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IN VITRO SHOOT INDUCTION OF *BATIK* BAMBOO USING BAP AND NAA

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Abstract

Bamboo has great potential to be used as building materials, raw materials for a paper (pulp), textiles, absorbing carbon, holding and storing water. This study aimed to obtain the proper media combined with the addition of 6-Benzyl Amino Purine (BAP) and Naphthalene Acetic Acid (NAA) in inducing *batik* bamboo shoots. Observation parameters, including the time of the shoot, appeared, percentage of total explants sprouting, number of shoots, the height of shoots, number of leaves, length of leaves, and percentage of contaminated explants. Descriptive analysis was used to analyze the data by identifying the variable observations. The results showed that the J1 (BAP 0 + NAA 0.5) was the best combination for *batik* bamboo tissue culture in inducing the number of shoots, the height of shoots, number of leaves, and length of leaves.

Keywords **:** Bamboo, NAA, BAP, shoot induction.

Introduction

Bamboo is a type of grass that belongs to the family Poaceae and a part of non-timber forest product commodities. Bamboo has the potential as a wood substitute material because it can continue to produce, as long as the harvesting is controlled and planned. Bamboo has several advantages over wood because it has a small shrinkage ratio, can be curved or elastic and has a decorative value (Wong, 2004; Paembonan and SHL, 2019).

Research conducted by Astuti (2014) on the induction of shoots and roots of yellow bamboo (*Bambusa vulgaris*) with in vitro method by using Murashige and Skoog (MS) media with a combination of 6-Benzyl Amino Purine (BAP) 1.0 ppm and Indole Butyric Acid (IBA) 2.5 ppm gave better results in the speed of the shoots emergence and the number of yellow bamboo shoots. While the addition of 6-Benzyl Amino Purine (BAP) 2.0 ppm and 2.5 ppm IBA gave the highest results in yellow bamboo shoots for about 13.75 ± 1.50 mm.

Plant growth regulators have a vital role in controlling biological processes in plant tissues. The use of growth regulators in tissue culture depends on the intended purpose or direction of plant growth (Larekeng *et al*., 2020). The addition of auxin and cytokines on the culture media can increase the concentration of endogenous growth regulation substances in the cell. Thus it becomes a "triggering factor" in the process of tissue development. Ways to stimulate the formation of shoots, it can be done by manipulating the auxin and exogenous cytokinins doses (Lestari, 2011).

A critical factor for explant growth is the use of basic media and appropriate growth regulators. Growing media in tissue culture has a great influence on the growth and development of explants and the resulting seeds. The optimal growth of plants in growing media requires additional substances in the form of growth regulators. There are only a few organic compounds for growth on the plant; it is necessary to add hormones from outside. Synthesis hormones added from outside the plant are called growth regulators. These substances stimulate growth, such as root growth, shoots, germination, and so on (Lestari, 2011).

One type of auxin that is often used in tissue culture is Naphthalene Acetic Acid (NAA), which is a synthetic auxin that is very effective for callus induction (a group of amorphous/unformed cells which formed from cells that divide continuously through in vitro). In contrast, BAP is a synthetic cytokinin that is often combined with auxin. Giving NAA and BAP will stimulate cell defense and increase synthetic proteins that can affect callus growth (Wattimena, 1988).

This research is important to know the right media by adding BAP and NAA cytokinin in inducing bamboo shoots from shoot explants; thus, high-quality bamboo seeds are obtained by in-vitro. This research was using explants from *Batik* Bamboo because of its high economic value among the community of furniture makers.

Materials and Methods

Time and Place of Research

The research was conducted from February to June 2019. The research activities were carried out at the Laboratory of 2nd Regional Seed/Seedling Forest Office, Makassar.

Research Material

The plant materials used in this research were healthy *batik* bamboo explants shoots which not infected with pests and diseases. The media used in this research were MS media and its modification. Other materials used were plant growth regulators (PGR), BAP and NAA.

Research Procedure

Sterilization of plant material was performed in two stages, which were outside of LAFC and inside of LAFC, such as below:

Steps of sterilization outside of LAFC

- The explants were rinsed in sterile distilled water for a minute
- The explant shoots were soaked in a sterile distilled water with tween 80 for 20 minutes
- The shoot explants were rinsed by using sterile distilled water for three times.

- The explant shoots were soaked in a 2% of agrept for 60 minutes
- The shoot explants were rinsed by using sterile distilled water for three times.
- The explant shoots were soaked in a 2% of masalgin for 60 minutes
- The shoot explants were rinsed by using sterile distilled water for three times.

Steps of sterilization inside of LAFC

- The explants were soaked in 70% alcohol with two drops of tween 80 for a minute
- The explants were rinsed with sterile distilled water for three times.
- The explants were soaked in 20% sodium hypochlorite with two drops of tween 80 for 10 minutes
- The explants were inised with sterile distilled water for three times
- The explants were soaked in 10% sodium hypochlorite with two drops of tween 80 for 5 minutes
- The explants were rinsed with sterile aquadest for three times
- After drying, shoot explants would be ready to be planted on the media according to treatment.

Explant Planting : Planting was carried out in LAFC with the following steps:

- The explant material was inserted into a Petri dish containing a sterile filter paper.
- The edge of the explant was cut with a size of ± 2 cm by using a sterile scalpel knife with a slope of 45^o in the part that would be planted on the media.
- Shoot explant planted in culture bottles was closed to Bunsen with tweezers.
- The closed culture bottles that had been planted and glued together with plastic wrapping.
- The culture bottles were labeled with the date of planting, name of the media, and type of treatment given. After all the explants were planted, the bottles moved to the culture shelves that had been provided.

Research Design

The total media used in this study was 15 media, which was replicated six times. The total unit of observation was 90 units, with one explant for each unit.

Table 1 : Combination of BAP and NAA used for in vitro shoot induction on Batik bamboo

Treatment	Medium
J1	MS + BAP 0 ppm + NAA 0,5 ppm
J2	MS + BAP 0 ppm + NAA 1 ppm
J3	MS + BAP 0 ppm + NAA 1,5 ppm
J4	MS + BAP 1 ppm + NAA 0 ppm
J5	MS + BAP 1 ppm + NAA 0,5 ppm
J6	MS + BAP 1 ppm + NAA 1 ppm
J7	MS + BAP 1 ppm + NAA 1,5 ppm
J8	MS + BAP 2 ppm + NAA 0 ppm
J9	MS + BAP 2 ppm + NAA 0,5 ppm
J10	MS + BAP 2 ppm + NAA 1 ppm
J11	MS + BAP 2 ppm + NAA 1,5 ppm
J12	MS + BAP 3 ppm + NAA 0 ppm
J13	MS + BAP 3 ppm + NAA 0,5 ppm
J14	MS + BAP 3 ppm + NAA 1 ppm
J15	MS + BAP 3 ppm + NAA 1,5 ppm

Observation Variable

Observations were made after 90 days. Observed variables included:

- When shoots appeared, the observation was made once every two days. Shoot growth is a process of growth and development.
- Percentage of total explants sprouting per treatment (%), calculated at the end of the observation by using the formula below:

$$\frac{\Sigma \text{explants sprouting per treatment}}{\Sigma \text{total of explants planted}} \; x \; 100\%$$

– Number of shoots, ob

– served and counted every two days.

– Height of shoot (cm), measured at the end of the observation.

– The number of leaves (sheet), counted every two days.

– Leaves length (cm), measured at the end of the observation.

– Contaminated explant percentages (%), calculated per treatment with the formula below:

$$\frac{\Sigma \text{contaminated explants}}{\Sigma \text{total of explants planted}} \; x \; 100\%$$

Data analysis

The analysis used was descriptive analysis by observing and identifying each explant growth based on the parameters.

Results and Discussion

Time of Shoot Appeared

Batik bamboo tissue culture research was carried out for 150 days. The observations result showed that the types of media gave different responses to the time the shoot started to appeared. The growth was characterized by the appearance of a light green cone-shaped bulge with a length of about 1 mm and, then elongated which later called shoot and then followed by leaf formation. The appearance of the shoot is one important factor that indicates good growth in plant propagation by tissue culture methods. Roux (2004) (Roux, 2004) stated that the percentage of explants forming shoot could be used to measure the regeneration ability of explants. The result of observations on the average time of shoot appearance is shown in Figure 2.

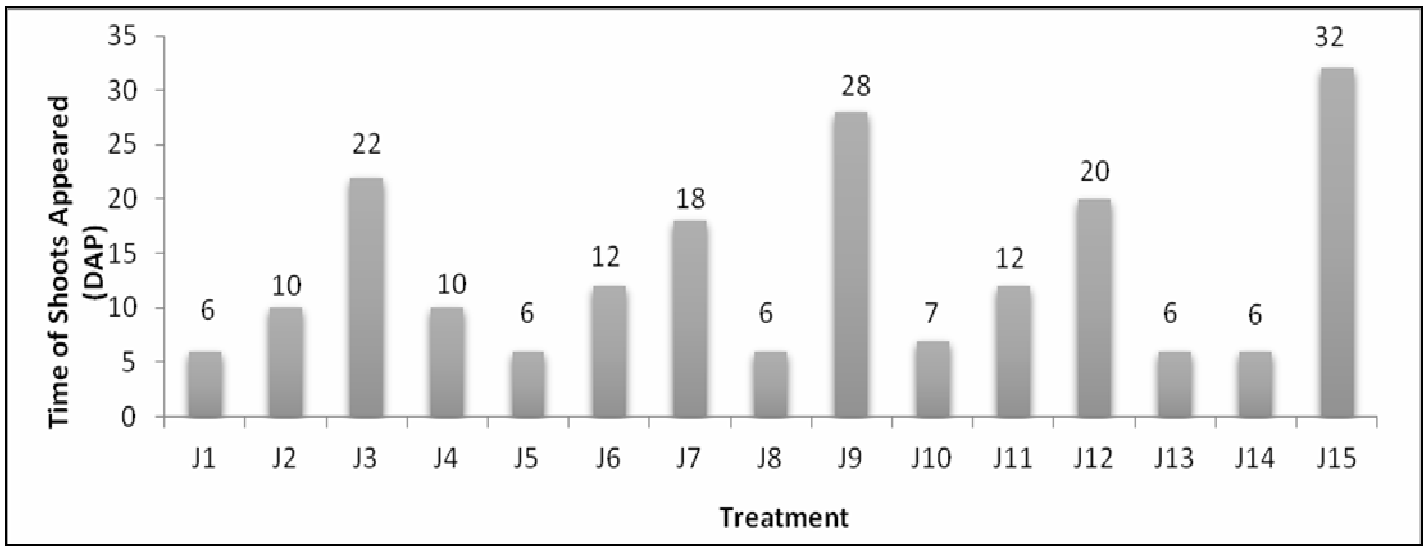

Fig. 2 : Time Charts of *Batik* Bamboo Shoots Appeared (DAP).

In Figure 2, bamboo had a growth response that began to sprout at 6 DAP. These results indicate that BAP and NAA have a better influence on stimulates shoot. The emergence of shoots is characterized by changes in the size of the node and an increase of size on the next day. The fastest shoots formed were on the J1, J5, J8, J13, and J14 media, which was 6 DAP, followed by J10 for 7 DAP, then J6 and J11 sprouted on 12 DAP, J7 sprouted 18 DAP, JA12 sprouted 20 DAP, J3 sprouted 22 DAP, J9 sprouted 28 DAP, and J15 sprouted on 32 DAP. These results mean that the BAP and NAA as growth regulators are suitable in stimulating the shoot appearance through different concentrations.

Based on observations, it showed that *batik* bamboo had a rapid growth response, which occurred during the first week after planting. NAA is an auxin group as a regulator of cell enlargement and triggers cell extension at the end of the meristem area. Low auxin concentrations will increase the formation of somatic embryogenesis. It is a process of forming new plants through embryonic resistance. The embryo has a bipolar structure that trigger shoots and root growth (Fitri, 2007).

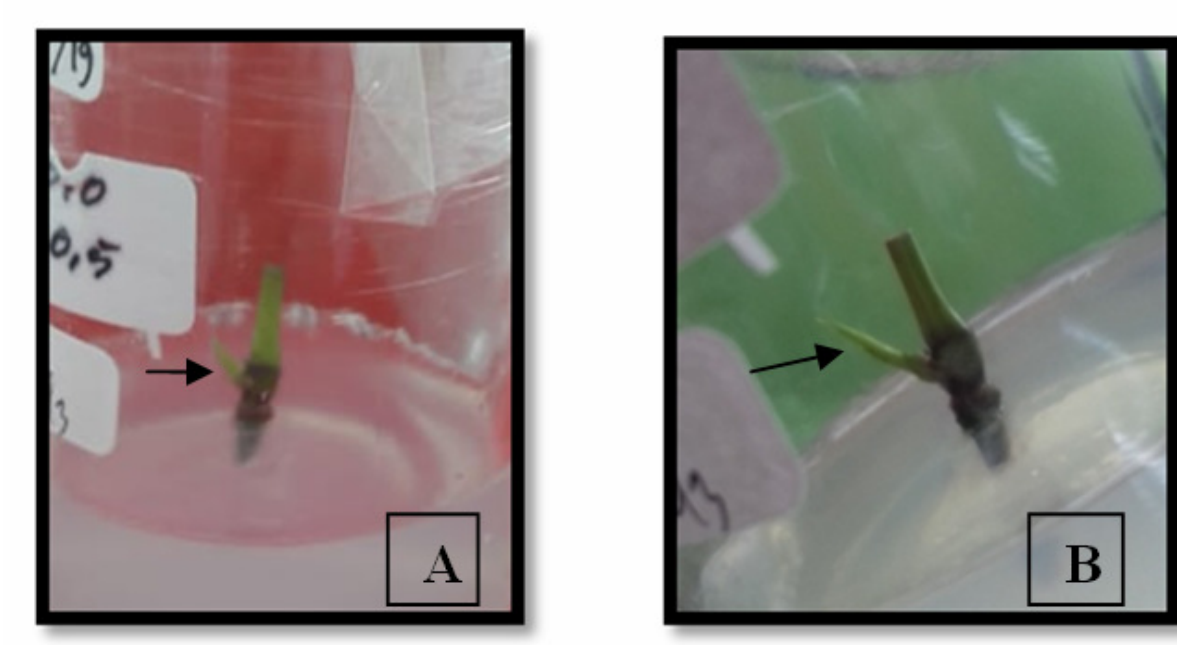

Fig. 3 : A shoot of *Batik* Bamboo A. 7 Days After Planting (DAP) and B. 14 Days After Planting (DAP).

Figure 3 presents *batik* bamboo that was able to grow and developed into a new plant. The growth of cultured *batik* bamboo is categorized as good by the formation of shoot and leaf.

Percentage of total explants sprouting

The best combination of growth regulators for shoot formation was on J1 (BAP 1.0 + NAA 0.5). From observations for 90 days, the percentages of explants that were sprouting between 10% - 50%. The percentages in the graph can be seen in Figure 4.

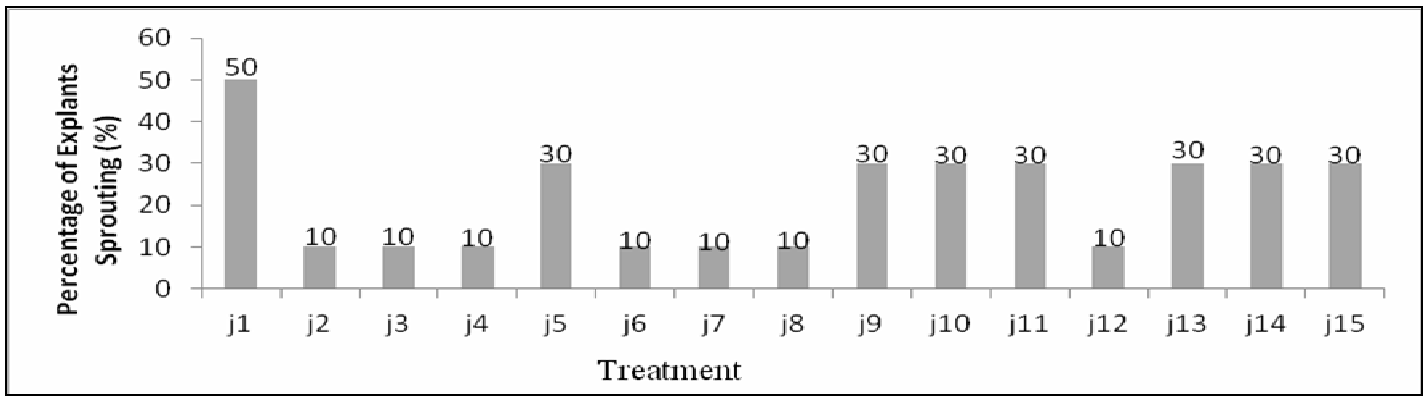

Fig. 4 : Average Percentage of *Batik* Bamboo Explants Sprouting

J1 (BAP 0 + NAA 0.5) produced the highest sprouting explants with a percentage of 50%, this value was determined from the results of the calculation per treatment of six replications and the MS base media with growth regulator concentration gave the best response. Based on observations from 15 treatments (90 bottles), the average percentage of explant sprouting was 27%.

Number of shoots

The number of shoots is the most important factor in plant multiplication by tissue culture. Calculation on the number of shoots is from the elongation of the shoot. Culture multiplication can be done to produce a new shoot in large quantities.

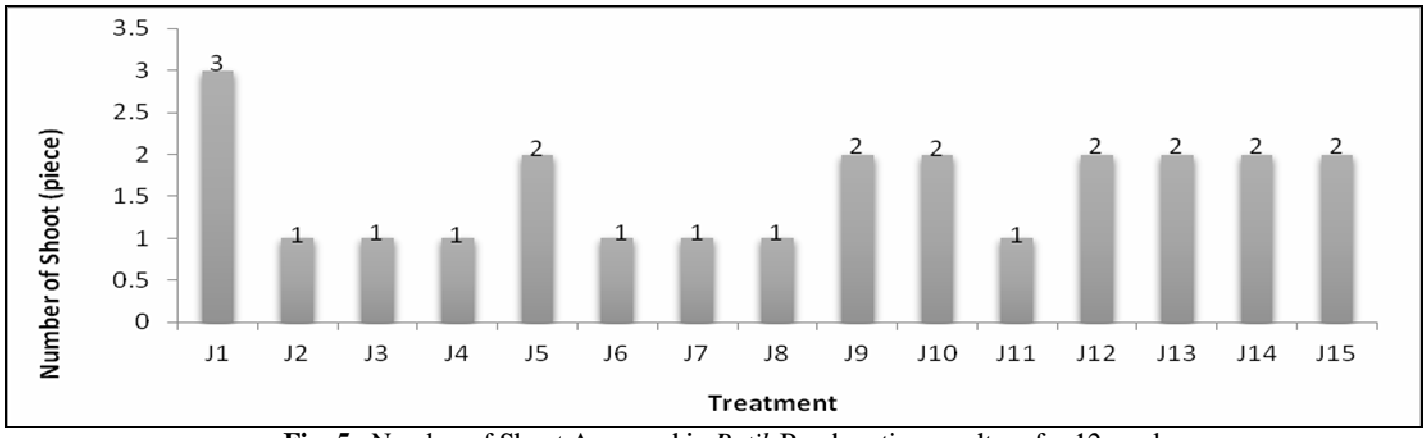

Fig. 5 : Number of Shoot Appeared in *Batik* Bamboo tissue culture for 12 weeks

Figure 5 depicts the highest average number of shoots that were obtained on J1 (BAP 0 + NAA 0.5) was 3. Media J2 (BAP 0 + NAA 1), J3 (BAP 0 + NAA 1.5), J4 (BAP 1 + NAA 0), J6 (BAP 1 + NAA 1), J7 (BAP 1 + NAA 1.5) , J8 (BAP 2 + NAA 0), and J11 (BAP 2 + NAA 1.5) produced the least number of shoot, which was 1. Different number shoots were influenced by the ability of the explants to absorbed nutrients in the MS media and the growth regulators given.

The positive response of plants toward the application of growth regulators is influenced by several factors, including plant types, plant growth phases, types of growth regulators, concentration, and how to apply growth regulators (Fitri 2007)—the low number of shoot caused by under optimal conditions of explants used. Contamination occurred in several trials because the sterilization process was not optimal.

Height of shoots

The height of the shoot was approximately 0.3 cm to 1.8 cm. Treatment J1 (BAP 0 + NAA 0.5) produced the highest average shoot height, which was 1.8 cm, while J3 (BAP 0 + NAA 1.5) produced the lowest average shoot, which equal to 0.3 cm. The average shoot height result is shown in Figure 6.

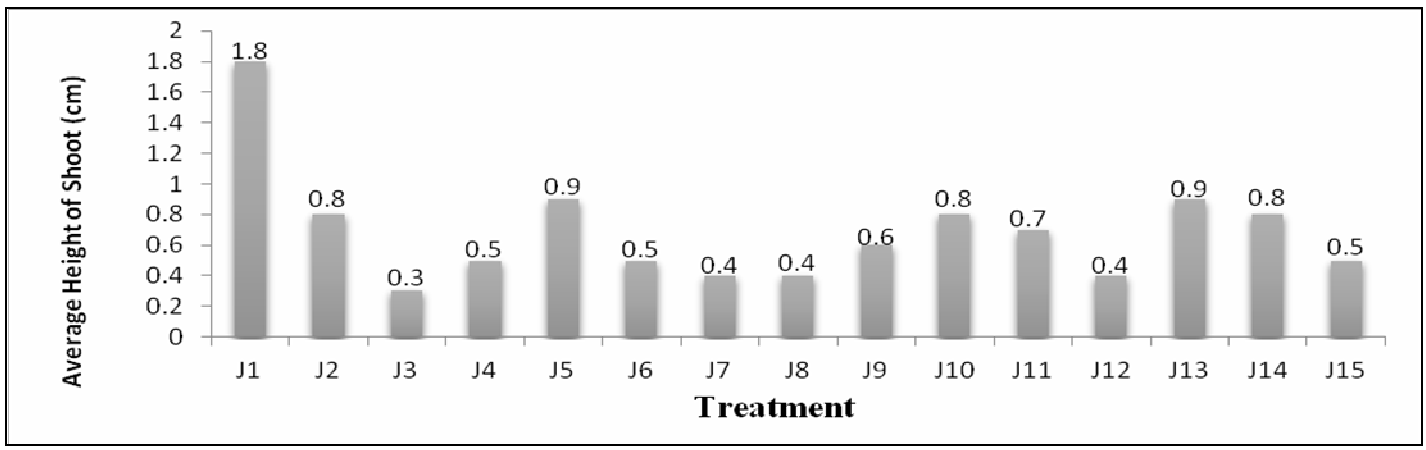

Fig. 6 : Average Height of Shoots of *Batik* Bamboo

In this study, it could be seen that BAP and NAA gave different shoot growth effects. The difference in shoot height was because of the concentration combination of auxin PGR, namely NAA. The less addition of NAA, the higher the shoots produced. The BAP growth regulator did not significantly influence shoot height because some BAP concentrations used in this study showed that shoot height had more influence on NAA concentration.

Number of Leaves

The leaf's characteristics are green sheets, either still curled or have been opened (Nurhayati 2014). The calculation on the number of leaves was begun at the time of the leaves formation until the 12th week, with the number of leaves formed in each treatment were different in every culture bottles. The growth of *Batik* bamboo leaves can be seen in Figure 7.

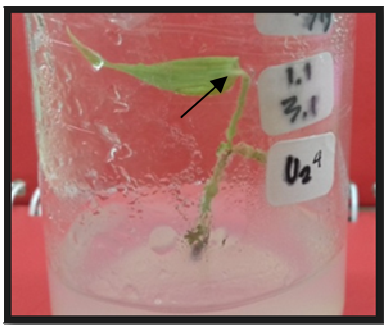

Fig. 7 : Leaves formed after the 8th week

The observations showed that the leaves began to grow in the 8th week after planting. However, not all explants showed any leaf, due to a combination of PGR concentrations in each treatment media and also some explants were contaminated. The number of leaves in each treatment is presented in Figure 8.

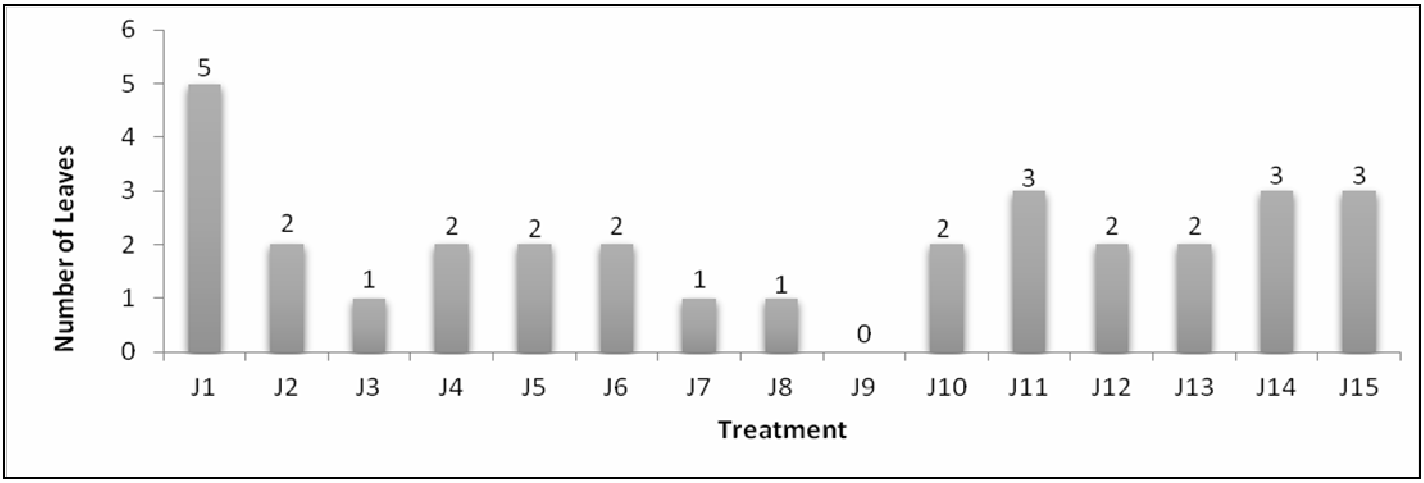

Fig. 8 : Number of Leaves of *Batik* Bamboo

J1 (BAP 1.0 + NAA 0.5) produced the highest number of leaves with five sheets, while J9 (BAP 2 + NAA 0.5) did not produce any leaf. The results of the calculation of the number of leaves showed that media treatment with auxin NAA with low concentrations and BAP with high and low concentrations were the best in producing leaf. The addition of the cytokinin hormone (BAP) induces leaf cells to be meristematic, to defend and able to develop into plant buds. Hence, it forms whole plant organs through the process of organogenesis (Rohma, 2012).

Length of leaves

The length of the leaves in some treatments showed different responses. The treatment with the addition of PGR at different concentrations gave different influences on the length of leaves. The average length of leaves is depicted in Figure 9.

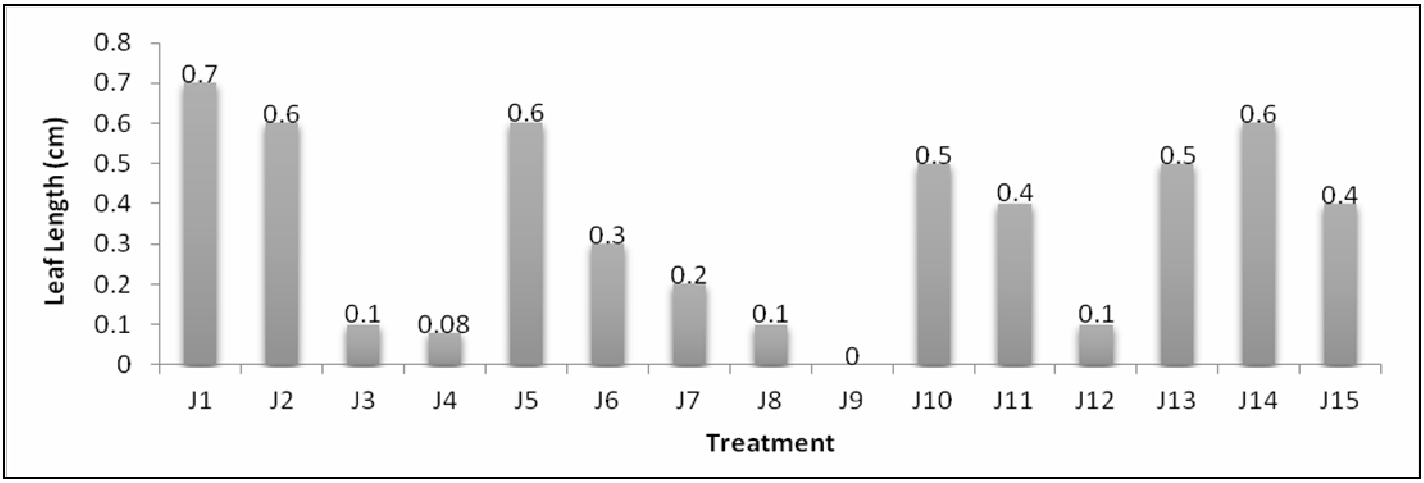

Fig. 9 : Average Length of *Batik* Bamboo Leaves

Figure 9 displays J1 (BAP 1.0 + NAA 0.5) had the highest average leaf length, which was 0.7 cm, while the lowest leaf length on J4 (BAP 1 + NAA 0), for 0.08 cm. J1 (BAP 1.0 + NAA 0.5) was a media with the growth regulator concentration, which able gave the best response on the highest increase in leaf length. A study by Behera et al (2018) (Behera S, Kamila P K, Rout K K, Barik D P, Panda P C and Naik S K 2018) showed that leaf length had a relationship with the number of leaves in explants, good leaf growth produces an average longest leaf length.

Percentage of contaminated explant

The results showed that some explants got contaminated. Bacteria and fungi caused the contamination; both types of contaminations could be seen from the physical characteristics that appeared in explants and culture media. When contaminated by fungi, the plant was drier, and fungal hyphae appeared on the affected plant and can be characterized by the presence of lines (like thread) with white to gray colors. When contaminated with bacteria, the plant got wet and produced mucus.

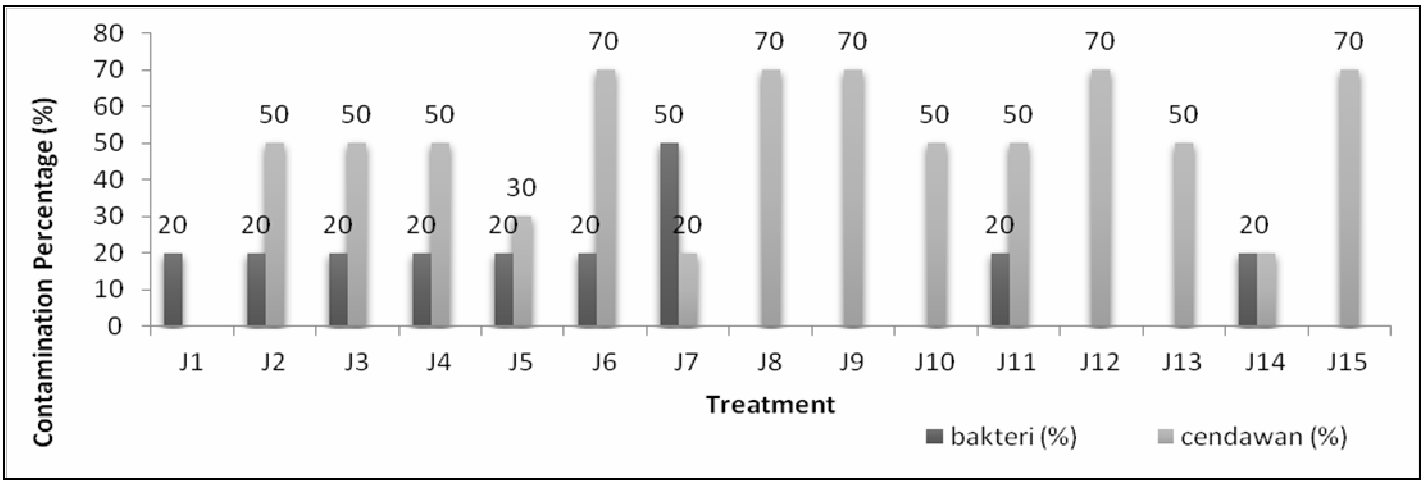

Fig. 10 : The average percentage of *Batik* Bamboo Contamination

Figure 10 shows that there was a high level of contamination in all treatments. The highest contamination occurred on J6, J8, J9, J12 and J15. The contamination of bacteria and fungi on *batik* bamboo was causing a stunted plant growth process. Fungi and bacteria live and breed in the surface of bamboo nodes, making it difficult to eliminate them completely without causing any damage to plant parts. Contamination also caused by explant parent plants that had been contaminated with pathogens. Examples of fungal and bacterial contaminations are presented in Figure 11.

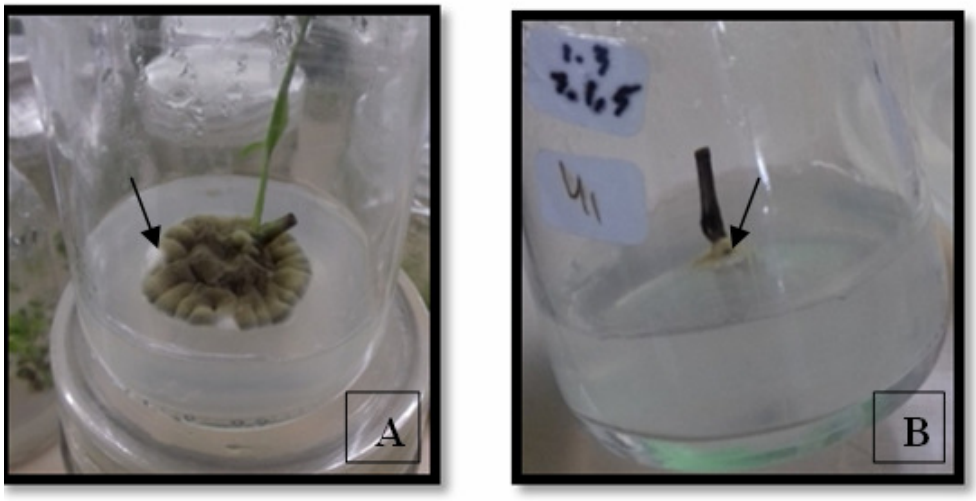

Fig. 11 : Contaminated *Batik* Bamboo Explant (A). Fungi ; (B). Bacteria

Gunawan (1992) explained each plant material has a different level of surface contamination, which depends on the growth environment and explant conditions. Based on observations, it was generally caused by fungi that begun to attack the surface of the explant and spread throughout the bottling area. Bamboo tissue plant culture can be succeeded if contamination does not occur. Contamination occurs because the explant's surface sterilization process was not effective in removing microorganisms that can cause contamination; in this case, fungi and bacteria.

Conclusion

The study showed that J1 (BAP 0 + NAA 0.5) was the right combination for *batik* bamboo tissue culture in inducing the number of shoots, shoot height, leaves length, and a number of leaves.

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