Selection of Good Quality Mother Plant to Be Used as Sources of Explants for in Vitro Propagation of Sumatera Benzoin (Styrax Benzoin Dryander)

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Abstract: Sumatera Benzoin (*Styrax benzoin* Dryander) is one of the forest trees that need attention, especially in increasing the production of non-timber forest products. The benzoin sap contains bioactive compounds which are used in medical and cosmetics ingredients. One strategy that can be done to increase the production of benzoin sap is through providing good quality mother plant for in vitro propagation. The aim of this study is to select good quality mother plants with high production of benzoin sap to be used as sources of explants for in vitro propagation of the Sumatra Benzoin, as a strategy to provide good quality seedling for industry and forestry purposes in Indonesia. The study is carried out in the forest area at Kabupaten Pakfak Bharat, North Sumatera Indonesia. The procedures are consisted of identification and selection of healthy *Styrax benzoin* trees that producing high quantity and good quality benzoin sap. Identification is carried out based on phenotype appearance and the selection was done through production forensic given by the incense farmers in the forest area. Good quality mother plants of Sumatra Benzoin (*Styrax benzoin* Dryander) have been obtained at different forest locations. The selected parent trees grown well and fertile, healthy and producing very good quality incense sap. The selected trees are suited to be used as a source of explants for in vitro propagation of Sumatra Benzoin (*Styrax benzoin* Dryander) as a strategy to obtain good quality plant seedling.

1 INTRODUCTION

Plant breeding, especially forest plants that produce bioactive compounds for medicinal raw materials, is needed to avoid excessive plant exploitation. Woody forest plants generally grow and develop in a relatively long age, so that excessive use of plants can lead to the extinction of plants from the forest area. One of the forest plants that needs attention is Sumatra Benzoin (*Styrax benzoin* Dryander) (Nurwahyuni and Sinaga, 2019; van Wyk, 2009; Nurwahyuni and Elimasni, 2006). This plant grows well in tropical forest areas in Indonesia. Sumatra Benzoin (*Styrax benzoin* Dryander) needs to be preserved as a non-timber production plant because it produces incense sap containing bioactive compounds as raw materials for medicine and cosmetics (Singh, et al., 2017; Fan, et al., 2017). Sumatra Benzoin plants grow well and become a leading commodity in Pakpak Bharat Regency, North Sumatra, Indonesia (BPS, 2018). The population of Sumatra Benzoin (*Styrax benzoin* Dryander) in forest areas in North Sumatra tends to decrease because it generally grows naturally and not much is cultivated. To increase non-timber forest production such as incense sap, *Styrax benzoin* plants need to be conserved and cultivated so that the population of these plants can increase and existing plants in the forest area are not cut down for wood production because the incense sap has a higher selling value than wood products (Warren-Thomas, et al., 2018;).

The problem faced is the limited number of Sumatra Benzoin (*Styrax benzoin* Dryander) seeds available for crop cultivation and for the purposes of planting community forests and industrial forests. The small number of plant seeds is caused by the difficulty to obtain the plants seedling since they are generally obtained from seeds that grow naturally in the forest area near the parent tree. Efforts to plant incense seedlings from seeds also experience difficulties because very few of the seeds can

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germinate, and also requires a very long time to produce sprouts. Another problem faced with generative plant cultivation is the difficulty of obtaining plants that have uniform quality, especially in the production of incense sap. One alternative that can be done to produce seeds of Sumatra Benzoin (*Styrax benzoin* Dryander) is through in vitro propagation. This technique is very appropriate in providing plants in large quantities, uniform and with the quality production of the incense is expected to be similar to its mother plants (Mirani, et al., 2017; Pniewski, et al., 2017; Martínez-Estrada, et al., 2017; Thiem, et al., 2017; Gashi, *et al.,* 2015). This in vitro propagation technique requires a mother plant as a source of explants that will be planted in culture media (Baskaran, et al., 2017; Cardoso, et al., 2017; Wu, et al., 2014).

The parent plant must have criteria as a superior plant, which is growing well, is relatively resistant to plant pests, and has a large amount of frankincense production accompanied by high-quality frankincense gums. To fulfill this requirement, a survey was conducted to identify and select suitable mother plants to be used in propagation of plants in vitro in a further study of this research stage. The purpose of this study was to select and determine a very good quality Sumatra Benzoin (*Styrax benzoin* Dryander) parent plant, which is a healthy plant, well-developed, relatively resistant to disease, and produces large amounts of incense sap with very good quality. This parent plant will be used as a source of explants in the propagation of plant seeds technically in vitro. In vitro propagation is an alternative supply of very good quality *Styrax benzoin* seeds as a step to meet the needs of seeds for breeding of community forests and industrial forests. In the long term this strategy is one of the efforts to increase the production of incense sap as a non-timber product and to tackle illegal logging in North Sumatra.

2 RESEARCH METHODS

2.1 Materials and Method

The work was conducted in the Department of Biology, Faculty of Mathematics and Natural Science, Universitas Sumatera Utara. Survey for choosing of good quality plants of Sumatra Benzoin (*Styrax benzoin* Dryander) was carried out in the forest at Pakpak Bharat Regency , North Sumatera, Indonesia, with selection of four Regencies from eight Regencies, those are cover the forest areas in the region. The information for the existence of the *Styrax benzoin* plants in the forest location are made by the help of experience farmer followed the parameters given for good mother plants. To ensure the quality of selected *Styrax benzoin* plants meet the criteria as a parent plant for in vitro propagation purposes, the productivity of the target plants is assessed based on the quality of the incense sap and the estimated incense sap production that has been obtained from the tapping of plants in one harvesting period.

2.2 Identification and Selection of Good Quality Mother Plant

The identification and selection of Sumatra Benzoin trees was conducted in four forest locations, those are situated in four districts (*Kecamatan*) in the regency, followed by the guidance given by the farmers with consideration of plant population density of *Styrax benzoin* trees. The selection of *Styrax benzoin* trees has been carried out by registering of healthy and productive *Styrax benzoin* trees based on the experience of harvesting the incense sap in the previous year. An objective assessment is also carried out by a team of expert researchers to ensure that the selected *Styrax benzoin* trees meet the criteria as a mother plant that can be used as a source of explants at the micropropagation stage in subsequent studies. The main criteria used as consideration for choosing good quality parent plants are a combination of health level, plant fertility and incense sap productivity. Plants that meet these requirements are classified as good quality plants and can be used as a source of explants in vitro propagation.

2.3 Technique to Produce Incent Sap

Traditional technique is used to produce benzoin incense sap from *Styrax benzoin* trees, and the tapping process is done followed the procedures given by experience farmer (Tambun Berutu, 2019: Personal communication). The equipments used for the purposes of tapping of the incense sap from *Styrax benzoin* trees are consisted of rope stairs, metal chisel, sharp metal sickle, and bamboo basket as listed in Figure 1. Rope stairs are made the farmer easy to carry and having flexibility in the applications on difficult situation in forest area (Figure 1a). A sharp metal sickle has stalk that is helping the farmers to clean the stem area for

tapping of the benzoin incense sap (Figure 1b). A dull steel chisel is used to injure the skin surface of the incense stem and used to secure the position of the skin after tapping process has been conducted (Figure 1c). All benzoin incense products are stored in the bamboo basket during the harvest time in the forest area (Figure 1d).

Figure 1. The equipments that are used on tapping of incense sap: (a) Nylon rope stairs, (b) metal chisel, (c) Metal sickle with dull, and (d) bamboo basket.

Tapping process is done after first cleaning the stems of *Styrax benzoin* plants from impurities by using of sharp steel sickle until the surface is free of mold. The next step is injuring the bark by using a sharp steel chisel, then prying the bark and lifting the bark until the bark is separated from the wood but still attached to the tree trunk, and continued with the tapping process by using the back of a blunt chisel so that the wood part reunites with the bark. Furthermore, the scars are left to trigger the production of frankincense, which is the product from an attempt to repair the wound, and then the frankincense is allowed to harden until a period of three months before harvest. The time to injure the plant's skin is done according to the condition of the plant, that is, when the plant looks healthy which is indicated by the lush plants and has finished fruiting. Harvesting of the sap is conducted through removing of the bark containing incense gum. Removing of the incense gum from the bark is performed by naturally drying in suns until the raw material of incense sap product is obtained.

3 RESULTS AND DISCUSSION

3.1 Survey for Good Quality Mother Plant of Sumatra Benzoin

A survey of the Sumatra Benzoin (*Styrax benzoin* Dryander) plant was carried out in a forest area in Pakfak Bharat Regency. Mature plants have a stem size between 20-30 cm in diameter, mature tree height of about 10 to 25 meters, with a straight trunk structure, and a slight branching. A description of the survey location and information on the condition of the Sumatra Benzoin plant are summarized in

Table 1. There are 247 plants selected as healthy plants that are distributed in forest areas in four districts in the regency, and 97 trees are classified as very good with each of them are producing frankincense sap of more than one kilogram in each the harvest period. The availability of many very good quality plants gives researchers the flexibility to use mother plants as a source of explants in in vitro propagation steps to produce large quantities of good quality seedling.

Table 1. The descriptions for the existence of Sumatra Benzoin (*Styrax benzoin* Dryander) plant population in four districts of Pakfak Bharat Regency, North Sumatera Indonesia.

No	Forest Location (District / Regency)	Area of Survey (± m^2)	Healthy trees produce sap (*n*)	Number of Plants producing incense sap in a harvest period (*n*)		Short information on the condition of the forest location
				> 1 kg	< 1 kg	
1	Pergetteng Getteng Sengkut	10,000	58	21	37	mountainous and lowland lands
2	Siempat Rube	10,000	75	33	42	mountainous and lowland lands
3	Sitellu Tali Urang Julu	10,000	62	23	39	mountainous and partially flat, and partially peat areas
4	Tinada	10,000	52	20	32	mountainous and lowland lands
	Total	40,000.0	247	97	150	

Good quality Sumatra Benzoin (*Styrax benzoin* Dryander) plants have been selected that met the criteria as mother plants to be used as source of explants for in vitro propagation. The selected plants are characterized with having of very good appearance in the growth and development of the plants that are visually been seen from the phenotype descriptions. The selected plants in plain sight look healthy, grown fertile, have branches, twigs, leaves, and perfect fruit as shown in Figure 2. The target plants have also been categorized as productive trees, which has produced a relatively large amount of incense sap with having the sap classified as super quality. Sumatra Benzoin (*Styrax benzoin* Dryander) population is distributed in groups in the forest area. The existences of the *Styrax benzoin* plants are growing naturally from seeds, so that the plant population is close to one another. To facilitate identification, the forest area is limited (about ±10 000 m^2) in each district of the forest area. In general, the Sumatra Benzoin plant in Pakpak Bharat Regency looks healthy. Based on information from farmers, it is known that the selected plants have a high-volume of incense resin with high quality sap. Very good quality of *Styrax benzoin* trees can be used as a source of explants for micropropagation procedures.

3.2 Production of Benzoin Sap

The selection and designation of mother plant of Sumatra Benzoin (*Styrax benzoin* Dryander) tree is merely based on the condition of the plant and the quality of incense gum product. Production of the gum becomes a crucial parameter in an effort to increase non-timber forest production. In this study, identification of several Sumatran Benzoin plants which were classified as healthy was carried out to see the potential production of incense sap that is produced in each tree at harvest time. Incense production techniques are carried out by traditional tapping as is generally done by farmers in forest areas. This technique is believed to not damage plants and produce optimum sap. Frankincense farmers always try to maintain *Styrax benzoin* trees in a healthy condition because incense product has become the main income for some farmers. Tapping of the incense sap from adult plant stems is done traditionally. The incense sap produced in the tapping process is a result of the plant's efforts to repair wounds made at the tapping procedures. The quality of the incense varies as shown in Figure 3. The grouping of frankincense is based on the selling price in the market. After the tapping process is carried out, the sap of frankincense will slowly fill

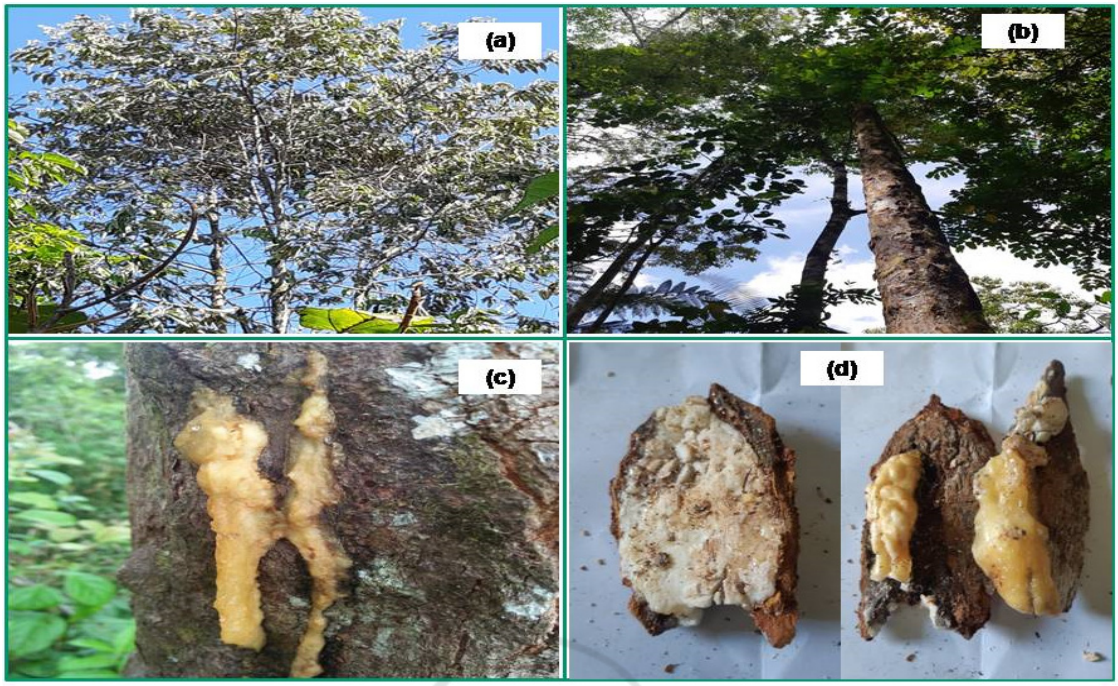

Figure 2. A selected mature Sumatra Benzoin (*Styrax benzoin* Dryander) tree with high quality incense gum production to be used as a source of explants for in vitro propagation technique: (a), Healthy tree in the forest area (b) The location of tapping of sap in the stem, (c) Closed view of the stem with high quality gum, (d) The benzoin incense gum product.

Figure 3. Typical quality of Sumatra Benzoin incense sap based on selling prices in the market: (a and b) super quality incense sap (*sidungkapi*), (c) incense sap quality below super (*barbar*), (d) incense sap type of ash (*Tahir / Kikisan*).

the cavity of the bark and will slowly harden into incense products with very good quality or super quality (sidungkapi). The texture of high quality incense sap is white crystals (Figures 3a and 3b). The excess of the incense partly come out of the skin cavity to produce good quality latex, called as good quality or lower super (barbar). This type of incenses is yellowish-colored crystals probably caused by the oxidation of the compound in exposing to open air for long time (Figure 3c). Some incense that is not categorized as very good quality and is classified as a mixture of incense, generally in the form of powder is called as an incense of ash (Figure 3d).

The determination of good quality mother plants is ensured after the physico-chemical properties of the incense are carried out, which is shown from the production of the incense sap. Incense production parameters in terms of the quantity and quality of incense are needed in addition to visual observations on the presence of Sumatra Benzoin (*Styrax benzoin* Dryander) trees. Adult incense trees that produce large amounts of frankincense sap along with very good quality incense gum are selected as parent plants for in vitro propagation in further research. From the results of a forensic study through information provided by incense farmers, it was found that the quantity and quality of the incense sap produced by each plant differed, although it was thought to come from relatively similar seed sources (one mother plant). This may be due to genetic variation in plants by cross-breeding and producing generative seedlings. It should be noted that almost all incense plants that grow in the forest in Pakfak Bharat Regency are derived from seed propagation. Thus in order to increase the production of incense in the future, plant seeds obtained by in vitro techniques become very relevant to produce uniform plants in the production of incense in the same high amount as their parent plants. In this study, several prospective parent plants have been obtained which will be used as sources of explants for in vitro propagation procedures to obtain good quality seedlings. The selected parent plant is a plant that grown very fertile, healthy, and produce big quantity of incense sap that is classified as very super quality.

4 DISCUSSION

The selection of parent plants which are used as sources of explants for in vitro propagation is crucial in the success of supplying large and uniform plant seedling. Several factors must be considered so that in vitro techniques for the propagation of woody plants work well, including the source of explants, the composition of growth regulators, and types of plants (Mozafari, et al., 2015; Nurwahyuni, et al., 2018; Nurwahyuni, et al., 2017; Nurwahyuni and Sinaga, 2014). Propagation of Sumatra Benzoin (*Styrax benzoin* Dryander) is very strategic in the effort to increase the production of non-timber forest types (Potter and Loffler, 2010). If the incense plants can produce large amounts of the sap, the farmers will be interested in cultivating Sumatra Benzoin (*Styrax benzoin* Dryander), so that the presence of these plants will no longer only grow naturally but will be planted and maintained by farmers who are near the forest area. Incense sap is known to have a very high selling value because it has a bioactive compound that contains raw materials for drugs and cosmetics (Wangchuk, et al., 2016; Chen, et al., 2016). By increasing the productions of incense sap, the farmers will be able to maintain the forest well as the main source of livelihood (Nambiar, 2015). Automatically illegal logging in forest areas will be reduced because the production of incense sap can meet the economic needs of the farmers (van den Boog, et al., 2018; Dattagupta and Gupta, 2014; Jarangchi and Sangma, 2019). Therefore, it is necessary to supply good quality seedling of Sumatra Benzoin (*Styrax benzoin* Dryander) that are produced by in vitro propagation (Cui, et al., 2019). This technique has been confirmed to be able to produce seedling the same quality as the mother plant. Therefore, the steps taken to select the best mother plants to be used as a source of explants for in vitro techniques that will be carried out in this research phase are very appropriate. The strategy is an effort to produce high quality Sumatra Benzoin (*Styrax benzoin* Dryander) seedling to supply the need for domestic forestation and industrial forest in North Sumatera.

5 CONCLUSION

A survey to identify and select very good quality incense plants has been carried out. There are 97 healthy trees that have very good growth with very large quantities of gum production. Traditional tapping techniques have been successfully used to confirm and select very good quality *Styrax benzoin* plant. Good quality parent plants of Sumatra Benzoin (*Styrax benzoin* Dryander) have been obtained at different forest locations. The plant has fulfilled the criteria as mother plant for in vitro propagation. The selected mother trees grown well and fertile, healthy and producing good quality

incense sap. The traditional techniques to obtain the benzoin incense sap from the parent trees are demonstrated. The selected parent trees are suited to be used as a source of explants in the propagation of Sumatra Benzoin (*Styrax benzoin* Dryander) to obtain good quality plant seedling.

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