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Short Communication: Macropropagation – An important tool for conservation of North Sumatran endangered tree species, *Dryobalanops aromatica*

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Abstract. *Susilowati A, Kholibrina CR, Rachmat HH, Elfiati D, Aswandi, Raeni IM. 2018. Short Communication: Macropropagation - An important tool for conservation of North Sumatran endangered tree species, Dryobalanops aromatica. Biodiversitas 19: 1672-1675.* *Dryobalanops aromatica* or locally known as *kapur* is a tree producing borneol used for pharmaceutical purposes. Due to illegal harvesting for wood and borneol, depauperate population with low and rare reproductive mother trees and land conversion to oil palm plantation, its population decreases every year. *Ex situ* and *in situ* conservation efforts are needed to prevent this species from extinction. One of the *ex situ* conservation efforts that can be done is propagating this species vegetatively with shoot cuttings. Shoot cutting is technically simple and inexpensive method to produce new planting stock for further purposes such as production and conservation. This method has also been successfully used for the propagation of endangered and highly economically valuable species. Despite many applications of shoot cutting for clonal forestry, there was still lack of information about the successfulness of this method for *Dryobalanops aromatica*. Therefore the objective of this research was to get data about the successfulness of *kapur* cuttings using different media and growth regulator treatment. A factorial experiment using a Completely Randomized Design with two factors was conducted for this research. The first factor was cutting media, consisting of three types, namely (i) sand, (ii) combination of sand and soil, and 3) combination of sand, soil and rice husk. The second factor was plant growth regulator (PGR), consisting of two levels, namely (i) without PGR addition and (ii) with PGR addition. The parameters observed were survival percentage, rooting percentage, number of primary and secondary roots, length of primary and secondary roots and adventitious root formation. Results showed that rooting percentage of cutting using this technique varied from 30 to 60% and thus this technique was prospective to be developed as a tool for propagating *kapur* trees. Adventitious roots originated from the wounded area near the cambium which was later followed by the formation of callus and root primordia.

Keywords: adventitious roots, camphor, cuttings, medium, PGR, rooting

INTRODUCTION

Kapur, *Borneo camphor*, *camphor tree*, or *Sumatran camphor* is an emergent canopy tree species with hermaphroditic bee-pollinated flowers. This species is distributed in the lowland dipterocarp forests of Malaya, Sumatra, including Riau archipelago, and Borneo (Kitamura et al. 1994). *Dryobalanops aromatica* C.F.Gaertn. (Syn. *Dryobalanops sumatrensis* (J.F. Gmelin) A.J.G.H. Kostermans) or locally known as *kapur* is commonly found in mixed Dipterocarpaceae forests up to 300 m in altitude on hillsides with sandy soils. Its distribution includes Peninsular of Malay, Sumatra and Borneo. In Sumatra, it is distributed in the western part of Singkil, Natal River, between Sibolga and Padang Sidempuan to Airbangis, the south of Rokan River to the north of Batanghari. In the east part of Sumatra, it can be found in the Riau Island, in the west on Mursala Island, but not in Simalur, Nias, or Batu islands (Heyne 1987; Subiakto and Rachmat 2015; Rachmat et al. 2018). The timber has been logged by local people as it is known to have high quality of wood for construction and it is also extracted for resin production. The timber is categorized as Class I for its strength and durability, meaning that it can be used as major timber for construction and building.

Its resin is also well known in commercial market and has potential use in a wide range of medicinal purposes. *Kapur* crystal resin is present in the axial parenchyma tissues of the stem (Yamada and Suzuki 2004), and it is often harvested by cutting and splitting the stems. The resin can also appear on the bark of broken or injured stems. These white crystals contain borneol compounds, a form of terpene alcohols ($C_{10}H_{18}O$), which are widely used in the manufacture of fragrances, antiseptic and others (Huo 1995). In China, it is known as *Bing pian's* that serves as anti-inflammatory medicine and analgesic. Currently, it is also popular to be used in bio-panty which reduces pain during menstruation, reduces muscle and joint pains, helps cleanse the blood clots, and prevent the proliferation of germs (Duke 2005). Borneol originated from *Dryobalanops* is one of the most effective medicines for blood clots or blockage of blood vessels in human heart and brain (Dharmananda 2003).

The high utilization of *kapur* both for its wood and resin has not been followed by intensive conservation efforts including habitat protection. Habitat alteration due to conversion into palm oil plantations, settlements and agricultural purposes caused the decrease of its population both in numbers of trees and also the size of the trunks. The very apparent evidence of extreme condition for this species was described by Rachmat et al. (2018) in Mursala Island where big mature trees almost disappeared. Fruiting period that usually occurs once in 4-year interval may contribute further to its higher risk of extinction.

Conservation status for this species based on IUCN listing is Vulnerable (VU) (Barstow and Randi 2018). Therefore, a rapid effort is needed in order to conserve this species, and one of the approaches is by creating a breeding shortcut through vegetative propagation.

Vegetative propagation techniques are the first step in tree species domestication and offer opportunity of avoiding the problem of recalcitrant seeds. Vegetative propagation also facilitates the transfer of genetic potential as well as the non-additive variance of the parent to the new plant and offers availability of superior individuals in a short period of time for large-scale commercial plantation. One of promising vegetative propagation techniques is macrocutting. Compared to other techniques (e.g., microcutting, grafting) macrocutting is easier, cheaper, faster and economically more beneficial.

Root formation is a critical phase in determining the success rate of rooted cuttings. The root formation of cutting is a complex mechanism and influenced by physiological, environmental and genetic factors. Physiologically, the cutting becomes stressed after severance from the stock plant, and if there is little water or nutrient uptake, the cutting usually reduces stomatal conductance, until the root formation has been developed (Druege and Kadner 2008, Pop et al. 2011). Environmental factors, especially temperature, light, and relative humidity also affect the root formation (Sakai and Subiakto 2007). When environmental factors do not support the cutting, it will be unable to promote root formation. From the genetic aspect, individual cutting has its own ability to form the adventitious root because this phenomenon is related to gene activation after cutting process (Tiberia et al. 2011). Therefore it is necessary to find combination of some factors that will support root cutting formation (Susilowati et al. 2017) and produce new seedlings with high-quality root system (De Klerk et al. 1997).

MATERIALS AND METHODS

The cutting materials were originated from orthotropic shoot of 1-year-old naturally regenerated seedlings available at Aek Nauli Forest Research Agency, Pematang Siantar. The cutting media were sand, topsoil and carbonized rice husk (1: 0: 0; 1: 1: 0, 1: 1;1 v/v in ratio). Commercial auxin powder growth regulator was used for promoting root formation. Propagator box, shading net, pot tray and cutting scissors were also used as tools for this research.

Cutting experiment was carried out based on KOFFCO technique (Subiakto and Subiakto 2007) with minor modification. The cutting materials were taken from orthotropic branches and cut about 7-10 cm and immediately stored in container water. The cuttings were then repeatedly washed using sterilized water. The cuttings were planted in pot-tray containing sterilized medium and placed in propagation boxes according to the treatment. The propagation boxes were stored in a greenhouse with light intensity reduced to about 50 %. Watering was done twice daily, once in the morning before 10.00 a.m. and once in the late afternoon after 4.00 p.m to ensure the seedlings received enough water during the initial growth stage.

The factorial complete block design with two factors was used in this cutting experiment. The first factor was cutting media (I), consisting of I_1 (sand), I_2 (sand: topsoil) and I_3 (sand: topsoil: carbonized rice husk). The second factor was plant growth regulator treatment (A), consisting of A1 (without PGR addition) and A2 (PGR addition). Each combination was replicated into 10 pot-trays. Observation was done at 20 weeks after planting, parameters observed were cutting survival percentage, rooted cutting 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 more new roots were formed. At the end of each month, the number of rooted cuttings, number of roots and length of roots were recorded.

RESULTS AND DISCUSSION

The successfulness of cutting propagation varied with the plant species, some of which were easy to propagate, and others were difficult. The results of medium and auxin treatment on *kapur* cuttings after 20 weeks are presented in Table 1. At the end of observation period (20 weeks), all cuttings were healthy as indicated by their green leaves. Survival rate varied from 50 to 80%.

The high survival percentage of *kapur* cuttings at the end of observation was probably caused by the juvenility of cutting source materials. Similar research conducted by Maura-costa and Lundoh (1994) found the higher survival ability (80%) by using juvenile materials of *D. lanceolata*. In *Diploknema butyracea,* Zargar and Kumar (2018) found the decrease in survival percentage from 92.2% to 37.20% by using mature donor plants. According to Mitchelia et al. (2004), maturity stage of donor plants has negative influence on the performance of rooted cuttings. Hartman and Kester (1983) also stated that the juvenility of the stock plant can also be an overriding factor in root formation especially for plants which are difficult to root. The effect of juvenility on rooting ability may be related with low levels of rooting inhibitors as well as high levels of photosynthates, but as the plant grows older, the inhibitor levels increase (Kontoh 2016). A general decline in rooting ability, root quality, and survival has been associated with donor plants having reached a stage of reproductive or ontogenetic maturity (Hacket 1985).

The rooting ability of *kapur* varied from 30-60%. Lower rooting ability of *kapur* cutting may be caused by terpenoid present in cutting materials. The highest rooting percentage occurred in sand media and PGR treatment (60%) and the lowest rooting percentage occurred in sand: rice husk (1: 1) with no auxin addition (30%). The variance analysis (Table 2) showed that media and interaction between media and auxin did not significantly affect all observed parameters of cuttings, while the auxin addition only affected the length of primary roots. The same results were also found in *D. oblongifolia*. Brodie (2003) found that interaction between media and exogenous auxin treatment did not influence the rooting ability of cuttings and suggested other factors (e.g. individual condition of stock plant) were more important for rooting in *D. oblongifolia*.

Auxin addition significantly affected the length of primary roots in this research. Auxins play a critical role in the formation of adventitious roots by increasing initiation of the root primordium and growth via cell division (Fogaca and Fett Neto 2005). Auxins promote starch hydrolysis and mobilize sugars and nutrients to the cutting base (Das et al. 1997). During cell division and auxin transport, auxins act 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). In this research, we used commercial auxin powder containing a mixture of auxins (IBA and NAA). Davies and Haissig (1990) stated that mixtures of root-promoting substances are sometimes more effective than either compound alone, and adding a small percentage of certain *phenoxy* compounds to either IBA or NAA increased rooting and produced root systems better than using *phenyl* compounds alone.

Adventitious root formation was also observed in this study. The results on *kapur* morphological observation of roots are described in Figure 1. The formation of adventitious roots is a high energy requiring process, which involves cell division, in which predetermined cells switch from their morphogenetic path to act as mother cells for the root primordia; hence more reserve food material is needed for root initiation (Aeschabacher et al. 1994). It is known that root formation is a critical phase that determines the success of propagation by cuttings. Physiologically the formation on cuttings depends on endogenous auxin in plant material and other synergistic components such as *diphenol*. The synergistic component promotes the synthesis of ribonucleic acid (RNA), which stimulates root initiation (Hartmann et al. 2002).

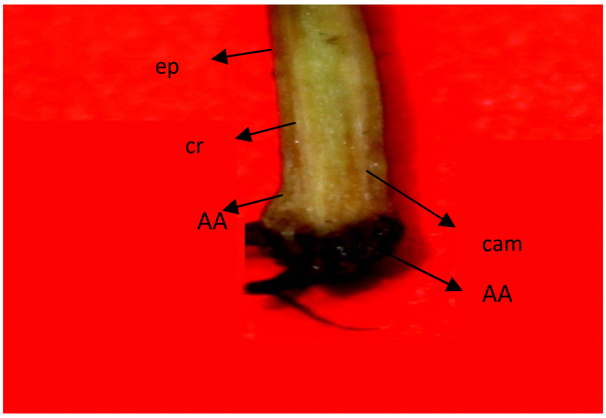

Figure 1. A root of *kapur* cutting (5x magnification). Epidermis cell (ep), cortex (cr), cambium (cam) and an adventitious root (AA)

Table 1. The average value of *kapur* cutting parameters (20 weeks after cutting)

Parameters	Treatment					
	I_1A_1	I_1A_2	I_2A_1	I_2A_2	I_3A_1	I_3A_2
Survival percentage (%)	70	80	50	60	60	70
Rooting percentage (%)	50	60	30	40	40	40
Number of primary roots	2	3	2	2	2	3
Number of secondary roots	24	29	16	28	20	27
Length of primary roots (cm)	1.76	2.81	2.45	4.50	2.81	8.42
Length of secondary roots (cm)	5.57	2.17	2.59	5.83	2.93	1.79

Table 2. The summary of variance analysis for cutting rooting ability

Variable	Treatment		
	Media	PGR	Media* PGR
Number of primary roots	0.88^{ns}	0.92^{ns}	0.86^{ns}
Number of secondary roots	0.90^{ns}	0.69^{ns}	0.64^{ns}
Lenght of primary roots	0.33^{ns}	0.05^{*}	0.79^{ns}
Lenght of secondary roots	0.67^{ns}	0.83^{ns}	0.34^{ns}

Note: ns = not significant at 5% level probability

The bud or leaf produces a complex compound different from auxin which stimulates the formation of roots. The compound found by Bouillenne and Went (1933) is called *rhizocaline,* a complex compound consisting of three components: (i) Specific factors translocated from leaf with chemical properties as ortho-dihydroxy phenol, (ii) Non-specific factors (auxin) transited and found in low biological concentrations, and (iii) The enzymatic factor residing within the cellular tissue, possibly polyphenols. The root initiation process occurs when *ortho-dihydroxy phenol* reacts with the addition of auxin and enzyme, resulting in acceleration of the respiratory process and cell mitotic division leading to cell and tissue differentiation.

Generally, adventitious root developments occur outside of the central portion of vascular tissue (Hartmann et al. 2002). The results of histological observations showed that root formation in *kapur* cuttings started from the outer bark outside the meristem on the cambium. Root formation began with the formation of callus, followed by root primordial formation and ended with the appearance of an adventitious root. Symptoms of rooting at *kapur* cuttings occurred at 18 -20 weeks of cuttings, indicated by the callus formation on the cuttings. In the conifer, Hartmann et al. (2002) found 4 stages of adventitious root formation process that begins with cell proliferation at the basal of cuttings, followed by differentiation of vascular tissue and periderm in the wounded area, differentiation of cutting areas around the cambium and phloem to further form prospective roots and end with meristem formation at the root. Furthermore, Tiberia et al. (2011) stated that physiologically, root formation was the role of auxin-responsive genes in controlling cutting response to auxin addition. This process is also affected by environmental factors and donor plant condition.

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