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Effect of BAP (6-Benzyl Amino Purine) and NAA (Naphtalen Acetic Acid) on the Induction of Axillary Shoots in Sandalwood (*Santalum album* L.)

Imaniah Bazlina Wardani
Kiai Haji Ahmad Siddiq State Islamic University, Indonesia
Correspondence author, imaniahwardani@gmail.com

Abstract Sandalwood (*Santalum album* L.) is a native species of East Nusa Tenggara that is facing a population reduction in its natural environment as a result of exploitation. In a relatively short amount of time, a large quantity of seeds is required. One of the methods used is vegetative propagation through tissue culture techniques. The inclusion of growth regulators such as BAP and NAA to the culture medium is crucial to the successful induction of shoots via tissue culture. This study's objective was to examine the influence of BAP and NAA on the induction of axillary shoots in Sandalwood (*Santalum album* L.). Using a completely random design with a factorial pattern consisting of 16 treatments and 3 replications, the study used a design that was fully randomized. The first component was BAP concentrations (0 mg/l, 0.5 mg/l, 1 mg/l, and 2 mg/l), while the second factor was NAA concentrations (0 mg/l, 0.1 mg/l, 0.25 mg/l). l and 0.5 mg/l). The findings of the research demonstrated that the addition of BAP and NAA affected the day of emergence of axillary shoots, the number of axillary shoots, the average length of the shoots, and the number of leaves. The most efficient combination of concentrations for generating axillary shoots in Sandalwood (*Santalum album* L.) was BAP 2 + NAA 0 mg/l.

Keywords: 6-Benzyl amino purine, Naphtalen acetic acid, Induction of axillary shoots, Plant tissue culture, *Santalum album*

INTRODUCTION

Sandalwood tree (*Santalum album* Linnaeus) is an important tree species for the people of East Nusa Tenggara (NTT) due to its great economic value and status as the world's best indigenous species (Pareira et al., 2019). Sandalwood is a source of essential oils and a possible non-timber forest product commodity; it is classed as luxury because to the distinctive quality of its core wood and the aroma-specific oil it contains.

The wood trunks, branches, roots, and branches of sandalwood trees may be used to produce sandalwood oil. The commercial value of sandalwood is derived from the wood's distinctively scented oil content (santalol). Sandalwood essential oil is a family of sesquiterpenoid chemicals that includes α-santalol dan β-santalol (Ariyanti & Asbur, 2018).

The qualities of sandalwood have led to excessive exploitation of this plant without concern for its sustainability, resulting in a precipitous decline in the sandalwood population in its natural environment. Sandalwood is an essential wood species that requires protection and preservation. This is shown by the inclusion of Sandalwood on the International Union for the Conservation of Nature (IUCN) Redlist from 1998 to the present in the category of vulnerable (vurnarable), indicating a high danger of extinction in the wild.

The establishment of sandalwood plantations has not received sufficient attention. The sandalwood planting initiative is still conducted in a restricted region, therefore the extraction rate of sandalwood in wild populations tends to outpace the replanting effort. This demonstrates that salvaging sandalwood is a significant concern that must be aided by effective culture methods as a step toward widespread regeneration (Herawan et al., 2015). The inability of forest plants to regenerate naturally necessitates human involvement for artificial regeneration, which may be accomplished by environmental manipulation or vegetative propagation. The advancement of plant cultivation technology necessitates the use of micropropagation or tissue culture for vegetative propagation (Jayusman, 2015).

Tissue culture is the process of growing plant components in the form of protoplasts, cells, cell groups, tissues, and organs in sterile or aseptic conditions. This approach has the benefits of producing the same tillers as the parent, producing mature plants relatively soon, maximizing land utilization, and being unaffected by the season (Harahap et al., 2019; Mastuti, 2017). Inducing the axillary branches of sandalwood plants is thus a method for achieving rapid mass regeneration. The benefit of axillary shoot proliferation is that the shoots are genetically more stable and there is little or no genetic variation (Ngezahayo & Liu, 2014).

Regulatory chemicals of the body added to the growth medium have a significant impact on the success of tissue culture. Cytokinins and auxins are two often used growth regulators. Benzyl Amino Purine (BAP) is a growth regulator belonging to the cytokinin family that is often employed because it is very efficient in promoting shoot

development and leaf formation, is readily available and reasonably affordable (George et al., 2007). NAA is a growth regulator from the auxin group that promotes root development, callus production, cell division, and elongation (Wattimena, 1988). This research aims to examine the influence of BAP and NAA on the induction of axillary shoots in Sandalwood (*Santalum album* L.).

METHOD

The research was carried out for four months at the tissue culture laboratory of UIN Maulana Malik Ibrahim Malang.

Tools and Materials

The following instruments are utilized: measuring cup, erlenmeyer, petri dish, stirring rod, culture bottle, dissection tools (scalpel, tweezers, scissors), LAF (Laminar Air Flow), analytical balance, oven, autoclave, Bunsen lamp, alcohol sprayer (spray), pH meter (pH indicator), refrigerator, culture rack, AC (Air Conditioner), lamp, hot plate and magnetic stirrer, culture rack, tissue, aluminum foil, plastic wrap, and a heating pan.

Sandalwood (*Santalum album*) one-year-old seedling stems are used as explants to be planted in the medium. Sterilization materials include 1 g/l of bactericide, fungicide, detergent, sterile distilled water, technical ethanol 70, HgCl, and clorox. MS Medium (Murashige & Skoog), 7% agar, and sugar were utilized as the media material. BAP and NAA were used as growth regulators (PGRs).

Research Design

This is a completely randomized design (CRD) research with two treatment components, namely the BAP and NAA concentrations. The combination of BAP and NAA concentrations included as many as 16 treatments with three replications. The design is shown in table 1 below.

Table 1. Research treatment combination.

NAA mg/l	**BAP mg/l**			
	0	**0.5**	**1**	**2**
0	B0N0	B1N0	B2N0	B3N0
0.1	B0N1	B1N1	B2N1	B3N1
0.25	B0N2	B1N2	B2N2	B3N2
0.5	B0N2	B1N2	B2N2	B3N2

FINDINGS AND DISCUSSION

The Emergence of Axillary Buds

The ANOVA test revealed that the combination of BAP and NAA concentrations influenced the development of Sandalwood axillary shoots (*Santalum album* L.). So, it

was proceeded with the DMRT 5% test, which revealed that the B2N3 treatment (BAP 1 mg/l + NAA 0.5 mg/l) was a concentration combination with the lowest average value, indicating that it has the fastest time in initiating the appearance of axillary buds, particularly for five days after planting (Figure 1).

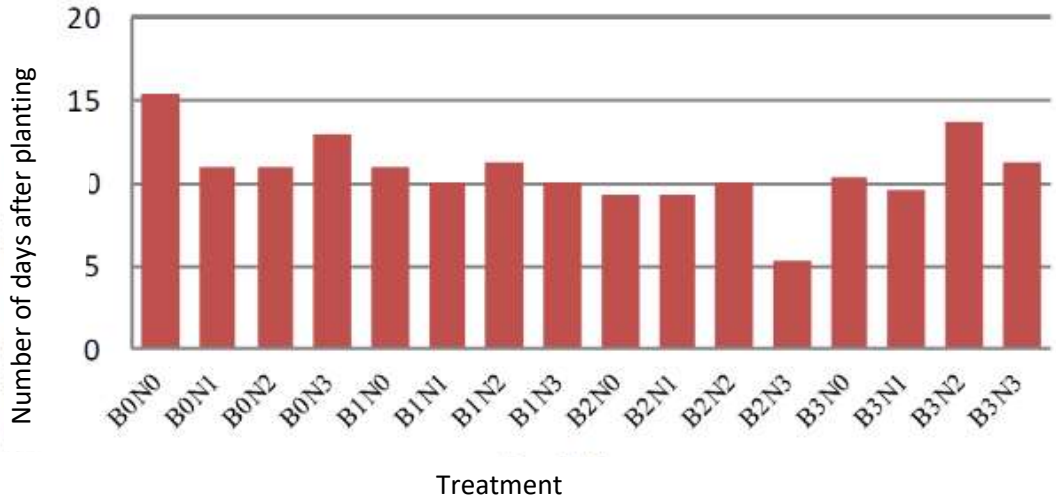

Figure 1. Effect of combination of BAP and NAA on the emergence of axillary shoots of sandalwood (*santalum album* l.) in vitro.

According to the scientific literature, the combination of cytokinins (BAP) and auxins (NAA) may accelerate morphogenesis during shoot production (Flick et al, 1993). Hormones called cytokinins have a significant effect on the development of shoots in the combined therapy. According to Yusnita (2003), BAP is a cytokinin that is often utilized to accelerate shoot development. Cytokinins have the ability to influence the KNOX (Knotted Like Homeobox) gene. The KNOX gene encodes a protein that stimulates the development and maintenance of the stem tip meristem, so ensuring that the cells are constantly proliferating (Wijayani and Solichatun, 2007).

Eventually, auxin will assist the differentiation of constantly dividing cells, resulting in the formation of shoots. The addition of BAP and NAA at the proper concentration may thereby hasten the development of shoots. The rapid emergence of shoots from explants is caused by the interplay between endogenous hormones and exogenous hormones (Yanti & Isda, 2021).

Number of Axillary Shoots

The quantity of branches is the most essential component in tissue culture plant proliferation. As additional shoots are produced, the culture may be multiplied to produce an increasing number of new shoots. The number of shoots was determined by counting the axillary buds, or the shoots that formed at the nodes of the plant.

The combination of BAP and NAA concentrations had an influence on the number of axillary shoots, as shown by the ANOVA test. The Duncan Multiple Range Test (DMRT) 5 percent additional test on the number of axillary buds in Sandalwood revealed that the B2N3 treatment (BAP 1+ NAA 0.5 mg/l) was an effective combination of concentrations for generating the number of shoots. According to the table, the mix of concentrations had different impacts on each explant. Higher BAP and NAA concentrations did not necessarily enhance the number of shoots (Figure 2).

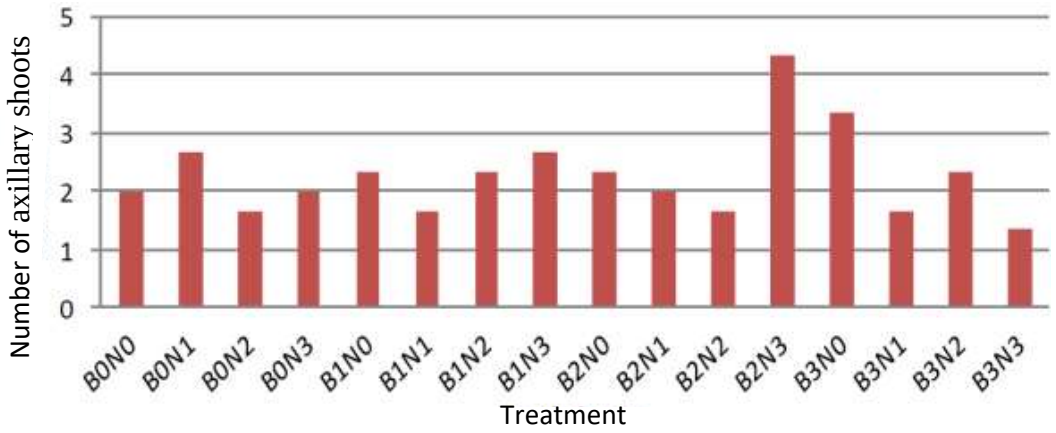

Figure 2. Effect of combination of BAP and NAA on the number of axillary shoots of sandalwood (*santalum album* l.) in vitro.

The combination of BAP and NAA at various concentrations affected the number of shoots produced in each treatment. According to Wattimena et al. (1992), the presence of certain combinations of growth regulators may impact the rate of cell division. Enzymes play a crucial part in metabolism, and a single molecule of growth regulator may impact how enzymes function; thus, several molecules of growth regulator can produce physiological changes in plants (Wattimena, 1991). The correct concentration of cytokinin and auxin in combination may enhance the number of shoots produced (Winarsih and Prayogo, 2000).

Axillary Shoot Length

The length of sandalwood shoots was affected by the combination of BAP and NAA concentrations, as shown by the ANOVA test on sandalwood bud length. The Duncan Multiple Range Test (DMRT) 5 percent additional test on the parameters of Cendana shoot length revealed that the treatment of BAP 0 + NAA 0 mg/l had no discernible influence on the behavior of other concentration combinations. This indicates that endogenous hormones in sandalwood explants may affect shoot length.

So that its action when combined with exogenous hormones is not considerably altered. However, in terms of average shoot length, the addition of 2 mg/l BAP and 0.5 mg/l

NAA is the most effective concentration combination. Nevertheless, the highest average cannot be used as a measure of the efficacy of hormone usage since the combination of effective amounts can only be identified by statistical analysis (Figure 3).

The elongation of the cells causes the extension of the shoot. The presence of the hormone auxin substantially influences the elongation of the cells. The primary purpose of auxin, according to Campbell and Reece (2012), is to encourage the elongation of cells in growing new shoots.

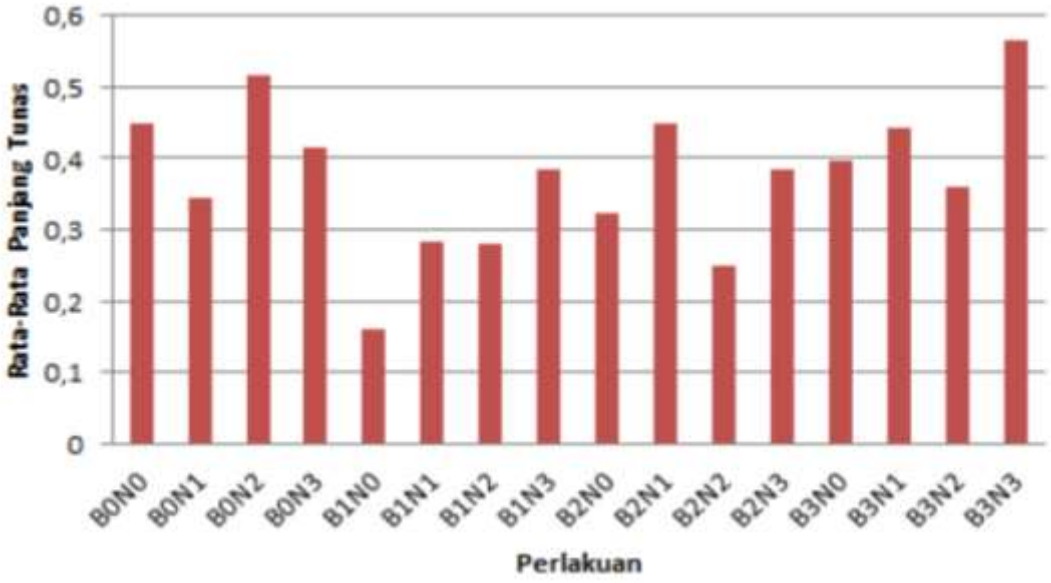

Figure 2. Effect of combination of BAP and NAA on the axillary shoot length of sandalwood (*santalum album* l.) in vitro.

Auxin may alter cell elongation by activating enzymes that reduce the pH of the cell wall. These enzymes function to disrupt the polysaccharide bonds in the cell wall, resulting in fast development. The primary auxin response takes place in the epidermis. Auxin activates genes in the epidermis by widening the epidermal wall; hence, the epidermis grows faster (Salisbury and Ross, 1995)

CONCLUSION

The addition of growth regulators BAP and NAA had an effect on the day of emergence of axillary shoots, number of axillary shoots, average shoot length, and number of leaves. Although the B2N3 treatment (BAP 1mg/l + NAA 0.5 mg/l) showed the highest average value for the parameters of the day of shoot emergence and the number of axillary shoots, the B3N0 treatment (BAP 2mg/l + NAA 0 mg/l) was more effective to

trigger axillary shoot induction because the results were not significantly different from the B2N3 treatment.

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