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Multiplication Of Pisang "Agung" (*Musa Paradisiaca* L.) With In Vitro As A Local Product For Food Security

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Abstract: Pisang "Agung"(Musa paradisiaca L.) is one of the banana varieties originating from Lumajang Regency. Pisang Agung is famous for its large fruit and sweet taste. The conventional way of propagating Pisang "Agung" takes a long time, produces few shoots, is not uniform and is not guaranteed to be disease-free, these obstacles can be overcome by using tissue culture. The design that will be used in this study is a Non-Factorial Completely Randomized Design (CRD) consisting of 100 ppm BAP (B) which consists of 4 levels, namely B0 (0 ppm), B1 (3 ppm), B2 (6 ppm), B3 (9 ppm). The parameters observed were the age of shoot emergence, shoot height, number of shoots. The results showed that the treatment of various concentrations of BAP gave a significant effect on all observation parameters with the best treatment B3 age of bud emergence (9.25 days), bud height is B3 (7.35 cm) and the number of buds is B0 (3.90 pieces).

Keywords: Agung Banana, Tissue Culture, Growth Regulator BAP (Benzyl Amino Purine).

INTRODUCTION

Agung Banana (Musa paradisiaca L.) is one of the banana varieties originating from Lumajang Regency. Pisang Agung is famous for its large fruit and sweet taste. Based on the data from BPS Dinas Pertanian Lumajang Regency, banana production from 2019 to 2021 in Lumajang Regency has increased. The highest increase occurred in 2021 as much as 1,231,216 ku. Meanwhile, the average productivity in 2021 was 190.10 ku/ha. From the increase in data, it can be concluded that the importance of banana plantains for the food security of the Lumajang community. The method of propagation of pisang Agung is the same as in general, namely conventionally using banana stumps or saplings that can only produce 5 - 10 seedlings, the time required is relatively long, not uniform and not guaranteed to be disease-free. These obstacles can be overcome with seedling propagation techniques using tissue culture. This technique is an advantage of plant propagation, through tissue culture techniques it is possible to obtain planting material in large quantities, in a short time and uniform nature with its parent.

In tissue culture growing media there is the addition of growth regulators. Plants need natural growth regulators (phytohormones) for the growth process, namely auxin and cytokonin growth regulators. The type of ZPT used in this study is BAP from the cytokinin group. Benzyl Amino Purine (BAP) is a ZPT from the cytokinin group that functions to induce the formation of adventitious shoots from banana explants. BAP is a type of ZPT that has a wide range (distance) in stimulating (stimulator).

METHOD

The tools used in this research are Laminar Air Flow (LAF), autoclave, plastic, culture bottle, analytical balance, tweezers, magnetic stirrer, hand sprayer, micro pipette, scapel, gnting, banana, warping paper, pH meter, filter paper, tissue, aluminum foil, oven, cuvette, bunsen lamp, glass jar, rubber band, rubber gloves and stationery.

The materials used in this study were Agung banana explants, MS base media, 70% alcohol, 96% distilled water, ZPT BAP and Kinetin, agar, sucrose, NaOH, HCL and spirtus.

The design that will be used in this study is a Non-Factorial Completely Randomized Design (RAL) consisting of the provision of BAP (B) 100 ppm consisting of 4 levels, namely:

B0 : Without Kinetin = 0 ppm/100 ml

B1 : Kinetin 3 /100 ml

B2 : Kinetin 6 /100 ml

B3 : Kinetin 9 /100 ml

The research was carried out in several stages, including sterilization of tools and materials, sterilization of space, making media, making zpt, sterilizing explants, planting explants and sub-culturing.

Sterilization of tweezers, scapels, Petri dishes, filter paper, tissue is done by wrapping it using aluminum foil paper and then put in plastic and tied using a rubber band, then sterilized using an autoclave for 20 minutes with a temperature of 1210C and a pressure of 17.5 Psi.

Laminar airflow sterilization is done by cleaning with alcohol using cotton and turning on the UV lamp for 60 minutes before multiplying. Room sterilization is done by cleaning the dirt in the room and spraying the room with formalin, then incubated for 24 hours. The media incubation rack and the place to grow the planlets were cleaned using alcohol.

Making ZPT BAP using 100 ml with the calculation of BAP 100 ppm; 10 g. Then homogenized using a magnetic stirrer and store in a dark place or not exposed to sunlight.

Media preparation is done by putting stock solutions A, B, D, E each 5.6 g, Stock C and vitamins each 7 ml into a glass jar. Then distilled water 500 ml, then homogenized using a magnetic stirrer and Ph meter. Good media has a pH between 5.8 - 6.0. If the pH is less than 5.8, a few drops of NaOH are added and if the pH is more than 6.0, 1 N HCL is added to lower the pH of the media. After that, add sucrose and agar to the media. Then after everything is well mixed, cook the media on a hot plate. After boiling, pour the media into 16 bottles with a volume of 25 ml each. After that, each media is added to the ZPT according to the treatment using a micro pipette and tightly closed and then given a plastic wrap.

The next stage is that the bottle containing the media with each treatment is sterilized using an autoclave for 20 minutes with a temperature of 121oC and a pressure of 17.5 psi. After sterilization, the media was incubated for 3 days to determine the presence or absence of contaminants.

Explant sterilization was carried out by taking the young stem of Agung banana, then brushing it clean and soaking it in soapy water (sun light), scrubbing it for 1 minute. After that, soak the stem in fungicide and bactericide liquid for 1 hour. After 1 hour of soaking, cut the banana stem layer by layer until the stem size becomes small. Then move to the laminar airflow room, rinse the stem using alhocohol and cut the banana stem until you see young shoots or potential explants that we will multiply. Move the explants using tweezers then soak the explants in 30% clorox for 30 seconds then soak the explants in 20% byclyn for 20 seconds and finally rinse using alcohol after that distilled water. Drain the explants on filter paper (3 layers), if it is dry plant the explants in the media bottle that has been prepared and close it tightly using plastic wrap on the neck of the bottle then place it on a shelf in the incubation room.

Sub-culturing of agung banana plantlets was carried out in a laminer airflow using a petri dish base. Planlets were taken from old multiplication bottles using tweezers, then the leaves and roots that grew were cleaned using a scapel, then measured using a ruler placed under the petri dish. Next, the planlets were put into bottles containing media that had been made with a total of five planlets each. After that, the bottle was tightly closed and given a plastic wrap on the neck of the bottle, then placed on a shelf in the incubation room.

RESULTS AND DISCUSSION

3.1 Age of Shoot Emergence (Days)

Based on the results of data analysis of the Sprouting Age parameter, the main administration of BAP concentration significantly affects the sprouting age of grand banana explants. Table 1. Average Age of Sprouting (HST) of Banana Agung Explants with BAP concentration treatment

Concentration BAP (ppm)	Age of Shoot Emergence (Days)
B0	10,56 b
B1	9,40 a
B2	11,20 b
B3	9,25 a

From the table it can also be seen that the provision of BAP B3 (9 ppm) and B1 (3 ppm) is significantly different from B0 (without BAP) and B2 (6 ppm). The best treatment for the age of shoots is found in the K3 treatment which is 9.25 days in this study a high concentration of BAP can accelerate the growth of shoots and shoots produced with a concentration of 9 ppm 9.25 days, B1 (3 ppm) 9.40 days, producing sturdier shoots when compared to treatment B0 (10.56 days). While the B2 treatment (6 ppm) the growth of the shoots was disturbed because the BAP given was lower and the shoots produced were shorter, it is suspected that the concentration of BAP 6 ppm was not able to cell division, it is clearly seen that in the tissue culture of agung banana requires a relatively high concentration of 9 ppm, this indicates that the higher the provision of BAP, the more the growth of the shoots of banana agung explants will increase.

The shoots that appeared on the agung banana explants were because the cells in the meristem continued to actively divide. This can be observed in the increase in explant size at the bottom and the presence of fractures at the tip of the explant. According to Sadat et al. (2018), the emergence of shoots on the explants can be characterized by the size of the explants that experience swelling then followed by the fracture of the explant tip, the prospective banana micro shoots can be formed at the fracture of the explant tip which is characterized by the appearance of small green shoots.

According to Bella et al. (2016) the ability of banana explants to sprout is influenced by plant genotypes. Shoot multiplication is also influenced by the type of cytokinin and the concentration used. The influence of exogenous concentrations is a major factor in shoot multiplication activities to obtain optimal results. BAP is a cytokinin hormone that can be added for cell division so that it can spur the formation of agung banana shoots

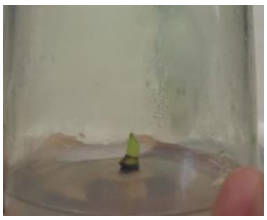

Figure 1. The beginning of shoot emergence

3.2 Shoot Height (Cm)

Based on the analysis of the data from the observation of shoot height parameters, the BAP treatment significantly affects the shoot height of banana aguung. Average shoot height (cm) of agung banana Explants with BAP concentration treatment.

Concentration BAP (ppm)	Shoot Height (cm)
B0	5,24 c
B1	5,90 B
B2	5,80 c
B3	7,35 a

The provision of BAP concentration in B3 treatment (9 ppm) is significantly different from B1 (3 ppm), B2 (6 ppm) and B0 (without BAP). The highest BAP treatment is B3 which is 7.35 cm followed by B1 with a shoot height of 5.90 cm and followed by B2 with a shoot height of 5.80 cm and the lowest is B0 (without BAP). The shoot height with a BAP concentration of 9 ppm shoot growth is very good and the growth of banana explant leaves is long and neatly arranged. Shoot height with a concentration of BAP - 3 ppm shoot growth tends to lead to the side and after that just go up, when compared with BAP 6 ppm shoot growth is a little good. This is thought to be due to osmotic pressure that is too high, causing the rupture of cell walls so that it is more directed to cell division. Without BAP, the banana explants will be inhibited in forming buds.

The success and speed of explant growth in in vitro culture is highly dependent on the availability of mineral nutrients and growth regulators available in media with certain concentrations. The completeness of culture media with several growth regulators plays a role in regulating the permeability of cell walls so as to facilitate the entry and exit of water containing nutrients needed for protein synthesis and other organic compounds needed for further growth of explants.

Ramesh and Ramassamy (2014) stated that plant height is influenced by the number of buds that appear, so the fewer buds that appear, the higher the plant height, and vice versa. This is because the ZPT hormones needed for shoot elongation are used for the formation of other shoot candidates, so that shoot height can be inhibited. Fitramala et al. (2016) also stated that explants with single buds or explants with a small number of buds do not increase in number but extend quickly.

Figure 2. Explants in B3 treatment

3.3 Number of Shoots

Based on the analysis of the data from the observation of the number of shoots parameter, the single treatment of BAP has a significant effect on the number of shoots of Agung banana. Table 3. Average number of buds (fruit) of agung banana explants with BAP concentration treatment.

Kosentrasi BAP (ppm)	Number of Shoots
B0	3,90 a
B1	3,35 b

B2	2,73 c
B3	3,03 bc

The concentration of BAP in treatments B0 (without kinetin) and B3 (9 ppm) was significantly different from B1 (3 ppm) and B2 (6 ppm). Judging from the numbers, the treatment with the highest number of buds was B0 with 3.90 buds and followed by B3 with 3.03 buds then B1 with 3.35 buds and the least number of buds was B2 with 2.73 buds. The best treatment is found in the K0 treatment (without BAP) this is thought to be phytohormones contained in the plant is able to form buds even though not added growth regulators.

Cytokinin plays a role in spurring the synthesis of RNA and protein in tissues which in turn can encourage cell division. In addition, it can also encourage tissues to absorb water from the surroundings so that the process of protein synthesis and cell division can run well.

Ferdous et al. (2015) also added that the higher the concentration of cytokinin given to plants can produce a large number of shoots and the provision of a single cytokinin produces the maximum number of shoots, but at certain concentrations or not according to the capacity needed by the shoots will produce abnormalities in the shoots obtained.

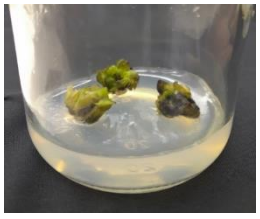

Figure 3. Shoots that appear in one explant

CONCLUSION

The provision of BAP treatment has a real effect on the parameters of shoot emergence age, namely B3 (9.25 days), shoot height, namely B3 (7.35 cm) and the number of shoots, namely B0 (3.90 pieces).

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