

In-Vitro Shoot Induction of Pring Tutul (*Bambusa maculata*) through in Various Plant Growth Regulators (PGR)

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Submission date: 18-Mar-2021 07:42AM (UTC-0700)

Submission ID: 1536187884

File name: Larekeng_2020_IOP_Conf._Ser.__Earth_Environ._Sci._575_012139.pdf (925.28K)

Word count: 3757

Character count: 18013

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To cite this article: S H Larekeng *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **575** 012139

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In-Vitro Shoot Induction of Pring Tutul (*Bambusa maculata*) through in Various Plant Growth Regulators (PGR)

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Abstract. Bamboo is one of the plants in the family of grass (*Poaceae*). The need for bamboo is increasing, but it has not been matched with innovative cultivation. Thus the population of bamboo in nature is decreasing. Induction of bamboo shoot can be used as an alternative method for plant multiplication in a short time through tissue culture. Additional plant growth regulators such as Benzyl Amino Purine (BAP) and Indole Butyric Acid (IBA) are needed to induce bamboo shoots. The data were analyzed using the R Statistics software. Furthermore, the results showed that Murashige & Skoog (MS) media with the addition of 1 ppm of BAP and 0.5 ppm of IBA (M5) was the best combination to induce the number of shoots, shoot height, leaves length, and number of leaves in pring tutul.

1. Introduction

Bamboo is one of the plants in the family of grass (*Poaceae*) [1]. Bamboo has excellent potential to be developed as building materials, raw materials for making paper (pulp), textiles, absorbing carbon, holding, and storing water. Bamboo roots and trunks also have benefits for producing medicines. The need for bamboo is increasing, but it has not been matched with innovative cultivation [2]. Thus the population of bamboo in nature is decreasing. One type of bamboos that is widely used and developed is pring tutul (*Bambusa maculata*) [3].

The need for seedling cannot be fulfilled by only relying on generative plant propagation due to limitations such as limited fruiting time, varied hereditary traits, required large space, and a limited number of seeds produced. Tissue culture can be used as an effort to accelerate the supply of quality seeds and can be beneficial for the conservation area to save pring tutul [4].

Propagation of plants through the induction of tissue culture shoot is far superior to conventional propagation since it can create new plants in a short time, produce healthy and good quality seeds, same physiological and morphological characteristics as the parents, and save energy, places and time. The four factors that influence growth in in-vitro culture are the explants used, culture media, growth environment, and growth regulators [5].

Tissue culture requires Plant Growth Regulators (PGR) that can stimulate the growth of roots, shoots, and germination. Types of PGR that are often used in tissue culture, according to



Manokari et al [6] Marzuki et al [7] are auxin and cytokinin. Auxin is a growth hormone that cannot be separated from the growth and development of a plant. Indole Butyric Acid (IBA) plays a role in root formation. IBA has low auxin activity, but it is more stable under conditions with light variation, temperature, and can be active longer than other types of auxin while cytokinin can increase cell division in plant tissue and regulate plant growth and development. Benzylaminopurine cytokinin (BAP) can induce the production of endogenous hormones in plant tissue; thus, division and formation of shoots occur rapidly [8]. When auxin concentration is higher than cytokinin, it will stimulate callus formation, whereas if the concentration of cytokinin is higher than auxin, it will stimulate shoot formation.

Astuti (2014) [9] reported the induction of shoots and roots of yellow bamboo (*Bambusa vulgaris*) with in-vitro using MS media with a combination of 1.0 ppm BAP and 2.5 ppm IBA gave better result regarding the number of bamboo shoot and speed of shoot emergence, while the addition of BAP 2.0 ppm and IBA 2.5 ppm produced the highest height of yellow bamboo shoot which was 13.75 ± 1.50 mm. Therefore, this research is essential to determine the right media for induction of pring tutul shoot, thus high-quality pring tutul seedlings can be obtained through in-vitro.

2. Research methodology

The explants used were healthy pring tutul explants which not infected with pest and disease and categorized as young tissue. The explants used were healthy pring tutul explants which not infected with pest and disease. The sterilization process was carried out with two stages consists of sterilization outside the laminar air-flow cabinet (L AFC), and inside the L AFC, the steps were as follows:

2.1. Sterilization Outside of L AFC

- a. The explants were rinsed using sterile distilled water for 1 minute.
The explants were soaked in distilled water with an additional of tween 80 for 20 minutes.
- c. The explants were rinsed using sterile distilled water for 3 times.
- d. The explants were soaked in 2% agrept solution for 60 minutes.
- e. The explants were rinsed using sterile distilled water for 3 times.
- f. The explants were soaked in a 2% masalgin solution for 60 minutes.
- g. The explants were rinsed using sterile distilled water for 3 times.

2.2. Sterilization Inside of L AFC

- a. The explants were rinsed using sterile distilled water for 3 times.
- b. The explants were soaked in a 20% commercial bleach solution and 2 drops of tween 80 for 10 minutes.
- c. The explants were rinsed using sterile distilled water for 3 times.
- d. The explants were soaked in a 10% commercial bleach solution and 2 drops of tween 80 for 5 minutes.
- e. The explants were rinsed using sterile distilled water for 3 times.
- f. Pring tutul explants were ready to be planted.

2.3. Explant Planting

Planting was carried out inside of L AFC. First, the explants were put into a petri dish containing filter paper by using a tweezer, and the plant shoots were cut using a scalpel knife. Then, the explant leaves were planted in a culture bottle using a tweezer and tightly closed and glued with plastic wrapping. Last, the bottles were labeled with the date of planting, media name, and type of treatment given.

2.4. Observation Variable

Observation variables were the time of shoot emergence (days after planting/DAP), percentage of explants sprouted (%), number of shoots (piece), height of shoots (cm), number of leaves (sheet), length of leaves (cm), and percentage of contamination (%).

2.5. Data Analysis

Data were analyzed using R Statistics software (R Development Core Team, 2013) and Microsoft Excel (Microsoft Corporation). Quantitative data were analyzed using Poisson regression. Pearson Chi-square test at 95% confidence level was used to test the statistical significance of the observed variables. While data that not normally distributed were analyzed using the Kruskal-Wallis Test. The value of "0" was changed to 0.000001. Kendall rank correlation test was used to see the correlation between the observed variables.

3. Result and discussion

3.1. Time of Shoot Emergence

The tissue culture research was carried out for 90 days. The observations showed that different types of media gave a different effect on the time of shoot emerged (Figure 1). Growth of pring tutul through tissue culture characterized by the appearance of shoot buds followed by leaf formation. Figure 1 shows that the pring tutul had the fastest shoot growth response at 14 Days After Planting (DAP). The results showed that BAP and IBA growth regulators gave a better effect to stimulate shoot buds. It started to appear with changes in node size and increased in size by the next day.

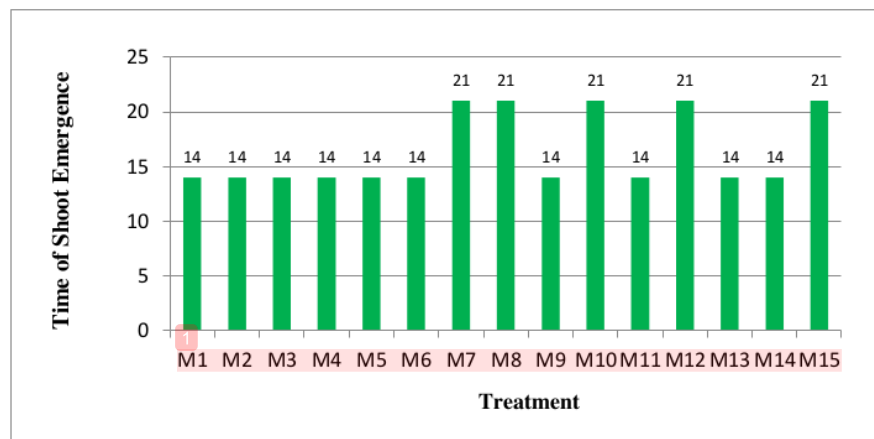


Figure 1. Diagram of Time of Shoot Emergence (DAP) of Pring Tutul (*Bambusa maculata*)

3.2. Percentage of Explants Sprouted

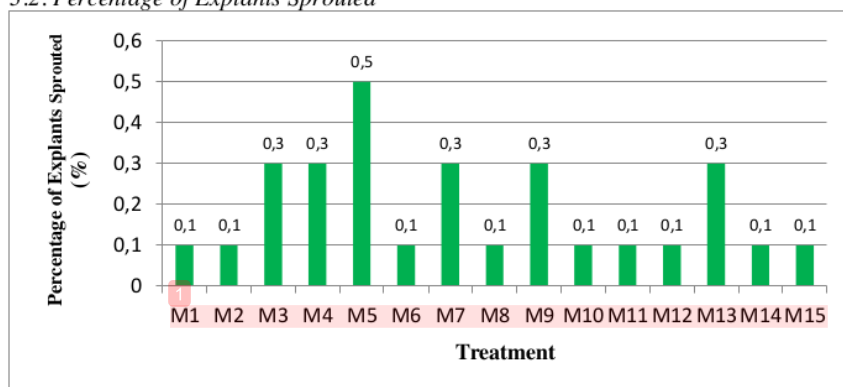


Figure 2. Percentage of Pring Tutul (*Bambusa maculata*) Explants Sprouted

Based on the observation result, the average percentage of explants sprouted was 0.1% in the M1, M2, M6, M8, M10, M11, M12, M14, and M15 treatments. The percentage of explants sprouted was 0.3% in the M3, M4, M7, M9, and M13 treatments. The percentage of explants sprouted was 0.5% in the M5 treatment (Figure 2). The highest value of explants sprouted was M5 (MS + BAP 1 ppm + IBA 0.5 ppm), with a percentage of 0.5%. MS, with the addition of PGR influenced the growth rate of shoots. However, the types and concentrations of PGR for each plant are not the same, it is all depending on the genotype and physiological conditions of plant tissue [10].

3.3. Number of Shoots

Calculation on the number of shoots was done on all shoots that arise from the elongation of the buds. The analysis results can be seen in (Table 1) showed that the M9 treatment (MS + BAP 2 ppm + IBA 0.5 ppm) significantly affected the number of shoots. The results of data analysis showed that the treatment media data had no significant effect on shoot height. Data were analyzed with the Kruskal Wallis statistical test. Kruskal Wallis statistic is one of the parametric methods. This procedure can be used for data that are not normally distributed and does not require the normality of data [11].

Table 1. The Poisson Test on Pring Tutul (*Bambusa maculata*) Number of Shoots.

Treatment	Estimate	Std.Error	z value	Pr(> z)
Treatment M1	1.792e+00	1.000e+00	1.792	0.0732
Treatment M2	3.055e-15	1.414e+00	0.000	0.0732
Treatment M3	1.099e+00	1.155e+00	0.951	0.3414
Treatment M4	1.099e+00	1.155e+00	0.951	0.3414
Treatment M5	1.386e+00	1.118e+00	1.240	0.2150
Treatment M6	1.099e+00	1.155e+00	0.951	0.3414
Treatment M7	1.099e+00	1.155e+00	0.951	0.3414
Treatment M8	7.724e-15	1.414e+00	0.000	1.0000
Treatment M9	2.079e+00	1.061e+00	1.961	0.0499 *
Treatment M10	3.851e-15	1.414e+00	0.000	1.0000
Treatment M11	5.956e-15	1.414e+00	0.000	1.0000
Treatment M12	6.931e-01	1.225e+00	0.566	0.5714
Treatment M13	6.931e-01	1.225e+00	0.566	0.5714
Treatment M14	3.077e-15	1.414e+00	0.000	1.0000
Treatment M15	6.931e-01	1.225e+00	0.566	0.5714

Note : *(Significant on the 95% confidence interval)

BAP can induce the production of endogenous cytokinin hormones in plant tissue; thus, division and formation of shoots occur quickly. Stimulation of shoot formation can be done by giving high cytokinin concentration and the influence of auxin, or even not giving auxin at all [12]. BAP is a cytokinin that is often used because it is most effective in stimulating the formation of shoots, more stable, and resistant to oxidation [6][13].

3.4. Height of Shoots

The increasing height of bamboo shoots showed the potential to develop further into new plants. The average shoot height in this research was around 0.25 cm to 1.46 cm. Figure 3 shows that M5 treatment produced the highest average shoot, which was 1.46 cm while the M10 treatment (MS + BAP 2 ppm + IBA 1 ppm) produced the lowest average shoot height, which was 0.25 cm. Ridzuan et al and Tikendra et al [14,15] stated that IBA auxin function as cell development could extend plant cells at a specific concentration.

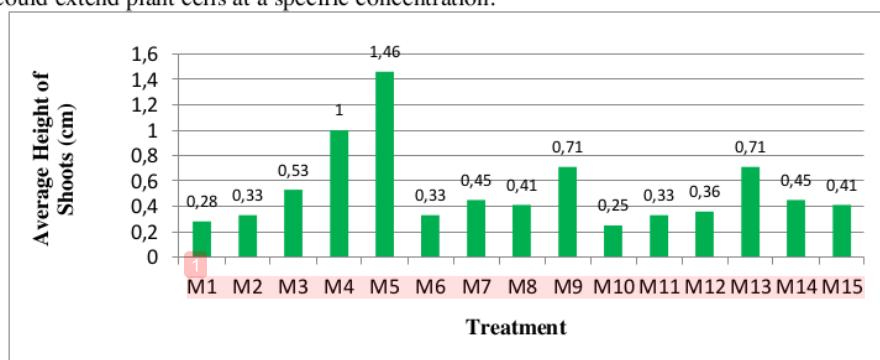


Figure 3. Average Height of Shoots of Pring Tutul (*Bambusa maculata*)

3.5. Number of Leaves

The formation of the number of leaves in each media showed different results. The lowest number of leaves was on the M7 treatment, which produced 0.16 number of leaves, while the highest was on the M5 treatment (Figure 4), it produced an average number of leaves, which was 1.33. While M2, M6, M8, M10, M11, M12, and M14 treatments were not able to produce the number of leaves. The addition of the cytokinin hormone (BAP) can encourage leaf cells to [16].

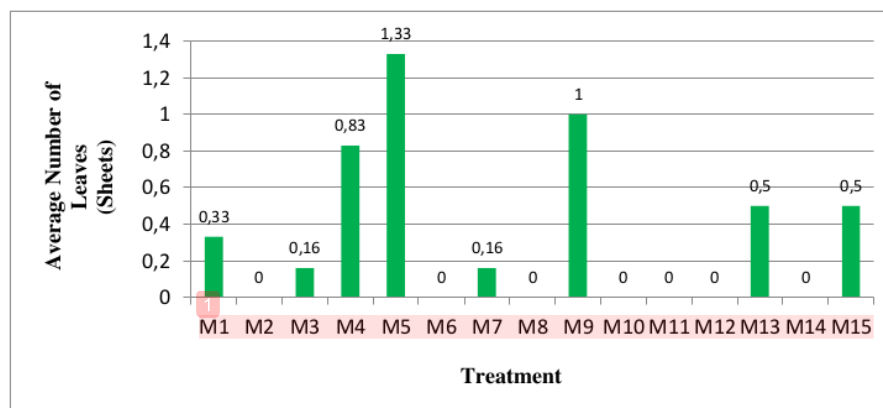


Figure 4. Average Number of Leaves of Pring Tutul (*Bambusa maculata*)

3.6. Length of Leaves

The length of leaves in several types of treatments gave different responses. The analysis results showed that the treatment media had no significant effect on the length of the leaves. Figure 5 shows that only M5 had the highest average value on the length of leaves, while the lowest was found in the M7 and M15 treatments (0.16 cm). M5 treatment showed as the best concentration of growth regulator (PGR), which was increased by 0.71 cm. Based on Rohmah (2012) that the length of the leaves has a relationship with the number of explant leaves, good leaf growth will produce the highest average of length.

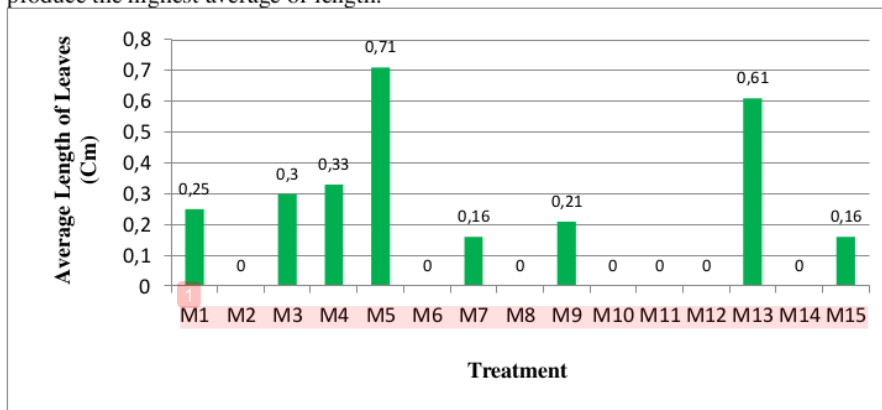


Figure 5. Average Length of Leaves of Pring Tutul (*Bambusa maculata*).

3.7. Percentage of Explant Contamination

The observation result showed that some explants experienced contamination. Bacteria and fungi can cause contamination, both types of contamination can be seen from the physical characteristics that appeared in explants and culture media. However, contamination caused by fungi is drier, and fungal hyphae will appear on plants that are attacked and can be characterized by the presence of lines (like thread) that are white to gray. While contamination by bacteria will cause the plant to be wet with the appearance of mucus around the explants.

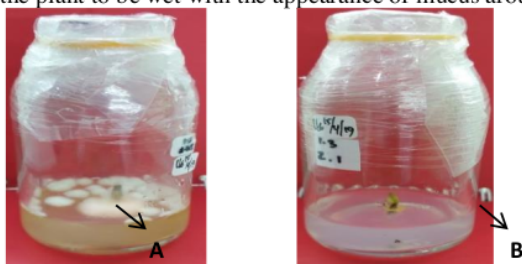


Figure 6. Contaminated Explants of Pring Tutul (*Bambusa maculata*); (A) Fungi and (B) Bacteria.

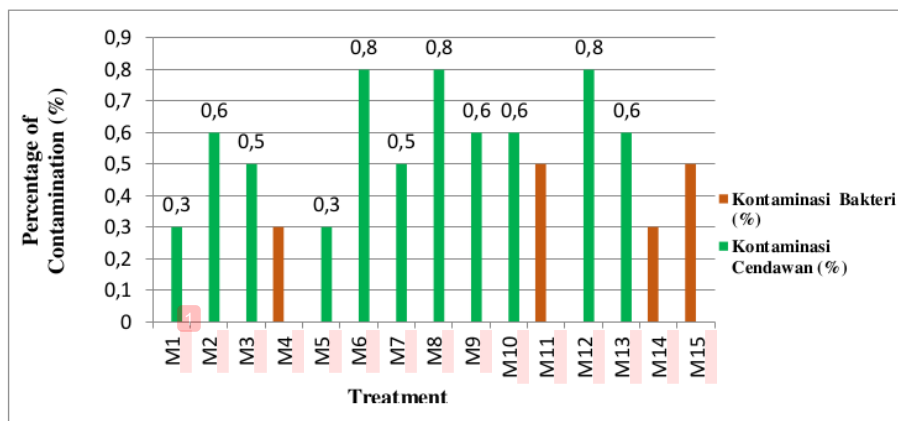


Figure 7. Average Percentage of Contamination of Pring Tutul (*Bambusa maculata*)

Figure 7 shows that contamination occurred in all types of treatment media. The highest contamination caused by fungi was found in treatments M6, M8, and M12 by 0.8% while the highest contamination caused by bacteria was found in treatments M11 and M15, which percentage was 0.5%. Contamination occurred because the surface sterilization process of explants was not effective in removing fungi and bacteria [16].

Fungi and bacteria usually live and multiply within the surface of the pring tutul node. It is difficult to eliminate fungi and bacteria without damaging the plant parts completely. Contamination can also be caused by explant parent plants that have been contaminated by pathogens. According to Murthy et al [17] each plant material has a different level of surface contamination, one of which depends on the growth environment and explant conditions. Based on the observation result, fungi began to attack the surface of explants and was spreading throughout the bottle surface. Pring tutul plant tissue culture can be thriving if it is able to prevent contamination.

The other versatility of bamboo tissues in the rhizome and culm of the skin has shown that they accumulate heavy metals in the cell walls, vacuoles and cytoplasm, such as *Phyllostachy Praecox*, with high resilience in soils polluted with metals, which enables significant absorption and accumulation of heavy metals. Excessive heavy metal concentrations can cause oxidative stress and bamboo plants harm [18].

Tissue culture is a technique also plays a pivotal role in industry, agriculture, and plant breeding as it complements crop production through micropropagation, somaclonal variation, hybridization, genetic transformation, pathogen eradication, and by preserving germplasm. The amendment of biotic, abiotic elicitors in media, precursor feeding, immobilization, the use of bioreactors in synthesizing and enriching phytochemicals and their commercial production in industry has also been highlighted [19].

4. Conclusion

The M5 treatment (MS + BAP 1 ppm + 0.5 ppm IBA) was the right combination in tissue culture to induce the number of shoots, shoot height, leaves length, and number of leaves in pring tutul, with the lowest contamination percentage that was 0.3%.

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