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Application of Liquid Organic Fertilizer on Cendana (*Santalum album* L.) Invitro from East Nusa Tenggara

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Abstract

Sandalwood (*Santalum album* L.), grown in East Nusa Tenggara, is an endemic plant known as hau meni. It is used as a base for carved artwork and perfume containing essential oils with a distinctive aroma. The owned superiority causes the selling price to be very high. This causes exploitation which results in a diminishing number of Sandalwood trees. Efforts to replant have been carried out through seedlings and nurseries, but the success rate is still low. Invitro attempts are expected to help conserve this plant to determine the effect of giving Liquid Organic Fertilizer (LOF) on the growth of Sandalwood and the best concentration in promoting germination. The study was conducted by adding different types of LOF with concentration variation. The addition of 2 mL Nasa is of which the optimal concentration to promote germination response at 14 Days After Planting on Murashige and Skoog (MS) medium, while the addition of lontar 2 mL concentration shows the response of the appearance of plumula. The addition of base type LOF on MS medium affected 82% germination percentage while lontar type LOF stimulated vegetative growth of Sandalwood plants

Keywords: Sandalwood; Nasa; lontar; germination; MS

Introduction

Sandalwood (*Santalum album* L.) is an endemic plant of East Nusa Tenggara. Naturally, this plant is spread in dryland areas such as Timor, Sumba, and Flores islands. In general, the people in Timor island are familiar with Cendana as "*Hau meni*" or "fragrant wood" for its distinctive fragrance. Sandalwood is one of the best species in the world due to the higher level of oil content inside the stem and how it produces a distinctive aroma frequently found in popular souvenirs such as prayer beads, fans, rosaries, soaps, aromatherapy, and carvings. The age of sandalwood affects the quality of the oil and the aroma produced. Its existence, especially in East Nusa Tenggara, continues to decline. This is indicated by the decreasing number of sandalwood trees due to unmanageable illegal logging. According to the IUCN Red List, sandalwood is the type of plant that was almost extinct (vulnerable).

The government has enforced various efforts to maintain the existence of this plant, one of which is replantation. The attained data shows that 17,546 sandalwood trees were successfully replanted in East Nusa Tenggara in 2015-2017. The success rate of replantation depends on the quality of the

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sandalwood seeds used. Good seed quality is able to determine the success of sandalwood expansion. Seeds provision through conventional propagation has been carried out, but it was inadequate to be widely developed; thus, the technology was needed to provide plants in large quantities and in a short period through vitro culture techniques (Indrianto, 2003). The use of an MS medium with a concentration of 2 mg/L NAA might induce Sandalwood germination (Solle et al., 2016). Application of 1 ppm BAP and one ppm kinetin in MS medium in the growth of apical tip explants of teak plants into callus and shoots (Lina et al., 2013). The use of conventional techniques has also been done to break dormancy in Sandalwood seeds in order to accelerate germination and growth. The said technique provides good results using the sanding method, which is as much as 30% can germinate, while the chemical method using KNO_3 gives a yield of 27% germination (Dolu, 2018). Seed germination testing is a test of a number of seeds through a percentage of the number of seeds that allow them to germinate in a period of time (Sari et al., 2017). The common method used to isolate plants aseptically on culture media to reproduce and regenerate completely is called *in vitro culture* (Indrianto, 2003).

In vitro culture or tissue culture, the use of primary media is something that needs to be considered to support the success of plant culture. MS medium was the basic medium that was used because the elements and compounds were complete. Besides the use of plant substance regulators, fertilization can also help the growth of plants themselves. Soil fertilization or through the medium is performed with both artificial and natural fertilizers. Natural materials such as Liquid Organic Fertilizer (LOF), an easily obtained and cheap fertilizer in the market, can be used as an alternative medium. In addition, LOF can also be produced from other natural materials, such as the mesocarp of the Lontar fruit (*Borassus flabelifer* L.). Mesocarp is part of the Lontar fruit containing a lot of fiber; therefore, it will be easier to use as the main ingredient of LOF. Nasa is a LOF sold cheaper and more affordable in the market, and it has full of NPK, which much beneficial for plants. LOF Nasa can be easily given to plants and mixed in water (Mebeng, 2016). The use of LOF has been widely used to repair soil conditions, as well as to help plant growth. It contains the background, rationale, and urgency of the research. References (relevant literature or research) need to be included in this section concerning the justification of the research urgency, the emergence of research problems, alternatives, and the solutions chosen.

Research Methods

The research population of this study was sandalwood plants in East Sumba Regency, East Nusa Tenggara. The sample used was sandalwood seeds with as many as 1000 grains, researched experimentally from March to September 2019. The tools used in this research were Autoclave, dissecting kit, knife, and petridish. Laminar Air Flow Cabinet (LAF), culture bottles, weigher, hot plate, pH meter, Bunsen, hand sprayer, pipette, micropipette, and aluminum foil. The ingredients used were Sandalwood seeds (*Santalum album* L.) from East Sumba Regency, Murashige and Skoog (MS) medium, Nasa LOF, Lontar LOF, and coconut water which were the natural cytokinins. Liquid detergent, fungicide (Dithane M), 70% alcohol, Sodium Hypochlorite, and sterile distilled used to explant the sterilization.

The research method used was experimental research. In this study, there were four treatments with four replications for each treatment. Dispose of variations in LOF concentration of LOF 0 mL, 1 mL, 2 mL, 3 mL, and 4 mL into MS medium mixed with LOF types of Nasa and Lontar. The research procedure of this study started with washing all the equipment needed with detergent, rinsing them thoroughly in running water, and then drying them. We divided the sterilization stage into two parts: equipment sterilization and explant sterilization. The equipment sterilization stage utilized aluminum foil-covered culture bottles, scalpels, and petridish and dry tweezers wrapped in brown paper; also, bottles contained sterile distilled water. The autoclave sterilized all tools at 121^0C and 15 psi for 15 minutes.

As in the explant sterilization stage, the sandalwood seeds were first washed in running water to clean the dirt on the seed coat. We then soaked them in clean water for 24 hours. After the soaking process, the seed coat was peeled and washed. The next step was seed sterilization using the fungicide Dithane, Sodium Hypochlorite, and 70% alcohol, then it was rinsed with sterile distilled water. Sandalwood seeds were ready to be planted using sterile tweezers; repeated each treatment four times. After planting, the culture bottles containing the explants of Sandalwood seeds were immediately closed and taken into the incubator room; we carried out incubation in a bright place. The next stage was making MS medium, 1000 mL Erlenmeyer filled with 500 mL of distilled water plus macronutrient and micronutrient components, dissolved one by one. It was assisted by a magnetic stirrer, adding iron and vitamin stock to accelerate dissolution.

Myo-inositol was weighed and put into Erlenmeyer before dissolving it. The same treatment went with sucrose. Next was the measurement of the pH of 850 mL of distilled water using universal pH. To weigh as much as eight g/L, put into an Erlenmeyer and was heated (while stirring) until it dissolved. LOF Nasa was added, along with the LOF Lontar, 150 mL of coconut water and LOF according to the concentration used 1mL; 2mL; 3mL; 4 mL, and Control without LOF. Then, pour the media into the culture bottle in the liquid state. The bottle was tightly closed with aluminum foil and labeled according to the treatment.

The process went by putting it into an autoclave and sterilizing it at 1210C for 15 minutes with a pressure of 15 psi. Store the sterile medium in the storage room. The final stage in this study was the observation; this stage went by observing Sandalwood's growth, including the number of seeds that germinated, percentage of germination, germination height, and root length for 90 HST.

The data collection writing was in paragraphs. It is conducted by morphology and anatomy observation of Sandalwood growth (*Santalum album* L.). The data will be analyzed by ANOVA when there is a significant difference with the DMRT test at the 5% level.

Research Results and Discussion

The observations of Sandalwood seeds marked by germination and morphological observations of Sandalwood at the age of 90 HST indicated the sprout height, root length, and color of sprouts. This study was also accompanied by anatomical observations of 4 weeks aged Sandalwood. The following are the parameters of the observation made:

Sandalwood Seed Germination

The results showed that the different addition of LOFs in contrasting MS medium provided a good response to sandalwood growth. Adding Nasa LOF with a concentration of 2 mL was the optimal concentration for stimulating germination. It responded at 14 HST, while the concentration of 2 mL of LOF lontar showed a response to the peak appearance (plumula). The presentation of the research data in the form of tables and graphs of observations is as follows Table 1.

Sandalwood seeds, as the main explants, were harvested in 2018 and showed physiologically as good seeds, indicated by the outer black and white seed coat inside and round seeds with a diameter of 0.5 – 0.8 cm. Sandalwood seeds are orthodox seeds that can be stored at low water content. Aims to maintain water content with a temperature of approximately 40C and a humidity of 40-50%.

Sandalwood is a type of seed plant that is grouped into the class of Magnoliopsida (Dikotil). In vitro germination was carried out to obtain explants with sterile conditions. Germination was the initial phase of plant development, especially seed crops (Solle et al., 2019). The number of Sandalwoods germinated was 1000 seeds, and Sandalwood seed was physiologically good with enzyme activity, biochemical reactions, and good seed respiration in response to germination with an average of 50 seeds planted on the growth medium.

Growth, division, renewal, and cell death were four stages that became the pillar of cell life (Oeke, 2016). Growth was needed by cells in young body tissues, which experienced regeneration. In multicellular organisms, a division is needed to repair the damaged or dead cells and increase the number of cells to enhance the size of the organism's body tissues.

The results of morphological observations showed that Sandalwood (*Santalum album* L.) seeds germinated in vitro and shown in Table 1 above showed a very good response to the use of the medium, POC, and the quality of Sandalwood seeds used. The viability of good sandalwood seeds was indicated by seed germination reaching 82% with the ability to grow normally in a suitable environment and absorb nutrients well. In comparison, the medium without LOF (Control) showed a lower percentage than the treatment of 58% of seeds that germinated. It showed that LOF application in a growth medium could also act as a growth regulator and help activate enzymes that support growth. One of the growth regulators that play a role in germination is gibberellins. If gibberellins were absent or less active, alfa amylase would not be formed, which would hinder the starch breakdown process so that germination would not occur. According to Salisbury and Ross (1995), natural auxins are synthesized in the shoot meristem, growing leaves, and fruit, which are transported from cell to cell in a basipetal direction (from shoots to roots).

Morphology and Anatomy of Sandalwood Sprouts

Morphological observations were carried out every day by taking care of the availability of nutrients in the medium, controlling the temperature and observing room cleanliness, and preventing contamination. Presentation of data in table 2. Based on Figure 2, it can be seen that the type of epigeal germination with characteristics such as the radicle that appeared first will form a hypocotyl, which is

believed to have a fairly high endogenous auxin content because it is located under the canopy and becomes a pathway for auxin to be passed. The hypocotyl will form a loop (indentation) that can elongate and bring the cotyledons to the surface of the medium. The increasing amount of N absorbed by plants will cause the meristematic tissue of the growing stem to be more active to grow taller. This is supported by the data sprout height was 13.34 cm at 2 mL MSN. Samaludin (2010) stated that plant height begins with the process of division and enlargement of plants in the shoot apical meristem; this process will occur when cells experience turgidity with the basic elements of water and nutrients. In MSN, 3 mL and 4 mL observation showed that there was no growth occurred because the ability of cells to distribute nutrients and growth regulators were different, and also, the high concentration of 3-4 mL of LOF tended to the inhibitor.

The appearance of 2 mL MSS of two young green leaves at eight weeks of observation was because LOF Lontar and coconut water can stimulate the new leaves. The addition of coconut water can help germination because it is a liquid containing nutrients and PGR; therefore, it can stimulate germination and growth. According to Yusuf et al. (2016), who claimed that coconut water contains cytokinin hormones 5.8 mg/L, auxin 0.07 mg/L, and gibberellins which are capable of restoring the germination and plant growth, as a stimulant in proliferation tissue, accelerate metabolism, and respiration. Atichart (2013) explained that cytokinin activity produces multiple shoots. LOF Lontar with gamal *(Gliricidia sepium)* leaves as a source of N plays a role in spurring the vegetative development of plants, such as leaf formation (figure 2f). Gamal *(Gliricidia sepium)* is a plant from the Leguminosae family with pretty high essential nutrients for plants. According to Febriana et al. (2018), gamal leaf tissue contains 3.15% N, 0.22% P, 2.65% K, 1.35 Ca, and 0.41% Mg. Endosperm in Sandalwood seeds contains a mass of starch (starch) protected by a layer called aleurone. The aleurone layer will diffuse with internal gibberellins where hydrolytic enzymes (α-amylase, protease, glucanase, and phosphatase are made) will diffuse into the endosperm sugars, amino acids, and others. Furthermore, gibberellins also increase proteinase enzymes that convert proteins into amino acids and lipase enzymes that convert fats into soluble fatty acids and glycerol (Asra, 2014). Changes in food reserves into substances that can be transported throughout the embryo so that the seeds can germinate.

According to Asra (2014), the formation of the alfa-amylase enzyme occurs at the beginning of germination by internal gibberellins. If the number of internal gibberellins is limited (not active yet), then the germination process will run slowly. LOF Nasa used high NPK elements with N 0.06%, P 0.01%, and K 0.11% (Yusuf et al., 2016). The addition of LOF, both nasa, and lontar, Sandalwood germination did not have a significant effect on root length. Even though the sprout height parameter showed that 2 mL MSN gave a yield of 13.34 cm, the root length was relatively small, 0.6 cm, perhaps because of inhibition of cell elongation due to disruption of the flow of water and nutrients from the xylem to other cells experience elongation and dilation. Besides, the use of LOF nasa was indicated to increase plant height, not for root as a means of absorption

of nutrients from the growth medium. It can also be said that growing Sandalwood sprouts have the ability to absorb nutrients and growth regulators endogenous in helping cell metabolism. The growth development that occurred in Sandalwood explants was due to metabolic processes in the cells. The function of the cell was to carry out the metabolic process and store the about-to-be-inherited information code. The use of NASA as one of the LOF's woods contains NPK elements that plants need. According to Puspadewi et al. (2016), fertilizers containing N, P, and K are needed for plant growth, especially in stimulating plant height and enlargement of stem diameter. Sandalwood's height and stem diameter require sufficient nutrients as a food supply that can support growth.

The tissues observed included (Figure 3): the epidermis, cortex, cambium, pith, and carrier bundle are the characteristics of dicotyledonous plants. There is an increase in size in the larger cells caused by the influence of endogenous hormones that diffuse into the explant cells. The epidermis is located in the outermost part and plays a role in preventing transpiration and protecting deep tissues from mechanical damage and disease. The area within the epidermis is the cortex, and inside, the cortex is bounded by the pericycle. Inside the endodermal cells, there are starch grains; therefore, this tissue is called the flour sheath or the starch sheath.

Vascular bundles are arranged in a circle consisting of the xylem, cambium, and phloem. The middle is composed of parenchyma tissue that has intercellular spaces called piths. The pith radius develops radially like a band in the secondary xylem. The pith radius develops from the pith radius cambium. In the stem of sandalwood sprouts, a cambium is located between the xylem and phloem, but the xylem, phloem, and cambium were not clearly visible because of the young age of the sprouts. The factors that influence the occurrence of cell division in vitro include adequate essential nutrients in the medium, cell density, specific growth factors, and lack of physical contact between cell membrane proteins and adjacent cells. Contact with solid surfaces and the availability of growth hormone was a signal major cell cycle. The cell cycle is a stage in an iterative process in the life span of a cell that begins with the growth of young cells into mature cells.

Table 1

Table Germination of sandalwood (Santalum album L.) seeds on MS medium with the addition of LOF nasa and lontar.

No	Treatment	Sandelwood seed (*Santalum album* L.)		average
		Planted seeds	Sprounted seeds	
1	MS_0	50	29.00 ± 2.43	58
2	MSN_1	50	12.00 ± 1.33	24
3	MSN_2	50	41.00 ± 4.55	82**
4	MSN_3	50	0.00 ± 0.00	0
5	MSN_4	50	0.00 ± 0.00	0

6	MS_0	50	29.00 ± 2.43	58
7	MSS_1	50	0.00 ± 0.00	0
8	MSS_2	50	11.00 ± 1.22	22
9	MSS_3	50	15.00 ± 1.66	30
10	MSS_4	50	0.00 ± 0.00	0

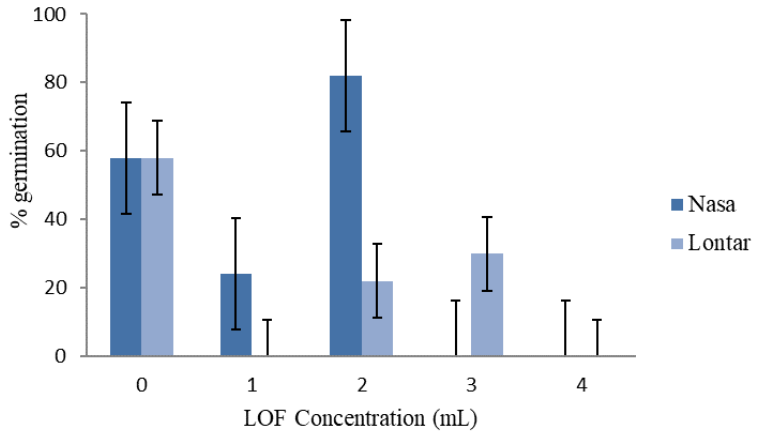
Information:
* Mean value ± Standard Deviation (SD)
** Highest germination percentage
MSN: Medium MS with the addition of Nasa
MSS: Medium MS with the addition of lontar

Table 2
Table Morphology of Sandalwood germination (Santalum album L.)

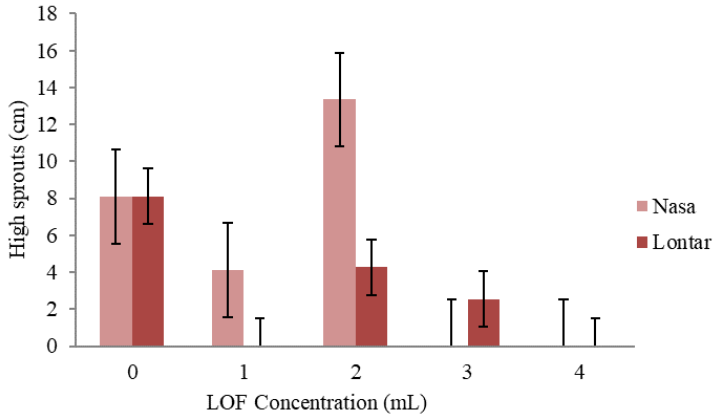
No	Treatment	Sandalwood seed (*Santalum album* L.)		
		germinate height	germinate length	germinate color
1	MS_0	8,1	0	hijau
2	MSN_1	4,13	0	hijau
3	MSN_2	13,34	0,6	hijau
4	MSN_3	0	0	-
5	MSN_4	0	0	-
6	MS_0	8,1	0	hijau
7	MSS_1	0	0	-
8	MSS_2	4,26	0	hijau
9	MSS_3	2,55	0,1	hijau
10	MSS_4	0	0	-

Figure 1
Figure Graph of high and germination percentage of 9 weeks old sandalwood (Santalum album L.*) germinate; a. Cendana Germination; b. germination high.*

a. Cendana Germin



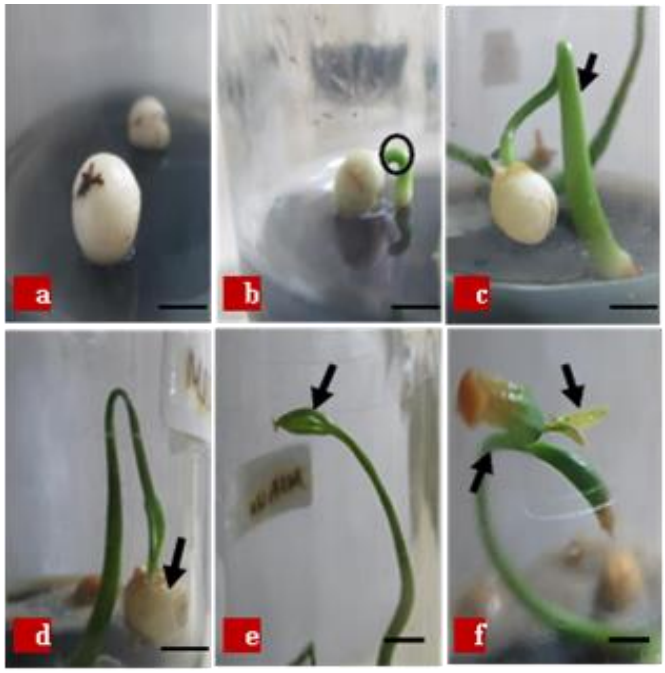
b. germination high



Sandalwood seed growth begins with a germination response, which is shown in Figure 2 below.

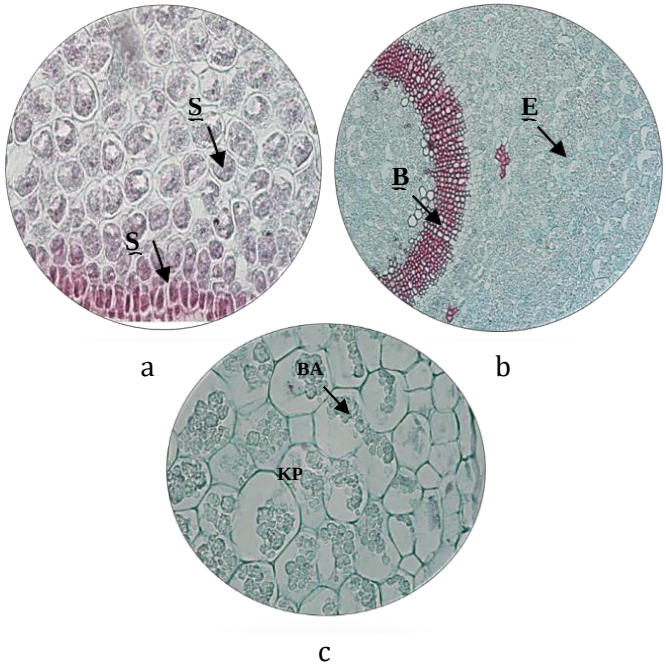
Figure 2

Figure Stages of germination of Sandalwood (Santalum album L.) in vitro; a) Sandalwood seeds grown on MS medium; b) Sandalwood hypocotyl forms a loop then elongates to bring the cotyledons to the surface of 2 mL MSN medium; c) Formation of 3-week-old sandalwood hypocotyl on 2 mL MSN medium; d) Cotyledons; e) The appearance of the first leaf MSS 2 mL; f) Development of 9 weeks old sandalwood and epicotyl plumules (shoots) on 2 mL MSS medium 1 mm bar scale.



Anatomical observations were made to see growth regulators play a role in metabolic processes in cells.

Figure 3
Figur Hypocotyl sprouts in vitro of Sandalwood (Santalum album L.) aged 4 in vitro; (a) cotyledons (b) 1 stem, transport bundle, 2. Endodermis; (c) Cortex layer on endodermis. SC: Scutellum; SE: epithelial cells; BP: Carrier File; BA: Starch granules; KP: parenchymal cortex; ED: Endoderm.



Conclusion

Giving LOF with a concentration of 2 mL was the best concentration in stimulating the germination of 82% MSN and MSS for the formation of plumules.

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