Effect of 6-BAP application on shoot production of *Melaleuca alternifolia* seedlings

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Abstract. *Melaleuca alternifolia* (tea tree) is one of the families of Myrtacea, which produces essential oils from their leaves, called *tea tree oil* (TTO) that is used as ingredients for cosmetics, antiseptics and pesticides. TTO leaves production of *M. alternifolia* plants can be increased by stimulating the growth of new shoots using 6-BAP hormone. The purpose of this study was to obtain appropriate dosage of 6-BAP to induce shoots in *M. alternifolia* seedlings. This research was conducted at the Dramaga Permanent Nursery in Bogor for 6 weeks from July-August 2019 with *M. alternifolia* seedlings originating from grafts over 4 months old. Completely randomized design (CRD) with different 6-BAP concentration treatments in 5 replicates was used. The 6-BAP (benzyl amino purine) with a concentration of 5 ppm, 10 ppm, 15 ppm, 20 ppm and without hormone (as control) were sprayed on the leaves and stem. The results showed that 6-BAP affected shoot length and number of leaves. Spraying with 5 ppm 6-BAP dose increased the length of shoots and the number of leaves after 6 weeks of application amounting to 1.9% and 11.4%, respectively.

1. Introduction

*Melaleuca alternifolia* (tea tree) belongs to Myrtacea family distributed from Australia to Asia, including Indonesia. This species grows in many areas in Australia, especially in Combell, Casino New South Wales [1]. The leaves of *M. alternifolia* is used as a source of tea tree oil (TTO) are produced by distilling tea tree leaves [2].

The use of tea tree oil (TTO) it is clear that TTO has many benefits for human health and care including derived from TTO. Among these uses are anti-bacterial [3, 4, 5] cure or relieve ulcers (acne) [6, 7], treatment of viral infections including herpes labialis, prevention of head lice, relieve symptoms associated with hypersensitive reactions, as an additional ingredient in toothpaste or mouthwash and anti-inflammatory [8]. Therefore it is necessary to find out the technology for stimulating leaves growth among others using cytokinin.

The demand that of TTO is dominated by the North American market (40%), Europe (30%), Asia (25%), Africa and South America (5%) [9] for natural and organic cosmetic products. Australia as the country's largest TTO with production reaches to 450-500 tons per year with total area of 3000 ha [10]. Based on the 2018 autumn market report from Ultra International B. V tea tree production in Australia from May to November was 600-700 tons with a market price of AUD 52.00 / kg. This shows an increase in market demand marked by increased production, so that the potential to be developed by countries other than Australia (Doran et al 2006). Demand for the TTO is used as a cosmetic raw material by some brands such as Boots (UK), The Body Shop, Elemis and Faith [11].

Cytokines are adenine-derived plant hormones that function to stimulate cell division and differentiation of mitosis, are synthesized at the root tip and translocated through xylem vessels. Natural cytokines are produced by tissues that are still active, especially in the roots, embryos and fruit. The
presence of cytokines will induce hormones activity to accelerate growth leaf buds [12]. Leaf biomass can be affected by the number of shoots and nutrients in increasing the number of leaves. Cytokinins are divided into two, namely: 1) the type of adenine produced in the roots, cambium tissue and parts of plants where cells are still actively dividing, for example kinetin, zeatin and 6-BAP, 2) this type of phenylurea is usually not formed by plants (synthetic) for example difeniluera, and tidiazuron (TDZ). Among the many types of hormones 6-BAP (benzyl amino purine) is a hormone to induce buds.

Quantitative increase in *M. alternifolia* plant production by accelerating the growth of new shoots by administering hormones. Application of the 6-BAP hormone as much as 60 ppm in tea leaves after 1 and 3 months increased the number of shoots, stem diameter, number of leaves and shoot length [13]. TTO production can be increased in quantity (biomass) and quality (genetics). The purpose of this research was to obtain the technique of induction of *M. alternifolia* buds by using the 6-BAP cytokinin hormone at various doses.

2. **Method**

2.1. **Study site**

The research was conducted at IPB Permanent Nursery Dramaga for 6 weeks of July-August 2019.

2.2 **Procedure**

2.2.1 **Materials**

Seedlings were obtained from grafting 9 months old coming from Cibodas, West Java as many as 75 plants. Growth media of husk charcoal, coconut fiber, and compost in a ratio of 1: 1: 2 (v / v / v). Factorial in was a of completely randomized design (CRD) was used. The seedlings were using 6-BAP with concentrations (1) 5 ppm, (2) 10 ppm, (3) 15 ppm, (4) 20 ppm and (5) without 6-BAP or control.

The 6-BAP solution was prepared by dissolving 100 mg of 6-BAP powder with 2 mL HCl 1% and diluted using distilled water until the volume became 100 mL. To get the 5, 10, 15, and 20 ppm hormone concentrations, 5, 10, 15 and 20 mL 6-bap solutions were needed which were diluted with distilled water until the volume reaches to 1 liter.

One week before treatment, trimming the shoots of plants was done. The application of 6-BAP hormone was done by spraying the leaves and stems of plants using a handsprayer. Each plant was sprayed using a 6-BAP solution of 10 mL every 2 weeks for 6 weeks. Hormones carried out in the morning at (06.00-08.00). After hormone treatments no watering for 24 hours.

2.2.2 **Data analysis**

Variables observed were new shoot number, number of leaves and shoot length were measured once every one week. Data is processed using SAS software 9.1.3. Analysis of variance (ANOVA) at a 95% confidence level followed by Duncan's test.

3. **Result and discussion**

The use of cytokines can be endogenously added to in vitro growth media and exogenously by spraying on plant parts. The addition of the BA cytokinin (benzyl adenine) hormone with a concentration of 1.1 μM in in vitro propagation increased the growth of *M. alternifolia* callus by 11.8 callus / explants after 60 days [14]. Exogenous cytokinin hormone at *M. alternifolia* is used to accelerate the growth of new shoots. Data on the effect of 6-BAP to *M. alternifolia* seedlings is presented in Table 1. Treatments cytokinins (6-BAP) as a whole are able to form buds and leaves at all dose levels but with different growth rates of new shoots.

| Dose 6-BAP (ppm) | New shoot | Number of leaf | Shoot elongation |
|------------------|-----------|---------------|-----------------|

Table 1. Effect of 6-BAP on *M. alternifolia* seedlings
Means within a value followed by the same letter in the same treatment and column shows no significant difference at P<0.05 by Duncan’s test.

Statistical results show that administration of 6-BAP was not significantly different from shoot growth. The average growth of shoots at doses of 0 ppm, 10 ppm and 15 ppm is the same, this can occur because of the lack of frequency of 6-BAP 6 times for 6 weeks. Application of BA 100-200 mg / L for 8 weeks on Dendrobium Hybrid plants with a frequency of giving once a week did not significantly affect the percentage of new shoots, number of new shoots and height of new shoots [15]. At a dose of 20 ppm, the average number of shoots increased by 3.73%. Cytokines are nitrogen-containing compounds namely adenine. Some cytokines are in the cells of all organisms, but their activity is found in plants. High cytokinin concentrations affect bud growth [16]. The use of cytokines can increase cell division and bud growth.

The shoot addition starts 1 week after spraying 6-BAP (Figure 1) which is marked by the formation of a bulge in the abdaxial or axillary part at the shoots and in the ovule. Inside the protrusion there is an organ called shoot apical meristem (SAM). The tissue is capable of producing various types of organs (leaves or flowers). The activity of SAM formation occurs very complex and is influenced by hormones contained in plant tissue. SAM growth activity to form new shoots is influenced by the hormone cytokinin. Cytokinins are involved in the processes of cell formation, bud growth, chloroplast formation and plant metabolism in response to the environment [17,18].

One of the factors that support the addition of shoots is mechanical treatment that is pruning shoots to regulate the balance of the cytokinin and auxin hormones that are auxillary buds and under the tip of the stem. Pruning shoots of M. alternifolia seedlings was done 1 week before the 6-BAP spraying treatment. At the shoot apical dormancy will occur so that no auxin supply occurs from the apical shoots which results in the activation of IPT (isopentyl transferase). IPT is an enzyme that is a biocatalyst in cytokinin biosynthesis. The resulting cytokines stimulate lateral shoot growth.

Synthesis of auxin which is not carried out causes the concentration of auxin to be low which decreases in the armpit of the leaf and stimulates the formation of the hormone cytokinin [19]. Low
cytokines and high induction trigger cell division and differentiation in the node to form lateral buds. The process of differentiation of the transport network in primordial branches affects the transport of nutrients and water from the stem to the primordial and stimulates the formation of lateral buds. The higher amount of cytokinin will stimulate shoot formation and the use of 6-BAP as much as 0.5 ppm is effective in stimulating shoot induction directly or adventitious in *Styrax benzoine* plants [19].

The increase in the number of shoots is followed by the increase in shoot length and number of leaves. The results of the analysis showed the average number of shoot growth and the highest number of leaves after 6 weeks (Figure 2) at a dose of 5 ppm were 1.95% and 11.4%. The effect of spraying cytokinin 6-BAP as much as 100 µM on wheat germ can increase the number of leaves [20]. Cytokinins wide influence on the physiological processes of plants one of which is capable of increasing the cytokinesis in cells, in addition to work in delaying the aging process in different types of plant leaves. The process of delaying the leaves occurs due to protein overhaul on the leaves. The aging process in leaves occurs due to the breakdown of proteins into amino acids by the protease enzyme so that in the presence of cytokinins, the performance of these enzymes is inhibited and protein life becomes longer. The role of cytokinins in delaying the process of leaf aging by inhibiting protein breakdown [21].

**Figure 2.** Increased shoot length after 6 weeks of 6-BAP spraying

Cytokines found in leaf exudates are commonly referred to as phloem gums and xylem gums which are used as regulators of motion in the process of cell division. Transportation of cytokines comes from going to the shoots. found the amount of cytokinins contained in the transport network, the availability of nutrients derived from the planting medium affects the length of the shoot. Planting media are able to influence the life needs of plants, namely providing space for root growth and development, providing air for respiration and providing water and nutrients needed by plants. Planting media in the form of husk charcoal: coconut fibers: compost (1: 1: 2) (v / v / v) can increase plant height, stem diameter, growth percentage and seed quality in *Eucalyptus pellita* [22]. Cytokinins influence the process of cell division in the lateral tip of the bud to convert into active meristem tissue thereby increasing the length of the shoot and the number of leaves. Sitoikin proper dosage coupled with the availability of water and nutrients obtained from the planting medium will affect the enlargement process and the leaf cell division and thus affect the size and number of leaves.

Leaf growth is also influenced by nitrogen and nutrient levels. Increased cytokinin levels are the effect of responses to nitrogen and phosphate content [23]. The element nitrogen is an important part of chlorophyll in the process of photosynthesis. Chlorophyll contained in the leaves affect the green color of the leaves. These nutrients act as protoplasmic constituents that affect cell size and numbers. Besides the factors of the number of cytokinins and nutrients, light intensity affects the photosynthesis process and affects the metabolic activity of plants. Carbohydrates resulting from photosynthesis are used as a food source for plant growth, besides the presence of the cytokinin hormone which supports cell division in apical meristem tissue affecting leaf size and shoot length.
Buds that are formed will develop into branches and shoots. Increasing the length of the shoots affects the number of sections and books in which the leaves grow. The length of the shoots causes maximum reception and absorption of sunlight. Maximum absorption of sunlight followed by nutrition, sufficient water, the photosynthesis process runs optimally. Photosynthetic capacity increases with an increasing number of leaves. The increased capacity of photosynthesis causes the distribution of photosynthates to run optimally, especially in the network of storage of food reserves including the stem, increasing the cell dividing response found in the shoots of the stem and causing the increase in shoot length. The increase in shoot length is directly proportional to the growth of the number of leaves.

4. Conclusion
The cytokinin hormone of 6-BAP can be used to induce shoots in *M. alternifolia* seedlings, it is evident that 6-BAP could stimulate the dormant buds to for new shoots and leaves in a small quantity. By spraying the leaves and stems of plants after at 6 weeks after spraying. At a dose of 5 ppm it was able to increase the number of leaves by 11.4% and 1.9%.

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