

Conservation of tropical tree biodiversity through macropropagation by shoot cutting of *raru* (*Cotylelobium melanoxyton*), a highly utilized dipterocarp in North Sumatra, Indonesia

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Abstract. Susilowati A, Hartini KS, Elfiati D, Rachmat HH, Kusuma YS, Sinaga MZE, Suhartati T. 2020. Macropropagation by shoot cutting of *raru* (*Cotylelobium melanoxyton*), a highly utilized dipterocarp in North Sumatra, Indonesia. *Biodiversitas* 21: 724-730. *Cotylelobium melanoxyton* (Hook.f.) Pierre, or locally named *raru*, is one of native tree species from North Sumatra that has many uses for its wood and medicinal purposes for anti-diabetic, anti laxative and blood coagulation. *C. melanoxyton* has been experiencing short supply due to its limited natural distribution, forest degradation, illegal harvesting for its wood and bark and uncertain fruiting season of the species. Along with these alterations, *C. melanoxyton* population has been decreasing. The objective of this research was to determine the effectiveness of *C. melanoxyton* propagation by shoot cutting. Randomized factorial design with 2 factors was employed in this research. The first factor (factor A) was the cutting media (i.e. topsoil only; topsoil and sand with proportion of 1:1 v/v), and the second factor (factor B) was commercial auxin addition (i.e. 1g/100cuttings; without auxin). The observed parameters were survival percentage, rooted percentage, number of primary and secondary root, length of primary and secondary root and root histology. The results showed that the interaction between media and auxin addition did not have any significant effect on all tested parameters. The average survival percentage was 70-90% and rooting percentage was 50-90%. Adventitious root formation on *C. melanoxyton* cutting was originated from cell differentiation on wounded area near cambium, followed by root primordia formation and root meristem formation. The success of artificial propagation technique for this species will provide basic practice of *C. melanoxyton* cultivation and may contribute to the effort of ex situ conservation for valuable and threatened tree species.

Keywords: *Cotylelobium melanoxyton*, *raru*, root ability, shoot cutting, vegetative propagation

INTRODUCTION

Cotylelobium melanoxyton (Hook.f.) Pierre, or locally named *raru*, is a small (less than 10 m) to large-sized tree up to 50 m tall. They are frequently have twisted bole, sometimes cylindrical and straight, branchless for up to 30 m but often forking at lower height with diameter up to 160-200 cm. The trees have small and rounded buttress roots. For a long time, *C. melanoxyton*'s woods have been used as raw materials by local people in Tapanuli District, North Sumatra Province, Indonesia for constructing houses, bridges, and boats (Susilowati et al. 2019) because they have high quality and they are very durable and resistant to termites and teredos. Beside their wood, *C. melanoxyton*'s bark is also used for the mixture of *Bataknese* alcoholic drink called 'tuak' to accelerate fermentation. The bark of *C. melanoxyton* contains *ampelopsin* F., *isoampelopsin* F., ϵ -*viniferin*, *vaticanol* A, E, G, *lyoniresinol* (Matsuda et al. 2009). The bark also contains *flavonoids* as antidiabetic, *tannins* and *saponins* compounds (Pasaribu et al. 2007) which are useful as an

antimicrobial (bacteria and viruses) and potential endophytic fungi as a source for medicine (Hasanah et al. 2017). *C. melanoxyton*'s leaves are also used to treat wounds by the local people. According to Soerianegara and Lemmens (1994), *C. melanoxyton*'s leaves and bark are also used as anti-diabetic drugs, and as medicine to treat diarrhea and malaria. In Thailand, *C. melanoxyton* has been used in traditional Thai medicine as an astringent, anti-laxative, and blood coagulation agent (Matsuda et al. 2009).

Different from other dipterocarp species, *C. melanoxyton* has specific geographic distribution because it is only found in Southeast Asia mainland (Thailand, Indonesia, and Malaysia). In Indonesia, *C. melanoxyton* can be found in Sumatera, Kalimantan, Maluku, and Papua. Particularly in North Sumatra Province, *C. melanoxyton* can be found in Central Tapanuli, Simalungun, dan North Tapanuli districts (Pasaribu 2007). The disjunctive distribution of this species has been also threatened by habitat fragmentation. Harvesting of its bark by illegally debarking and cutting woods without regeneration effort has decreased the population of this species (Susilowati et

al. 2019; Pasaribu 2007).

Cotylelobium melanoxylon has uncertain fruiting and flowering seasons. According to Appanah and Turnbull (1998), seeds of dipterocarp are produced in large quantities in periods of mass flowering once every 3 or 4 years. These periods are short, irregular and unpredictable. The seeds have a short viability period, a few weeks at the most, and are difficult to store or called recalcitrant seed. *C. melanoxylon*'s seed is also threatened by insect borers in their habitat. These conditions tend to cause the decline of *C. melanoxylon*'s population every year. The IUCN red list has classified *C. melanoxylon* with the Latin name of *C. melanoxylon* into a status of least concern (Barstow 2019), indicating that a rapid effort is required to protect this species from extinction. Artificial propagation of the species is one of the efforts to conserve *C. melanoxylon*.

The availability of appropriate propagation techniques is a major constraint to the domestication of some forest trees widely used by rural communities, including *C. melanoxylon*. Vegetative propagation techniques are the first steps in tree species domestication, offering opportunities for avoiding the problem of recalcitrant seeds (Susilowati et al. 2018; Rachmat et al. 2018) and producing clones in large quantity of the ortet for conservation purpose (Leakey 2004). In many dipterocarp species, vegetative propagation can provide an uninterrupted supply of high-quality planting stock at times when wildlings may be scarce because of the time gap since the last mass flowering (Priadjati and Rayyan 1998). Using part of plant, such as roots, stems, shoots, and leaves, will produce new planting stocks with similar genetic materials to their parent plant. Vegetative propagation using shoot cutting technique also facilitates the threatened species (Susilowati et al. 2019). The successful vegetative propagation using shoot cutting method as the effort of species regeneration has been reported in some species, such as for rare and endangered tree species (Rachmat et al. 2018), *Dipterocarpus cinereus* (Subiakto and Rachmat 2015), *Taxus sumatrana* (Susilowati et al. 2019) and *Dryobalanops sumatrensis* (Susilowati et al. 2018). There is no information regarding the artificial propagation of the species, yet mother trees as natural regeneration sources have been experiencing serious threats and declining rapidly in the wild. Shoot cutting is a method of plant propagation that is technically easy to do. This method can provide planting stocks of rare and endangered species, including *C. melanoxylon*, regardless of unregularly fruiting season and the scarcity of the mother trees. Based on this information, the use of shoot cutting technique is also expected to be able to contribute to solve *C. melanoxylon* regeneration problems. Therefore, this research is conducted to assess the effectiveness of *C. melanoxylon*'s propagation using shoot cutting technique.

MATERIALS AND METHODS

Materials

Approximately 1-year-old *C. melanoxylon* seedlings were collected from its natural population in Bona Lumban

forests, Central Tapanuli-North Sumatra, Indonesia (Figure 1). Information on the seedling age was based on local people information and the previous latest fruiting season that fall one year becomes the exploration conducted. Bona Lumban forest is located in Tukka Sub-district, Central Tapanuli District, North Sumatra Province, Indonesia and covers an area of 6.80 km². Central Tapanuli District has an average temperature of 26.40°C with the highest may reach up to 32.2°C while its lowest temperature is around 22.4°C. The average relative humidity was 82.50% and rainfall at around 12.1 mm/year (BPS 2016).

The forest in Bona Lumban Village is located in two types of land status, which are Protection Forest (*Hutan Lindung/HL*) and area for non-forestry uses (*Area Penggunaan Lain/APL*). The location of *C. melanoxylon*'s natural population in the Bona Lumban forest was generally hilly, with the distance among *C. melanoxylon*'s seedlings were far enough (Figure 2). To overcome the difficulties during their handling and transportation, *C. melanoxylon*'s seedlings were transported using bare-root technique. Bare-root technique has been reported to work for Dipterocarp family in order to overcome the long distance between cutting source and the greenhouse or nursery (Rachmat et al. 2018).

However, bare-root technique has some weaknesses. The risk of declining physiological quality of the seedling is substantially higher in bare-root than in containerized planting stock. It also requires a period of time for acclimatization. Due to this consideration, before they were used as cutting materials, the seedlings were initially conditioned in a greenhouse to avoid environmental stress due to differences in temperature and elevation between the research location and *C. melanoxylon*'s habitat. After 2 weeks of conditioning, *C. melanoxylon* seedlings were subsequently selected based on their health criteria, stem and leaf conditions. Healthy seedlings were indicated by being pests and diseases free, having a single stem with fresh green leaf. The cutting media were topsoil, and topsoil and sand (in ratio 1:1). Commercial auxin powder was used to promote root formation capacity. Shading net, propagator box, cutting scissors and pot tray were also used as tools in this research.

Methods

Shoot cutting technique and preparation were done according to KOFFCO technique (Sakai and Subiakto 2007) with minor modification in misting technique (Susilowati et al. 2017; Susilowati et al. 2018). The cutting materials were taken from orthotropic branches and made between two leaf segments (7-10 cm). The cutting materials were then immediately stored in water container. The leaves were trimmed into half or one-third of their original size to reduce water loss and stimulated root initiation. The basal of cut surface of the cuttings were cutting sideways (45°). The cuttings were then repeatedly washed using sterilized water. Before being used as treatment, auxin powder was diluted into distilled water until forming a paste with concentration of 10 mg/cuttings (Sakai and Subiakto 2007).

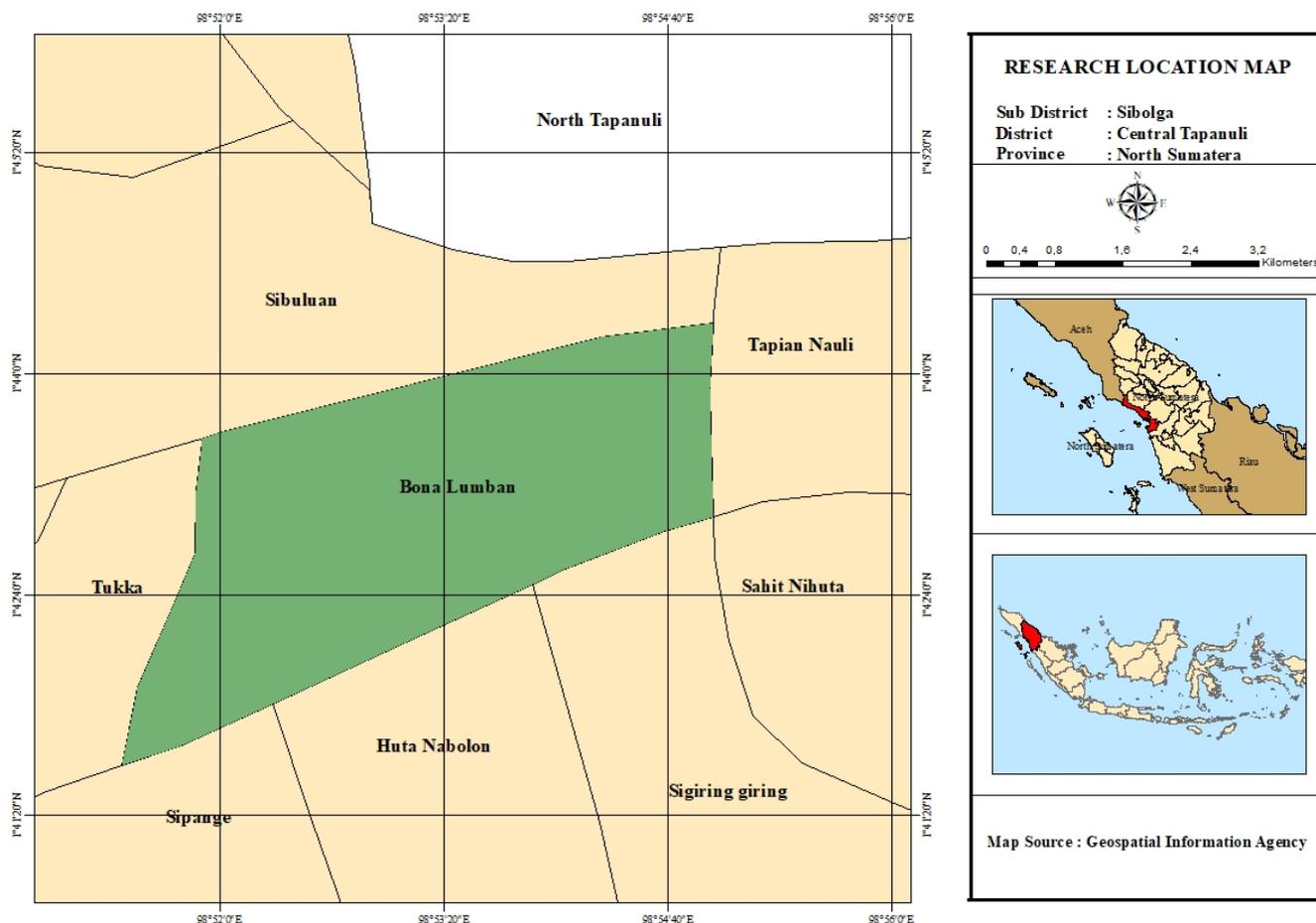


Figure 1. Cutting material (*Cotylelobium melanoxylo*) location in Bona Lumban Forest, North Sumatra, Indonesia

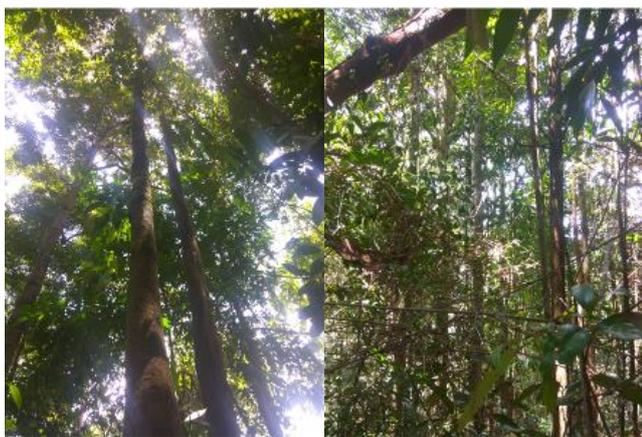


Figure 2. *Cotylelobium melanoxylo* tree and its natural population in Bona Lumban Forest, North Sumatra, Indonesia

The cutting materials were treated with commercial auxin paste then planted in pot-tray containing sterilized medium. All pot-trays with treated cutting were placed in propagation boxes according to the treatment. Watering was done twice in a week until 14 days after planting, once

a week for 14 days-30 days after planting. Cuttings media were sprayed with fungicide (i.e. Dithane M-45) before planting, to prevent fungal attacks.

Completely randomized design with two factors was set on this cuttings experiment. The first factor was cutting media consisting of A1 (soil) and A2 (sand: soil). While the second factor was plant growth regulator (PGR) consisting of B1 (auxin addition) and B2 (control). All treatments were done with 10 replications. Observation was done at 12 weeks after planting using parameters of survival percentage, rooting percentage, primary and secondary root length, and primary and secondary root number. Monthly assessments of cuttings were carried out for five months, i.e. until no new roots were formed. Fallen, infected and dead cuttings were removed daily to avoid spreading of diseases. Frequent weeding (once in a week) was done to avoid competition with the cuttings for nutrients. At the end of each month, the number of rooted cuttings, and the number of roots and length of roots were recorded. ANOVA values were obtained using statistical software.

RESULTS AND DISCUSSION

The effectiveness of cutting propagation is determined by the ability of root formation which varies within plant species. Some species show high rooting ability while others seem to have low ability of rooted cuttings. For dipterocarp species, Rachmat et al. (2018) stated that 67% of dipterocarps showed successful propagation through cutting techniques. The results of some cutting parameters of *C. melanoxylon* after 12 weeks are presented in Table 1. Until the end of observation, the cutting showed healthy indicators with new shoots and green leaves (Figure 3). The survival percentage of *C. melanoxylon* cutting varied from 70% to 90% and the rooting ability varied from 50% to 90% (Table 1). The high survival percentage and rooting ability of *C. melanoxylon* cutting in the end of observation could have resulted from the juvenility of cutting materials. Previous research conducted by Dumroese et al. (2011) demonstrated that the survival percentage, rooting ability, and the number of new roots and root lengths decreased as ramet ages increased. Cuttings from the juvenile parts of plant roots were much more easily done than those from adult plant tissues. Furthermore, Danu et al. (2011) stated that younger age of donor plants tended to contain high auxin and high C/N ratio that resulted in high rooting percentage. Kontoh (2016) also stated that the high number of developed shoots and roots of species could be attributed to their physiological age or juvenility. Hartmann and Hansen (1958) stated that better rooting results were obtained from cuttings taken from young trees at approximately 2-10 years of age as compared to the cuttings taken from trees that were over 40 years old. *C. melanoxylon* cutting material was originated from 1-year-old ramet. It may be considered as the cause for higher survival and rooting percentage of *C. melanoxylon* cuttings. Furthermore, the presence of carbohydrates in the cuttings helped the growth and development of roots as well as sustained the cutting while it was in the medium. Wiegel et al. (1984) have also reported that carbohydrates also enhanced metabolic activities that occurred at the base of the cuttings to aid cell division which brought about root initiation.

The highest rooting ability was produced by cutting inserted into soil media and treated with auxin (90%), while the lowest rooting ability was produced by cutting treated with soil media without auxin addition (50%). Variance analysis (Table 2) showed that there were no significant ($P > 0.05$) differences in interaction among media type and growth regulator, media and auxin addition, within all observed cutting parameters. The same findings were also found by Brodie (2003) in *Dryobalanops oblongifolia* and Susilowati et al. (2018) in *Dryobalanops sumatrensis* which stated that the interactions between media and exogenous auxin treatment did not influence the rooting ability of the cuttings and suggested that other factors (e.g. individual condition of plant stock) were more important for rooting. Aminah et al. (2006) also found that the juvenility of donor plants and cutting material diameters significantly influenced *S. parvifolia* and *S.*

macroptera cutting compared to media and auxin treatment interaction.

According to Leakey (2014), rooted cutting depended on the optimization of both pre-severance and post-severance factors. Post-severance factors for stem cutting were important to be mediated by photosynthesis, respiration, transpiration and carbohydrate mobilization. The other factors were the role of growth regulators like auxins, cutting size, leaf area, and physiological water stress. The physical and chemical characteristics of the medium such as pH, porosity, water holding capacity, and nutrient contents could be the factors stimulating root and shoot development. Physiological water stress was highly correlated with rooting media. These factors significantly influenced the rooting ability of stem cuttings of most ornamental plants. Generally, cutting inserted in soil media (A1) produces high value in cutting parameters (Table 1). The same results were also found by Magesa et al. (2018) i.e. red soil media produced the highest rooting percentage of the stem cuttings of hybrid coffee varieties. It might have resulted from the optimum moisture supply provided by the growing medium and the possible high carbohydrate to nitrogen ratio concentration in the cuttings. Another reason was that soil media had low pH of 4.0 and highest amount of micronutrients, such as iron, manganese, and zinc, which were known to stimulate rooting.



Figure 3. Cutting performance of *Cotylelobium melanoxylon* from different treatment after 12 weeks after cutting. A. A1B1, B. A1B2, C. A2B1, D. A2B2

Table 1. The average value of *Cotylelobium melanoxylon* shoot cutting parameters (12 weeks after cutting)

Parameters	Treatments			
	A1B1	A1B2	A2B1	A2B2
Survival percentage (%)	90	70	90	80
Rooting percentage (%)	90	50	80	70
Number of primary roots	2	2	3	1
Number of secondary roots	30	41	21	17
Primary root length	5.4	6.4	3.2	6.1
Secondary root length	0.37	0.28	0.33	0.48

Note: A1B1=soil media with auxin addition; A1B2=soil media without auxin addition; A2B1=mixed soil and sand media with auxin addition; A2B2=mixed soil and sand media without auxin addition

Table 2. The summary of variance number of primary and secondary roots, length of primary and secondary roots

Parameters	Treatments		
	Cutting media	Auxin	Cutting media × Auxin
Number of primary roots	0.796 ^{ns}	0.098 ^{ns}	0.446 ^{ns}
Number of secondary roots	0.291 ^{ns}	0.796 ^{ns}	0.614 ^{ns}
Length of primary roots	0.617 ^{ns}	0.446 ^{ns}	0.709 ^{ns}
Length of secondary roots	0.599 ^{ns}	0.837 ^{ns}	0.438 ^{ns}

Note: ns = not significant at 5% level of probability

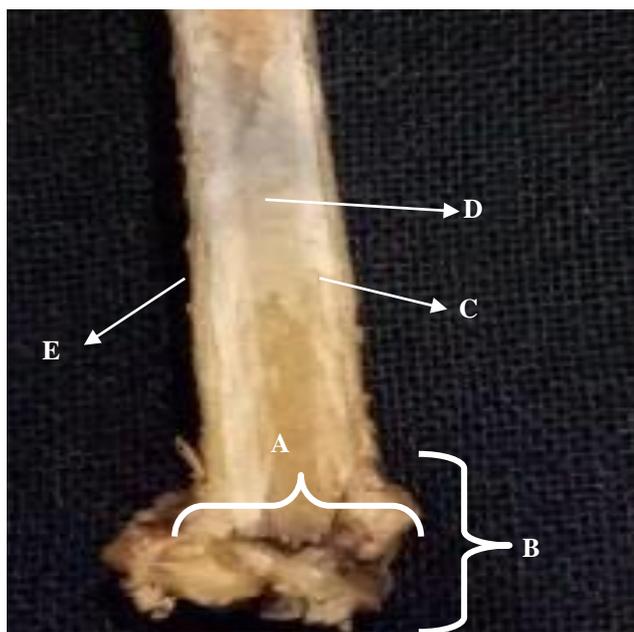


Figure 4. Root cross-section of *Cotylelobium melanoxydon* cuttings with 4.0x magnification. Note: A: basal of cut surface, B: roots zone, C: cambial zone, D: cortex, E: epidermis

Exogenous auxin treatment had been reported to increase the number of root primordia in the basal part of the cuttings, which led to increased rooting and root number. In this research, commercial auxin powder containing a mixture of auxins (IBA and NAA) was used. Davies and Haissig (1990) stated that mixtures of root promoting substances were sometimes more effective than either compound alone or adding a small percentage of certain phenoxy compounds to either IBA or NAA which increased rooting and produced root systems better than using phenyl compounds alone. Hormonal treatments had better-rooting responses than the control (without hormone). Auxins promoted starch hydrolysis and mobilized sugars and nutrients to the cutting base (Das et al. 1997). During cell division and auxin transport, auxins acted primarily through selective proteolysis and cell wall loosening with receptor protein transporting inhibitor response 1 and auxin-binding protein 1 (Da Costa et al. 2013). Although auxin influenced rooting ability of *C. melanoxydon*, this research also found that cutting without auxin treatment was also able to produce roots. The ability

of root formation without auxin addition in dipterocarp species also has been previously reported by Aminah (1990). Juvenility might be an important factor in the rooting potential of dipterocarp cuttings (Kurniawan 2011). Juvenile tissues of woody plants tended to have higher levels of endogenous auxins and were less differentiated and therefore were more prone to re-differentiation (Hartman et al. 2002).

Rooting was maximized when the cutting was photosynthetically active and produced assimilates for the development and elongation of root primordia (Leakey 2014). Adventitious roots of some easy-to-propagate woody plants were formed in longitudinal rows along the stem, with the positions being related to the anatomy of the underlying vascular tissue (Hartmann et al. 2002). Based on the histological observation, root formation of *C. melanoxydon* cutting was started with callus formation on wounded area, followed by root primordia formation, and ended with the appearance of an adventitious root. Root primordia were found in vascular tissue, followed by roots elongation through the cortex or emergence through the epidermis, and formed the adventitious root (Figure 4). In a study of six *Acacia baileyana* F. Muell genotypes, Schwarz et al. (1999) found that roots were initiated in internal callus and that all genotypes had similar stem anatomies, whereas the ability to form adventitious roots varied with genotype.

Physiologically, there are two major types of root formation in stem cuttings, i.e. preformed and wound-induced roots. Preformed or latent roots are initially present during stem development and lying dormant until stem cuttings were made and placed in the proper environmental conditions for further development and emergence as adventitious roots (Hartmann et al. 2002). While wound-induced roots are the major type of auxin response factor (ARF) in stem cuttings. Once the stem (or shoot) was removed from the plant (wounding), a series of wound responses occurred and de novo adventitious root regeneration proceeded (Hartmann et al. 2002). In the wounded areas, sealing off of the wound (protection from desiccation and pathogens) occurred by the production of suberized, protective cells. Cells begin to divide, and a layer of parenchyma cells (callus) is formed in the wound area. De Klerk et al. (2011) summarized the successive phases in rooting of apple microcuttings as dedifferentiation, induction, outgrowth in the stem, and outgrowth from the stem. The initial phase, dedifferentiation, is the activation of cells (to become competent) by wounding related compounds and auxin. The induction phase is the initiation of cell division (to become committed) where auxin stimulated the formation of root meristemoids. During outgrowth in the stem phase, meristemoids develop into typical dome-shaped root primordia (to become determined), and at this stage auxin (exogenously applied) becomes inhibitory. Root primordia elongate and develop during the differentiation phase and finally grows out of the stem during the last phase of the adventitious rooting process.

Based on adventitious root type and root formation process, *C. melanoxydon* is classified into wound-induced

type. Root formation was found in wounded areas. The process of wound-induced roots occurred immediately after cutting. In previous reports, it was determined that wound response signaling pathways were initiated at the base of the cutting (Schillmiller and Howe 2005), with a fast increase in jasmonic acid levels peaking 30 min after cutting (Rasmussen et al. 2015). The presence of this peak was correlated with adventitious root formation (Ahkami et al. 2009), and a short pulse of jasmonic acid increased adventitious root formation (Rasmussen et al. 2015). Adventitious root induction was promoted by high auxin and low cytokinin levels. Auxin levels could be regulated by biosynthesis, transport, conjugation, and degradation. Reductions in any of these also altered adventitious rooting (Ahkami et al. 2013). Auxin transport inhibitors significantly reduced adventitious rooting (Garrido et al. 2002).

In conclusion, one-year-old cutting materials of *C. melanoxylon* showed different rooting ability, ranging from 50 to 90%. The cutting medium, auxin addition, and interaction between cutting medium and auxin addition did not significantly influence all observed parameters of *C. melanoxylon* cuttings. Juvenility considered being the highly important factor in the rooting potential of *C. melanoxylon* cuttings. Based on the histological observation, adventitious root of *C. melanoxylon* classified into wound-induced roots. The process was initiated with callus formation on wounded area, followed by root primordia formation, and ended with the appearance of an adventitious root.

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