Effect of benzyl amino purine and indole-3-acetic acid on propagation of *Sterculia foetida in vitro*

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Abstract. *Sterculia foetida* is an oval seed plants that can be used as biofuel, which is one of the environmental friendly fuels. This plant is quite hard to find because not many peoples cultivate the plants. An in vitro propagation is one way to preserve the plant. This research aimed to determine optimum concentration of benzyl amino purine (BAP) and indole-3-acetic acid (IAA) to propagate *S. foetida in vitro*. The results showed that woody plant medium (WPM) added by 4 mg L BAP^{-1} and 0.5 mg L IAA^{-1} was able to produce complete plantlet, whereas those added by 4 mg L BAP^{-1} and 1 mg L IAA^{-1} generated the best growth of shoot and leaves.

1. Introduction

Sterculia foetida is a type of potential plant to be developed as a source of biofuel which has not been cultivated [1]. This plant can be grown in the tropics and sub-tropics (30°N-35°S). Oval-shaped seed is white with yellow seed coat [2]. The main composition of dry beans is the fats (51.78%), protein (21.61%), starch (12.1%), sugar (5%), cellulose (5.51%) and ash (3.9%) [3].

In this research, propagation of plant through tissue culture. Propagation by tissue culture is expected to provide seed mass, uniforms and all year round. Tissue culture also produces pathogen-free plant and germplasm storage [4]. Tissue culture media used, are woody plant medium (WPM) with plant growth regulator (PGR) benzyl amino purine (BAP) and indole-3-acetic acid (IAA). The BAP is a class of cytokines with function for cell division in meristematic tissue, stimulates the differentiation of the cells generated in meristem and encourage the growth of side shoots, leaves apical dominance and expansion. The IAA is a commonly used class of auxin in tissue culture serves to spur the process of cell elongation. This research aimed to determine optimum concentration of BAP and IAA to propagate *S. foetida* by *in vitro*.

2. Methods

Research was held at Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture Universitas Sebelas Maret Surakarta. The explants were maintained in the green house for three months. Materials used in the study were explant from stem of *S. foetida*, BAP (0, 2, 4, and 6 mg L^{-1}), IAA (0, 0.5 and 1 mg L^{-1}) and some nutrients used in the manufacture of WPM medium. The data were analyzed using descriptive methods that describe the results of observational studies.

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3. Results and discussion

3.1. Callus and shoot emergence

Callus induction from petiole explant was readily obtained within 2 weeks [5]. Formation of callus culture media is very important to observe because it is hopeful to promote callus differentiation. In this research, callus that first appeared in the initiation medium was white translucent with crumb texture. The results of BAP and IAA with varying concentrations to produce callus growth is uneven (Fig. 2a). The emergence of the fastest callus was found in the treatment of 4 mg L BAP^{-1} and 4 mg L BAP^{-1} + 1 mg L IAA^{-1} are two weeks after planting, while largely the result of interaction of BAP and IAA capable of producing callus on the 4th week after planting. The IAA concentration of 0.5 mg L^{-1} and 1 mg L BAP^{-1}can accelerate emergence of a callus. According Rao and Purohit [6] high concentrations of auxin stimulates callus formation and morphogenesis pressing.

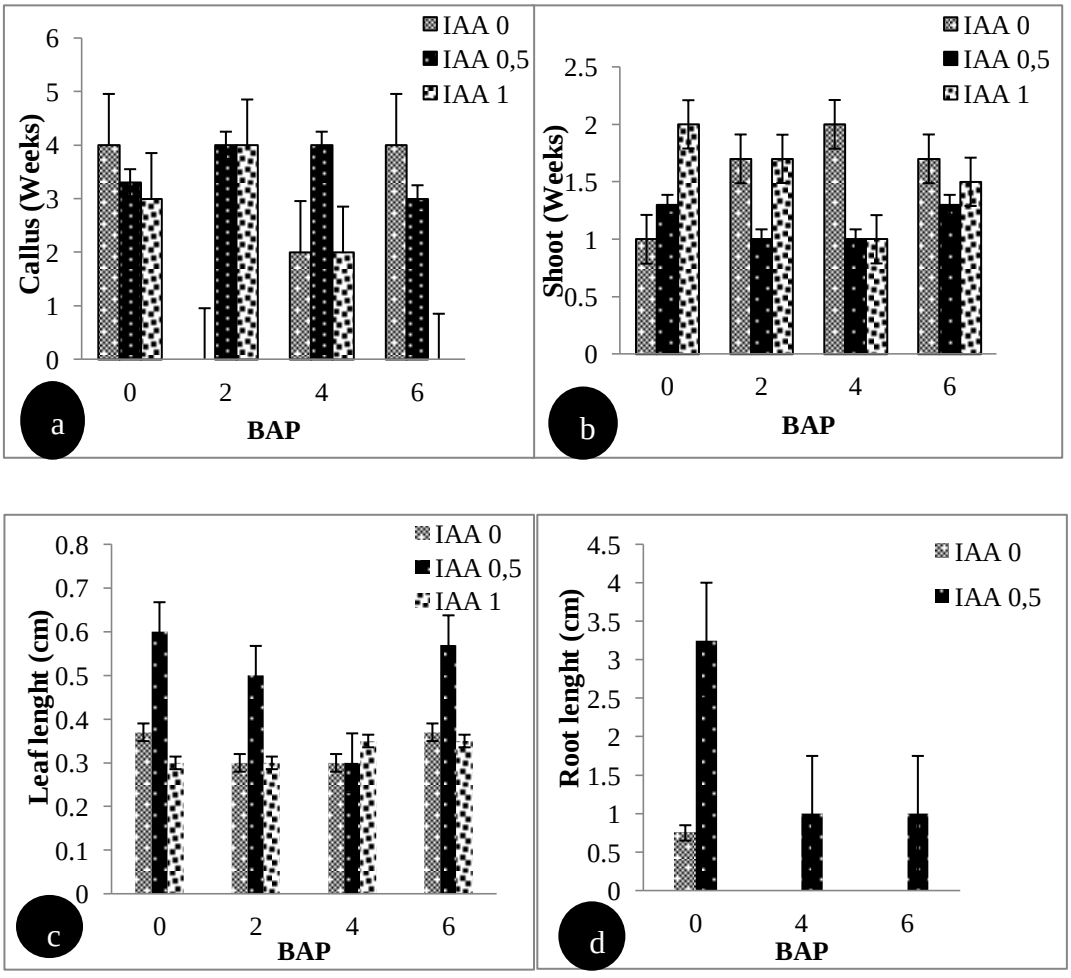

Figure 1. (a) Effect of BAP and IAA on the callus emergence of *Sterculia foetida* explant, (b) shoot emergence, (c) leaf length, (d) root length

The emergence of shoots characterized by their greenish bulge in the armpit leaves. Shoots growing from the meristem tissue composed of components promeristem or the basics of the new organs begin to form. The morphological diversity of plant organs depends on the developmental origin primordia, shoot meristem generate leaves [7]. The highest shoot regeneration potential was observed on stem and leaf segments [5]. In this research, the shoots appeared first in the treatment of 2

mg L BAP^{-1} + 0.5 mg L IAA^{-1}; 4 mg L BAP^{-1} + 0.5 mg L IAA^{-1}; 4 mg L BAP^{-1} + IAA 1 mg L^{-1}; and control (Fig. 2b). The most of the four treatments shoots appear in the first week. The addition of IAA at 6 mg L BAP^{-1} in the culture medium can accelerate the emergence of shoots, because the nature of the IAA at low concentration between 0.5-1 mg L^{-1} combined with high concentrations of cytokines can trigger the growth of shoots [8].

3.2. Leaf Length

Leaves for the plant have an important role because it the center of the leaf photosynthesis. Although the mechanism of formation of a crop that can perform photosynthesis by itself, conditions *in vitro* have not clearly known yet [9]. Number of leaves in this research was calculated based on observations of plantlets at 12 weeks after planting. The number of leaves was affected by the addition of plant growth regulators into the media.

The average number of leaves was ranged from 1 to 3 sheets. (Fig. 2b). Number of leaves that appear in visible explants varied. Variations in the number of leaves is possible because the endogenous hormone levels are not exactly the same so its response to the addition of plant growth regulator also varies [10]. Combination of BAP and IAA can successfully leverage in tissue culture if using specific concentration. Application of auxin with cytokinin (BAP) at the appropriate concentration can spur the growth of explants, especially in the formation of leaf, shoot and intensive segment [11]. The role of auxin in the plant growth of tissue culture, especially arranged leaf expansion [12].

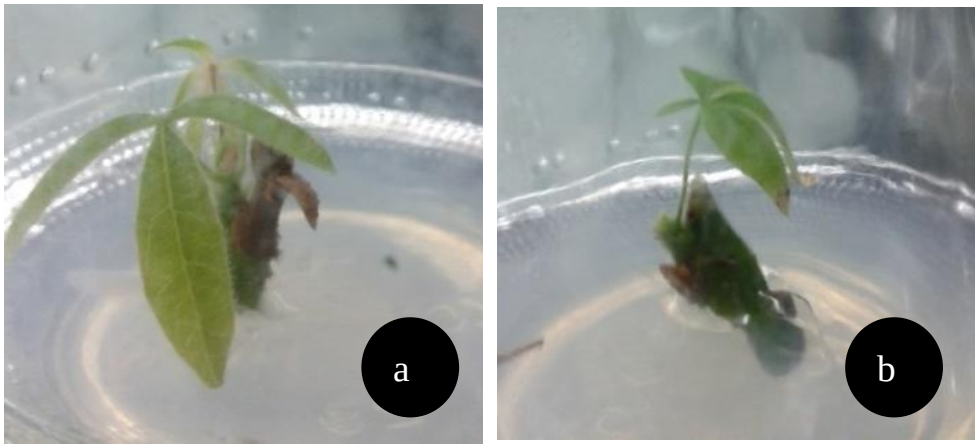

Figure 2. (a) Number of leaves with 0 mg L BAP^{-1} and 0.5 mg L IAA^{-1}. (b) 4 mg L BAP^{-1} and 0.5 mg L IAA^{-1}

In leaf morphogenesis, the control of cell proliferation seems to be related to the control of cell size [13]. Leaf growth is a process of differentiation leaf buds, with the addition of plant growth regulator such as auxin and cytokinin encourage the differentiation process. Leaf growth begins periclinal their division followed by cell growth in children and cause a bulge, is leaf primordia. Leaf surface area will increase caused by the activity of cleavage anticlinal [14].

The average length of the largest leaf obtained at 0.5 mg L IAA^{-1} treatment is 0.6 cm and a combination of 6 mg L BAP^{-1} and 0.5 mg L IAA^{-1} is 0.57 cm (Fig. 1c). High concentration of BAP combined with 0.5 mg L IAA^{-1} had leaves longer, while some combination of BAP and IAA treatment of most other produce leaf length of 0.3 cm. This is because the ability of each cell receiving a different PGR response and have optimal limit [15]. High concentrations of BAP were able to increase leaf area of explant [16].

In most of the higher plants, the root is the part that contained in the soil, the plant mainly serves the cantilever body and for the absorption of water and minerals [17]. Plantlets root formation is one

thing that is advantageous, because it can increase the growth during the process of propagation *in vitro*. Many roots that come out so much more facilitate and expedite absorption of nutrients and water to optimal plant growth.

3.3. Root Shoot

Adding BAP and IAA response for explant able to produce a single root. It also occurred in the research [18] that were capable of producing a single root from stem explant. Roots in explant representing 1. The roots can be directly formed on the explants, either from the network or callus, if the media is given with sufficient auxin. Low concentrations of auxin could stimulate root growth [19].

The roots of plants are an important part in the growth. The plant has root length also good position in the growth media. The fact that developmental stages of root cells are roughly correlated with distance from the apical meristem [20]. Root elongation process is accelerated by the presence of auxin in the media. Elongation is caused by enlargement of cells that are formed when the embryo is still developing in the mother plant [14].

The highest root length was obtained from the concentration of 0 mg/LBAP + 0.5 mg/LIAA is 3.25 cm (Fig. 1d). Control also produce roots that is 0.75 cm, but the results were higher than the results of additional treatment BAP 4 mg/Land 6 mg/L. The higher concentration of BAP generate root length. The effect of BAP on root elongation activity, concentrations of cytokines is thought to stimulate cell division effective root [21].

4. Conclusions

It can be concluded that: (a) WPM medium added by 4 ppm BAP and 0.5 ppm IAA is able to produce complete plantlet by forming buds, leaves and roots. (b) WPM medium added by 4 ppm BAP and 1 ppm IAA produce the best growth of shoot and leave. (c) Callus can be formed in almost any combination of BAP and IAA treatment.

Acknowledgements

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