Propagation for conserving endangered taxol producing tree

*Taxus sumatrana* through shoot cuttings technique

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Abstract. *Taxus sumatrana* or commonly known as Sumatran yew is native endangered taxol producing trees from Indonesia and promising drug for cancer source. Due to very limited and restricted natural growing habitat, small population size and the tendency of habitat deterioration, their sustainable occurrence is facing a serious threats. Urgent task for conserving this species was needed. Shoot cutting is promising methods for solving natural regeneration problems of this species. This research was conducted to determine appropriate shoot cutting technique for propagating the species and hence conserving Sumatran yew. Completely Randomized Design Factorial with 2 factors and 20 replications was used in this research. The first factor was cutting medium the second factor was auxin addition. The observed parameters were survival rate, rooting percentage, primary root number and primary root length. The result showed that the percentage of rooted cuttings were varied from 50 to 65 percent depend on the treatment. Cutting media and auxin treatment significantly only affected primary root number. Combination of soil and husk with auxin addition produced the highest rooting percentage.

1. Introduction
Genus *Taxus* is known as important source of taxane, a promising drug for cancer in the world. *Taxus* is interpreted to include 7-12 species based on morphological criteria and geographical distribution. But later [1], recognizes 24 types of parapatric with 55 varieties. However, until now the position of *Taxus*’s phylogeny is still very controversial. There is no agreement by botanists regarding the number of species included in the genus Taxus [2]. *Taxus* is predominantly distributed in mid-latitudes of the northern Hemisphere with some intrusion to tropical highlands, includes Europe, Asia and North America [3].

*Taxus sumatrana* or sumatran yew was one of member genus taxus that has disjunct distribution in South Sulawesi and some part of Sumatera Island. Previous study reported sumatran yew only found in mount Kerinci, protected forest in Mount Sibuaton and Mount Dempo [4, 5]. Local people harvested taxus for its wood for building material. Since the finding of of taxane from this species, the demand of taxus was increased [6]. Taxane was extracted from its bark and leaves, and demonstrated...
broad antitumor activity against the breast, liver, lung, blood, prostrate, head and neck, gynecological cancers, and Kaposi’s sarcoma-an AIDS-related cancer [7, 8].

Sumatran yew is unisexual and due to its long seed dormancy period and rapid loss of viability coupled with low survival percentage, its natural regeneration from seeds is very poor. Furthermore, based on molecular identification it was revealed that Taxus sumatrana growing in Sumatran Islands sequenced by using chloroplast DNA regions trnL-trnF, psbC-trnS and rbcL that yielded 2423 base pair in total showed no variation for all individuals and population, and their populations were not genetically structured [9]. Ironically, the extraction of the trees itself was not targeted for its timber but for the leaves and bark for which the anticancer yew trees are in the threat of extinction. Concerning the emerging threats of Taxus in almost all part of the world, Convention on International Trade in Endangered Species (CITES) listed T. sumatrana into Appendix II (CITES 2017), which means all traded Taxus and its derivatives was under strict control of CITES.

Conservation of this species become an urgent task to carry out. Any contribution to restore the population of threatened species is a milestone to conserve valuable species for the greater benefits of humankind. Although the regeneration of Taxus through seeds is possible [10] but it faces constraints mainly because of slow process, rapid loss of seed viability coupled with low survival percentage [11]. Conservation effort through vegetative propagation was needed to avoid this species from extinction.

Vegetative propagation techniques are the first step in tree species domestication and offers opportunity of avoiding the problem of recalcitrant seeds [12]. Vegetative propagation through shoot cutting technique also facilitates to threatened species [13]. It also promising way to achieve conservation and restoration goals as well as to meet the demand of plants for taxol or paclitaxel. The successful of shoot cutting for propagated some endangered and valuable species also reported by [14-16]. Therefore, our research was conducted to determine appropriate shoot cutting technique for propagating the species and hence conserving Sumatran yew.

2. Materials and Methods
Material for cutting originated from old trees taxus (approximately 40 year) in Simalem Resort. Situated in the clouds on a sprawling 206-hectare estate at the Merek, Karo Regency North Sumatra. There were 20 taxus trees was found in the estate. This location also nearby Sibuaton protected forest one of important habitat of taxus in Indonesia. Orthotropic shoot was taken from the trees and stored in ice box before planting. Cutting media were soil, husk and carbonized rice husk (1:1:1 and 2:1:0 v/v in ratio). Commercial auxin was used for improving root capacity. Propagator box, shading net, pot tray and cutting scissors were also used as tools for this research.

Cutting experiment and preparation was carried out based on KOFFCO technique [17] 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, later 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 growing stage.

The factorial complete block design with two factors was used in this cutting experiment. The first factor was cutting media (A), consisting of: A (soil:husk) and A2 (soil:husk:carbonized rice husk) The second factor was plant growth regulator treatment (B), consisting of B1 (without PGR addition) and B2 (PGR addition). Each combination was replicated into 20 pot-trays. Observation was done at 32 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.

3. Result and Discussion
Root formation is critical and important phase to determine the successful of rooted cuttings. The percentage of rooted cuttings were varied with the plant species, some of which were easy to
propagate, and others were difficult. The results of medium and auxin treatment on taxus cuttings after 32 weeks are presented in Table 1. Until the end of observation taxus cutting showed healthy condition, it indicated by the green leaf and new shoot formation. The survival rate was varied from 60-65%.

The lower survival rate of taxus cutting in this research might be caused by the juvenility of donor plant. Similar result was obtained on T. wallichiana [18], the survival rate of cutting was 56.25%. The donor plant of this research originated from old taxus tree (approximately 40 year old) in Simalem Resort Forest. Juvenility of cutting material was overriding factor for determined root formation in difficult rooted plant [19]. The juvenility 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 [20].

Table 1. The average value of taxus cutting parameters (32 weeks after cutting)

| Parameters                  | Treatment |
|-----------------------------|-----------|
| Survival percentage (%)     | 65        |
| Rooting percentage (%)      | 10        |
| Number of primary roots     | 10        |
| Length of primary roots (cm)| 1.75      |

The rooting ability of taxus varied from 10-30%. This result was lower compared to previous research on previous research on sumatran yew from kerinci [4] using KOFFCO technique. This condition might be caused by differences in greenhouse conditions when cutting process and maintenance. The greenhouse for kerinci's sumatran yew was fully equipped with temperature sensors and fogging system, so that the temperature in the greenhouse is always below 30° C. This condition is in accordance with the needs of taxus which requires low temperatures. While in this study, the greenhouses were not equipped with temperature sensors and automatic fogging systems. Manual spraying was done when the temperature increases with a sprayer. This difference in temperature modification is causing low root induction in this study.

Another reason for lower rooting ability of from old donor plant also have been stated by [21]. Maturity stage of donor plants have been reported to have negative influence on the performance of rooted branch cuttings. Cuttings made from the juvenile or young plants generally root best and produce roots of higher quality [22, 23]. The stem cuttings made from mature trees completely failed to root [24, 25]. It has been suggested that the reduction of root development may be caused by the presence of sclerenchyma tissue in the phloem of mature trees which obstruct the emergence of the root [26].

The variance analysis (Table 2) showed that interaction between media and auxin did not significantly affect all observed parameters of cuttings, while the auxin addition and media only affected the number of primary roots.

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

| Variabel                  | Treatment |
|---------------------------|-----------|
| Survival rate             | 0.076<sub>m</sub> | 0.076<sub>m</sub> | 0.076<sub>m</sub> |
| Rooting ability           | 0.314<sub>m</sub> | 0.076<sub>m</sub> | 0.076<sub>m</sub> |
| Number of primary root    | 0.04<sup>*</</sup> | 0.02<sup>*</</sup> | 0.100<<sub>m</sub> |
| Lenght of primary root    | 0.956<sub>m</sub> | 0.417<sub>m</sub> | 0.554<sub>m</sub> |

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

Auxin addition only affected the number of primary root. Auxins play a critical role in the formation of adventitious roots by increasing initiation of the root primordium and growth via cell division [27]. Auxins in promotion of ARF in stem cuttings of many plants including Taxus. It is a well-established fact that the application of auxins is necessary for the formation of roots in stem
cuttings as they have been found to stimulate cambial activity thereby resulting the mobilization of reserve food material to the site of root initiation [28]. Applications of auxins enhanced rooting and root quality in many tree species [19].

Media composition affected the number of primary root. An ideal propagation medium for successful rooting must be friable, sterile, have pH of 5.5 to 6.5 and well drained to ensure availability of oxygen and nutrients for seed germination, root initiation and seedling growth [29, 30, 31]. Soil: rice husk media produce higher number of primary root compared to soil:rice husk and carbonized rice husk. Rice husk is considered as a good planting medium due to its light weight and good chemical and physical properties [32]. Similar result also found in Vitellaria paradoxa [33, 34].

4. Conclusion
Forty years old T. sumatrana can be propagated by using shoot cuttings technique and resulted in low rooting ability (10-30%). The Soil:rice husk (1:1 v/v) media with auxin addition produced the highest rooting ability (30%). Auxin and media treatment only affected number of primary root of taxus cutting.

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