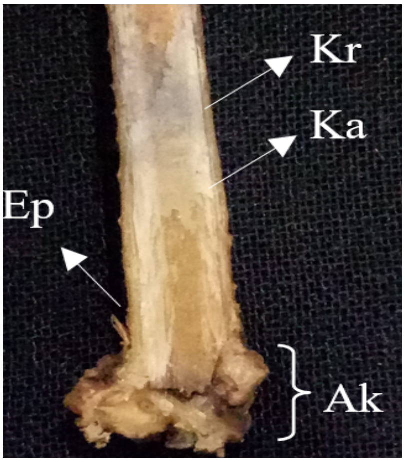

Figure 2. Histological analysis of roots on shoot cuttings of *Macadamia integrifolia*: cambium (ka), epidermis (ep), adventitious root (Ak).

The wound at the cutting site is the initial place for root growth, which ends with the formation of adventitious roots. According to [49], the rapid increase in jasmonic acid reaches its peak 30 minutes after cutting, where the presence of this peak correlates with adventitious root formation [50]. High levels of auxin and low levels of cytokinin promote the adventitious roots. Biosynthesis, transport, degradation, and conjugation can regulate auxin levels. The reduction of one of these factors is known to change adventitious roots [51]. Based on this, the different root growth on each cutting was thought to be caused by many factors, and internal factors in different cutting materials might have affected root emergence.

4. Conclusions

Macadamia integrifolia shoot cuttings showed varying rooting percentages from 6.66 to 40%. The interaction between cutting media and auxin addition did not significantly affect all parameters, but cutting media affected the length of the primary root and the number of secondary roots on macadamia shoot cuttings. The cutting media is considered a very important factor in the success of rooting. Histological observations of the roots showed that the adventitious roots of Macadamia belonged to the wound-induced root, which is a process that begins with callus formation and is followed by adventitious root formation.

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