The Effectiveness of Fruit Extract and Temperature for In Vitro Culture of Kepok Banana (Musa balbisiana)

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Abstract. Kepok Banana (Musa balbisiana) is beneficial in fulfilling nutrition for it has a high B vitamin complex. Additionally, it is of high economic value as a food processing material. Bananas as processed ingredients for industrial scale are constrained by unsustainable production. Thus, efforts are needed to supply quality, uniform seeds and meet cropping patterns. In vitro culture technique is an innovation in meeting the availability of standardized banana seed sources. This study aimed to examine the effectiveness of fruit extracts and temperature differences for in vitro culture in Kepok banana. Murashige and Skoog (MS) base media were used in the multiplication stage with the addition of melon, banana, and papaya extracts (150 g.L⁻¹). The explants were stored at various growth room temperatures (±25, ±28, and ±31°C). The results showed that the Kepok banana explants morphogenesis using MS media enriched with papaya extract gave no difference effectivity result with vitamin synthesis in the parameters of number of shoots (4), number of leaves (7), number of roots (7.3), and length roots (6.3 cm) and showed the highest results with parameter height of plantlets (9 cm). The best condition for the proliferation of Kepok banana explants was room temperature (±28°C).

1. Introduction
Kepok Banana (Musa balbisiana) is a tropical flora which is a type of processed banana which acts as an energy source and intake of various important vitamins in metabolism. Kepok banana is special compared to other types of bananas because its life is relatively longer, has a higher nutritional content, and can be processed into various types of food and drinks. Therefore, the Kepok banana has a high potential to be developed on a plantation or industrial scale to increase national fruit production. The development of industrial-scale or banana plantations in Indonesia not only requires land availability but also needs to pay attention to the main capital in the form of guaranteed availability of quality seed to optimize production. Efforts to ensure the availability of quality seeds in agro-technology can be done in vitro culture. In vitro culture technique produces uniform, healthy, large quantities of banana seeds that supply in relatively short periods depending on climate. Thus, the availability of quality seeds can be guaranteed in quality, quantity, and continuity.

Some of the success factors of in vitro culture in Kepok banana are influenced by growing environmental conditions and the media composition. According to Yusnita [1], the microclimate that determines the success of in vitro culture includes components, light, and temperature. Each type of plant...
requires a certain temperature for optimum morphogenesis. In general, the optimum temperature for plant incubation ranges from 20–27°C. Light intensity also provides an important role for the multiplication and morphogenesis of explants besides influencing tissue differentiation. Blue radiation and ultraviolet spectrum can stimulate bud formation. Meanwhile, root stimulation is stimulated by the red light intensity at the initiation stage is 0-1000 lux, the multiplication stage of 1000-10000 lux, the rooting stage of 10000-30000 lux, and the acclimatization stage of 30000 lux [1]. Based on the results of [2], optimization of the Kepok Amorang banana growth environment with photoperiodicity for 16 hours and a temperature of 30°C showed the best plantlets growth, namely the number of shoots and the number of leaves formed at the most.

The composition of nutrients, hormones, and vitamins in the basic medium of Murashige and Skoog (MS) is not enough to stimulate the optimum multiplication of explants. The role of ZPT includes regulating the multiplication or proliferation of networks and integrating these networks into individual plants. Each stage of growth or proliferation requires a different combination of cytokinin and auxins [3]. Auxin hormones such as: Indole-3-Acetic Acid (IAA), Indole-3-Butyric Acid (IBA), 1-Naphthaleneacetic Acid (NAA), and 2,4-D function in regulating the increase and reduction of cell permeability and cell osmotic pressure and they stimulate cell enlargement and elongation. IAA is the most abundant auxin produced or synthesized by various plants in a parallel path. IAA works from its own transportation mechanism and complex feedback metabolism, requiring complex and careful mechanisms for IAA homeostasis [4]. Meanwhile, cytokinin hormones such as zeatin, 2-iP, 6-Benzylaminopurine (BAP), Benzyladenine (BA), and kinetin act as catalysts during cell division, budding, and organ differentiation. Cytokines at low amounts is sufficient to induce shoots if endogenous hormones are adequate [5].

Fast micropropagation in Kepok Merah banana was reported by [6] that by using MS media and the addition of 0.5 mg.L\(^{-1}\) IAA and BA 5 mg.L\(^{-1}\) gave the best results, namely the growth of 6–17 shoots per explant. The addition of fruit extracts to enrich natural vitamins and hormones in explant growth media. A good concentration of papaya extract used for the dendrobium orchid plantlet micropropagation ranges from 150-200 g.L\(^{-1}\) [7].

In general, the influence of nutrition and the environment has a big role in the success of banana in vitro culture which also needs to be supported by optimum biological and genetic conditions. Based on the general description above, the research objective of this study was to examine the effectiveness of fruit extracts and temperature differences on the multiplication of Kepok banana, that the fruit extract formulations can be substituted for synthetic supplements. The result of this research is expected to streamline the supply of quality Kepok banana seeds.

2. Materials and methods

2.1. Material

This study was conducted in Universitas Sebelas Maret Plant Tissue Culture Laboratory from May to September 2019. Materials and equipment or instruments used in this study were explants of Kepok banana without leaves and roots, Murashige and Skoog (MS) basic medium, vitamin C, folic acid, melon extract, banana extract, papaya extract, coconut water, agar, IAA, BAP, disinfectant (70% alcohol, methylated spits, Dithane M-45, and sterile aquades), plastic warp, Laminar Air Flow (LAF), autoclave, analytical scales, stirrer hotplate, beaker glass, pH meter, volume pippete, petri dish, culture basket/container, culture bottle, LED light, sieve, glass stirrer, spatula, pipette, knife, alcohol lamp, and blender juicer. Regular explants were subculture every 4 weeks to obtain sufficient numbers of in vitro shoots of the experiment. Before subculture the laminar air flow is sterilized with 70% alcohol then the UV light and the blower are turned on ±30 minutes before use. During the subculture process, the sterilizer uses 70% alcohol to sterilize tweezers and knives with the immersion method.
2.2. Stock solution and treatment media

The treatment media were modified medium form MS added by fruit extract (150 g.L\(^{-1}\)) and coconut water (100 ml.L\(^{-1}\)). Fruit extract added to MS base medium after peeling, cleaning, cutting the middle and weighing 150 g of fruit. Then, it was mixed with distilled water, blended, and filtered. Furthermore, the fruit extract was put into the MS base medium solution and added distilled water until the solution reached 1000 ml. After the basic ingredients of MS and fruit extract were evenly mixed, pH was measured to reach the acidity of 5.7 to 5.9. If the pH of the solution was less than 5.7, NaOH 0.1 N was added. However, if the pH was more than 5.9, HCl 0.1 N was added. After the appropriate degree of acidity was obtained, as much as 7 g agar was added to 1 L of media solution. The measured media solution was then stirred and heated to boiling. After that, the media solution was poured into each culture bottle of 20 ml and immediately sealed. Bottles containing media solution were tightly closed and put in an autoclave to be sterilized. The media sterilization was carried out at a pressure of 1.5 kg.cm\(^{-2}\) and a temperature of 121\(^\circ\)C for 1 hour. The composition of chemicals and organics in the treatment media are shown in Table 1.

Table 1. The Composition of chemicals and organics in the treatment media

| No | Minerals          | MS  | MS + Synthetic Vitamins | MS + Melon Extract | MS + Banana Extract | MS + Papaya Extract |
|----|-------------------|-----|-------------------------|--------------------|---------------------|---------------------|
|    | Macro Nutrition (mg.L\(^{-1}\)) |     |                         |                    |                     |                     |
| 1  | NH\(_4\)NO\(_3\)   | 1650| 1650                    | 1650               | 1650                | 1650                |
| 2  | KNO\(_3\)         | 1900| 1900                    | 1900               | 1900                | 1900                |
| 3  | KH\(_2\)PO\(_4\)   | 170 | 170                     | 170                | 170                 | 170                 |
| 4  | MgSO\(_4\).7H\(_2\)O | 370 | 370                     | 370                | 370                 | 370                 |
|    | Micro Nutrition (mg.L\(^{-1}\)) |     |                         |                    |                     |                     |
| 1  | MnSO\(_4\).4H\(_2\)O | 22.3| 22.3                    | 22.3               | 22.3                | 22.3                |
| 2  | ZnSO\(_4\).7H\(_2\)O | 8.6 | 8.6                     | 8.6                | 8.6                 | 8.6                 |
| 3  | H\(_3\)BO\(_4\)    | 6.2 | 6.2                     | 6.2                | 6.2                 | 6.2                 |
| 4  | KI                 | 0.83| 0.83                    | 0.83               | 0.83                | 0.83                |
| 5  | Na\(_2\)MoO\(_4\).2H\(_2\)O | 0.25| 0.25                    | 0.25               | 0.25                | 0.25                |
| 6  | CuSO\(_4\).5H\(_2\)O | 0.025| 0.025                | 0.025              | 0.025               | 0.025               |
| 7  | CoCl\(_2\).6H\(_2\)O | 0.025| 0.025                | 0.025              | 0.025               | 0.025               |
| 8  | FeSO\(_4\).7H\(_2\)O | 27.8| 27.8                    | 27.8               | 27.8                | 27.8                |
| 9  | Na\(_2\)EDTA.2H\(_2\)O | 37.3| 37.3                    | 37.3               | 37.3                | 37.3                |
|    | Vitamins (mg.L\(^{-1}\)) |     |                         |                    |                     |                     |
| 1  | Thiamin-HCL       | 0.1 | 0.1                     | 0.05               | 0.05                | 0.05                |
| 2  | Myo inositol      | 100 | 100                     | 50                 | 50                  | 50                  |
| 3  | Nicotinic Acid    | 0.5 | 0.5                     | 0.25               | 0.25                | 0.25                |
| 4  | Pyridoxine        | 0.5 | 0.5                     | 0.25               | 0.25                | 0.25                |
| 5  | Glycine           | 2   | 2                       | 1                  | 1                   | 1                   |
| 6  | Ascorbic Acid     | -   | 10                      | -                  | -                   | -                   |
| 7  | Folic Acid        | -   | 0.001                   | -                  | -                   | -                   |
|    | Hormone (mg.L\(^{-1}\)) |     |                         |                    |                     |                     |
| 1  | BAP               | 2   | 3                       | -                  | -                   | -                   |
| 2  | IAA               | 1.5 | 1.5                     | -                  | -                   | -                   |
|    | Organic Compound  |     |                         |                    |                     |                     |
| 1  | Fruit Extract (g.L\(^{-1}\)) | -  | 150                     | 150                | 150                 |                     |
| 2  | Coconut water (ml.L\(^{-1}\)) | -  | 100                     | 100                | 100                 |                     |
|    | Agar (g.L\(^{-1}\)) | 7   | 7                       | 7                  | 7                   | 7                   |
|    | Sukrosa (g.L\(^{-1}\)) | 30  | 30                      | 30                 | 30                  | 30                  |
2.3. Grow room temperature

Explants were maintained in the incubator chamber or container with the conditions according to the treatment being tested, particularly balancing the light intensity between all treatment levels. The room temperature using the air conditioner (AC) was ±25°C with 40 watts fluorescence lighting (intensity of 1300 lux) while room temperature without AC was ±28°C with 10 watts LED lighting (intensity of 2650 lux). Outdoor temperature was ±31°C without lights (intensity of 2190 lux). The parameters observed were plantlets height, number of shoots, number of leaves, number of roots, and root length.

2.4. Experimental design

This study employed Completely Randomized Design (CRD) Split Plot with two factors namely main plot in the form of temperature (3 treatments: ±25, ±28, and ±31°C) and sub plot in the form of type of fruit extracts (5 treatments: without fruit extract, vitamin synthesis, melon extract, banana extract, and papaya extract) each combination was repeated 4 times.

To determine the effect on the propagation time of Kepok banana and their acclimatization ability, the data were analyzed with variance. If significant effect was found, the test would be followed by Duncan Multiple Range Test (DMRT) at 5% range. To find out the relationship between variables, a correlation test was performed.

3. Results

3.1. Multiplication of Kepok banana explants enriched with fruit extract

Multiplication of differentiates Kepok Banana explants began with micro shoots, followed by leaf growth and development before the root growth. The formation of micro shoots in Kepok banana explants relates to the role of auxin that induces the meristem cells rapidly by pumping protons into cell membranes that produce ATP-ase activation and increase cell expansion / cell size [8].

The fastest time of emergence of micro shoots was produced by explants grown on the media with the addition of melon and papaya extract which was also the same as the explants grown on the media with the addition of vitamins synthetic namely at 4 DAP. Whereas explants that grew on media without fruit extracts produced micro shoots 1 day later. The emergence of shoots from explants grown on media with the addition of banana extracts appeared the longest at 7 DAP (Table 2).

| Fruit Extract       | Shoots | Leaves | Roots |
|---------------------|--------|--------|-------|
| Without Extract     | 5      | 8      | 20    |
| Vitamin Synthesis   | 4      | 9      | 23    |
| Melon Extract       | 4      | 8      | 20    |
| Banana Extract      | 7      | 17     | 29    |
| Papaya Extract      | 4      | 8      | 19    |

The time required to form micro shoots to produce leaves was between 4-17 DAP. This is in line with the results of the study [9] in which the formation of shoots on banana explants took around 10-20 DAP. The positive correlation coefficient (r: 0.93; confidence level of 0.05) between the time of the emergence of shoots and leaves indicates the faster the emergence of micro shoots, the faster the appearance of leaves. The same thing also happened to the correlation between the time of appearance of the leaf and the root (r: 0.96; confidence level of 0.01). It indicates that the faster the time of the appearance of the leaf was followed by the appearance of the root. Meanwhile, there was no correlation between the emergence of micro shoots and the appearance of roots (Table 3).
Table 3. Correlation of the emergence of shoots, leaves, and roots

| Morphology | Analysis     | Shoots | Leaves | Roots |
|------------|--------------|--------|--------|-------|
| Shoots     | Pearson Correlation | 1      |        |       |
|            | Sig. (2-tailed)   |        |        |       |
| Leaves     | Pearson Correlation | 0.93*  | 1      |       |
|            | Sig. (2-tailed)   | 0.02   |        |       |
| Roots      | Pearson Correlation | 0.85   | 0.96** | 1     |
|            | Sig. (2-tailed)   | 0.07   | 0.01   |       |

*. Correlation is significant at the 0.05 level.
**. Correlation is significant at the 0.01 level.

The findings on the F test of the multiplication and morphogenesis stages of fruit extract type factors concluded that there was a significantly different effect on the parameters of the number of shoots, number of leaves, number of roots, and root length. Additionally, root length also gave the highest yield on the parameter height of the plantlets. The temperature factor and the interaction between the types of fruit extracts with temperature had no significant effect.

In general, the initiation of Kepok banana explants leaves and roots can be stimulated by MS medium enriched with melon, banana, and papaya extracts. However, the multiplication time and differentiation of explants depended on the microclimate and nutrients provided (Table 4). The effect of fruit extracts on the multiplication of Kepok banana which gave the best results and was not different from the synthesis of vitamin was caused by papaya extraction. The addition of papaya extract (150 g.L⁻¹) and coconut water (100 ml.L⁻¹) were able to replace the vitamin synthesis seen from the parameters of the number of shoots (4), number of leaves (7), number of roots (7.3), and root length (6.3 cm). It also showed the highest result for the height of the plantlets (9 cm). The addition of papaya extract as much as 150 g.L⁻¹ effectively replaced the function of vitamin synthesis resulting in chemical cost efficiency. Papaya extract and coconut water as natural hormones are able to work simultaneously to accelerate the propagation of Kepok banana explants. As stated by [10], auxin hormone cannot work optimally without interacting with other hormones such as cytokinins.

Table 4. Effect of adding fruit extract to MS media on Kepok banana explants growth

| Fruit Extract   | Number of Shoots | Number of Leaves | Plantlets Height (cm) | Number of Roots | Root Length (cm) |
|-----------------|------------------|-----------------|----------------------|----------------|-----------------|
| Without Extract | 4 b              | 8 b             | 6.0 b                | 9.7 b          | 10.1 c          |
| Vitamin Synthesis | 4 b         | 8 b             | 5.2 b                | 7.5 b          | 4.2 b           |
| Melon Extract   | 2 ab             | 5 b             | 7.0 bc               | 3.8 a          | 6.2 b           |
| Banana Extract  | 1 a              | 1 a             | 1.2 a                | 0.9 a          | 0.4 a           |
| Papaya Extract  | 4 b              | 7 b             | 9.0 c                | 7.3 b          | 6.3 b           |

Notes: Numbers followed by same letters in each column are not significantly different according to Duncan’s test at 5% sig level.

The addition of banana extract at a concentration of 150 g.L⁻¹ on the MS base medium greatly inhibited morphogenesis (Table 4). The addition of banana extract gave the lowest yield of all growth parameters. It might be caused by the content and concentration of the extract. Based on research [11], it is suspected that the addition of Raja Bulu banana extract on MS media to the propagation of banana explants increases the concentration of sugar which is a disruptive factor for shoot growth. High sugar concentration can bind with phenolic compounds to form glucosides which inhibit the cell division process [12]. Phenolic toxicity stems from the activity of oxidized phenolic compounds (can be in the form of sores and others) turned into other compound (polymerase) or quinon [13]. On the other hand it was revealed by [14] that the slow growth of explants in forming shoots could be influenced by endogenous factors in the explants.
Tissue performance of in vitro culture in the form of cell proliferation for dedifferentiation and organogenesis requires hormones or ZPR with certain concentrations at certain stages in the plant growth cycle. Thus, the mechanism of the proliferation of explant morphogenesis is not only determined by plant genotypes. Plant physiology conditions such as meristematic power of ability, cell metabolism, growth status of explant cells or tissues, availability of endogenous zpt, and the activity of genes that control the process of growth and development also determine the success of bud regeneration [15]. Some hormones such as zeatin, 2-iP, and kinetin are sometimes needed to optimize the differentiation [15]. Referring to the results of the research [16], 2 mg.L⁻¹ of BAP was more effective and efficient in increasing the number of shoots and plantlets height compared to 4 mg.L⁻¹ 2-iP and Kinetin or 0.08 mg.L⁻¹ TDZ in the propagation of Kepok banana. High cytokinin concentrations do not guarantee accelerating the propagation since there are endogenous hormones from the explant meristem influencing the process as well. In a separated research, the administration of 120 ppm BAP was able to spur an increase in tea shoots (8.88 shoots) within 3 months after pruning, meaning that the productivity of tea plants could be enhanced [17].

Vitamins are an essential organic substance that functions to stimulate tissue growth. Naturally, plants can produce vitamins for their own needs. However, in vitro culture techniques need to add a source of vitamins needed by plants. The nutrients contained in melon, papaya, and ripe banana are high enough to support the performance of meristem propagation, including carbohydrate and lipid metabolism, cell division, and respiration. In general, banana nutrition is higher than papaya and melon (Table 5). Thiamin (vitamin B1) is an essential vitamin that plays a role in accelerating cell division and the formation of chloroplasts [18]. Niacin (vitamin B3) or called nicotinic acid acts as a component of the coenzyme Nicotinamide Adenine Dinucleotide Phosphate (NADP) and Nicotinamide Adenine Dinucleotide (NAD) in carbohydrate and lipid metabolism [19].

Table 5. Nutrient composition of melon, banana, and papaya (per 100 g)

| Nutrition                        | Unit | Melon | Banana | Papaya |
|----------------------------------|------|-------|--------|--------|
| Protein                          | g    | 0.54  | 1.09   | 0.47   |
| Lipid total (Fat)                | g    | 0.14  | 0.33   | 0.26   |
| Ash                              | g    | 0.41  | 0.82   | 0.39   |
| Carbohydrate, by difference      | g    | 9.09  | 22.84  | 10.82  |
| Fiber, total dietary             | g    | 0.8   | 2.6    | 1.7    |
| including NLEA total (Sugars)    | g    | 8.12  | 12.23  | 7.82   |
| Sucrose                          | g    | 2.48  | 2.39   | 0      |
| Glucose (Dextrose)               | g    | 2.68  | 4.98   | 4.09   |
| Fructose                         | g    | 2.96  | 4.85   | 3.73   |
| Calcium (Ca)                     | mg   | 6     | 5      | 20     |
| Iron (Fe)                        | mg   | 0.17  | 0.26   | 0.25   |
| Magnesium (Mg)                   | mg   | 10    | 27     | 21     |
| Phosphorus (P)                   | mg   | 11    | 22     | 10     |
| Potassium (K)                    | mg   | 228   | 358    | 182    |
| Sodium (Na)                      | mg   | 18    | 1      | 8      |
| Zinc (Zn)                        | mg   | 0.09  | 0.15   | 0.08   |
| Copper (Cu)                      | mg   | 0.024 | 0.078  | 0.045  |
| Manganese (Mn)                   | mg   | 0.027 | 0.27   | 0.04   |
| Selenium (Se)                    | μg   | 0.7   | 1      | 0.6    |
| Ascorbic acid total (Vitamin C)  | mg   | 18    | 8.7    | 60.9   |
| Thiamin                          | mg   | 0.038 | 0.031  | 0.023  |
### Nutrition

| Nutrition                | Unit | Melon  | Banana | Papaya |
|--------------------------|------|--------|--------|--------|
| Riboflavin               | mg   | 0.012  | 0.073  | 0.027  |
| Niacin                   | mg   | 0.418  | 0.665  | 0.357  |
| Pantothenic acid         | mg   | 0.155  | 0.334  | 0.191  |
| Vitamin B-6              | mg   | 0.088  | 0.367  | 0.038  |
| Folate total             | µg   | 19     | 20     | 37     |
| Folate (food)            | µg   | 19     | 20     | 37     |
| Folate (DFE)             | µg   | 19     | 20     | 37     |
| Choline total            | mg   | 7.6    | 9.8    | 6.1    |
| Vitamin A, RAE           | µg   | 3      | 3      | 47     |
| Carotene, beta           | µg   | 30     | 26     | 274    |
| Carotene, alpha          | µg   | 0      | 25     | 2      |
| Cryptoxanthin, beta      | µg   | 0      | 0      | 589    |
| Vitamin A, IU            | IU   | 50     | 64     | 950    |
| Lycopene                 | µg   | 0      | 0      | 1828   |
| Lutein + zeaxanthin      | µg   | 27     | 22     | 89     |
| Vitamin E (alpha-tocopherol) | mg  | 0.02   | 0.1    | 0.3    |
| Tryptophan               | g    | 0.005  | 0.009  | 0.008  |
| Threonine                | g    | 0.013  | 0.028  | 0.011  |
| Isoleucine               | g    | 0.013  | 0.028  | 0.008  |
| Leucine                  | g    | 0.016  | 0.068  | 0.016  |
| Lysine                   | g    | 0.018  | 0.05   | 0.025  |
| Methionine               | g    | 0.005  | 0.008  | 0.002  |
| Cystine                  | g    | 0.005  | 0.009  | 0     |
| Phenylalanine            | g    | 0.015  | 0.049  | 0.009  |
| Tyrosine                 | g    | 0.01   | 0.009  | 0.005  |
| Valine                   | g    | 0.018  | 0.047  | 0.01   |
| Arginine                 | g    | 0.014  | 0.049  | 0.01   |
| Histidine                | g    | 0.005  | 0.077  | 0.005  |
| Alanine                  | g    | 0.044  | 0.04   | 0.014  |
| Aspartic acid            | g    | 0.088  | 0.124  | 0.049  |
| Glutamic acid            | g    | 0.153  | 0.152  | 0.033  |
| Glycine                  | g    | 0.016  | 0.038  | 0.018  |
| Proline                  | g    | 0.012  | 0.028  | 0.01   |
| Serine                   | g    | 0.023  | 0.04   | 0.015  |

Source: Food Data Central USDA, 2019.

According to [20], somaclonal variation in explant tissue is very likely to occur at the multiplication stage on media containing hormones. Some factors that cause a high frequency of somaclonal variation in banana in vitro culture include genotype of parent plants, number of subculture cycles, the concentration of various components of growth media, regeneration type, concentration and type of growth regulators, and the use of selective conditions of in vitro media. The previous study conducted by [21] concluded that it is very possible that differences in the phenotype of a child with a parent or somaclonal variation to occur due to certain character variations available among cell populations. For example, a somaclonal variation that occurs on in vitro mechanisms with the formation of callus. Therefore, an increase in the number of character variations in forming morphogenesis can be obtained.
through repeated explant subculture cycles because explants were grown over long periods often indicate the occurrence of chromosomal abnormalities that provide beneficial character variations.

According to study [22] on the type of Kepok banana, somaclonal variations that occur were able to eliminate or change one or more of the desired characters. Somaclonal variations that occurred in the Kepok banana had eliminated the superior character of the parent plant without male flowers to avoid attracting blood wilt disease. Thus, in this case, superior characters no longer dominate tiller phenotypes from the results of repetitive subculture cycles. Besides, it is possible to appear different characters from the parent.

Another study conducted by [23] with a repetitive subculture cycle on in vitro shoots on MS media + 2 mg.L\(^{-1}\) BAP showed a decrease in the rate of multiplication after the fourth subculture cycle. The same thing happened to Kepok banana explants that were repeated subculture with somaclonal variation in the small form.

3.2. Kepok banana explant growths at various temperatures

Growth and development of Kepok banana explant affected by the temperature of the incubation chamber. The findings showed that explants grown in a room with AC (±25°C) and room without AC (±28°C) were not significantly different from the growth of explants outdoors (±31°C) (Figure 1). The highest yield of Kepok banana exploratory morphogenesis grown in a room without AC (±28°C) only appeared on the number of shoots (3.6) and plantlets height (5.9). Meanwhile, the findings in the outdoor (±31°C) were only shown in the parameters of the number of leaves (7) and the number of roots (6.5). This indicates that Kepok banana explants can be cultured in a room without air conditioning or outdoors resulting in electricity savings.

![Figure 1. Effect of temperature on Kepok banana explants growth](image)

Kepok Banana explant growth at room temperature with AC gave the smallest number of shoots (2.2), the number of leaves (5), plantlets height (5.5), and the number of roots (4.9) compared to explant growth at room temperature without AC and outdoor. Only the root length parameter (6.2) gave the highest yield of explant growth at room temperature with AC. It is possible that temperature affected the rate of transpiration which resulted in increased absorption of water and nutrients to increase plant biomass in the form of shoots, leaves, and roots. According to [24], temperature plays a direct role in most growth functions by controlling the rate of biochemistry such as the rate of transpiration, the opening of stomata, water absorption,
nutrition, photosynthesis, and respiration. Meanwhile, its indirect role is to influence other factors especially water supply.

Chlorophyll biosynthesis requires temperatures of 30-40°C in C4 plants and a temperature of 10-25°C in C3 plants [25]. Banana is classified as a neutral day plant that can flower at any time of the year. However, banana can show seasonal variations in flowering and carrying out generative phases. Irradiation more than 12 hours in the mid-vegetative phase has a positive effect on accelerating the flowering rate [26].

4. Discussion

4.1. Effect of fruit extract on morphogenesis of Kepok banana
The addition of melon, banana, and papaya extracts along with coconut water was an effort to streamline production costs by reducing chemical components such as vitamins and hormones. The physiological process carried out by the tissue or explants formed was accelerated by the concentration of vitamins and natural hormones. It was seen that the morphogenesis of the explants in media enriched with melon and papaya extracts was not significantly different from the explants in media without fruit extracts. In general, melon extract, papaya extract, and coconut water effectively replaced vitamins and hormone synthesis. To find out the relationship between the growth of shoots, leaves, heights, roots, and root length influenced by vitamin and hormone synthesis as well as natural as a whole, analyzed Pearson Correlation analysis was conducted (Table 6).

Table 6. Correlation of number of shoots, leaves, roots, plantlets height and root length

| Morphology     | Analysis          | Number of Shoots | Number of Leaves | Plantlets Height | Number of Roots | Root Length |
|----------------|-------------------|------------------|------------------|------------------|----------------|-------------|
| Number of Shoots | Pearson Correlation | 1                |                  |                  |                |             |
| Sig. (2-tailed) |                   |                  |                  |                  |                |             |
| Number of Leaves | Pearson Correlation | 0.95*          | 1                |                  |                |             |
| Sig. (2-tailed) |                   |                  |                  |                  |                |             |
| Plantlets Height | Pearson Correlation | 0.70            | 0.71             | 1                |                |             |
| Sig. (2-tailed) |                   |                  |                  |                  |                |             |
| Number of Roots  | Pearson Correlation | 0.89*           | 0.96*            | 0.62             | 1              |             |
| Sig. (2-tailed) |                   |                  |                  |                  |                |             |
| Root Length      | Pearson Correlation | 0.59            | 0.77             | 0.70             | 0.83           | 1           |
| Sig. (2-tailed) |                   |                  |                  |                  |                |             |

*. Correlation is significant at the 0.05 level.

The correlation coefficient between the number of shoots and leaves was 0.95. That number indicates that the more shoots were followed by the addition of the number of leaves and vice versa. Other positive correlations that occur in the growth of Kepok banana explants were the correlation between the number of roots and shoots (0.89) and the correlation coefficient between the number of leaves and roots (0.96). Those correlations mean that the number of roots was followed by the addition of shoots and leaves (Table 6).

In addition to morphogenesis, Kepok banana explant also has failed to differentiate to form specific organs to become individuals. The regeneration pattern of organogenesis and embryogenesis was possible to occur directly or without the formation of callus (direct) and indirectly by forming callus (indirect). The percentage of Kepok banana explants differentiated directly and indirectly was 75.2% and 24.8% respectively. The most differentiated Kepok banana explants came from the media enriched with melon extracts (86%) and the undifferentiated explants originated from media enriched by banana extract (60%) (Table 7). In general, the proliferation that occurred in Kepok banana explant was influenced by nutrients, especially vitamins and hormones that regulated tissue performance. The 2,4-D hormone works during callus initiation along with BA in stimulating more adventitious buds [27].
Table 7. Percentage of differentiation in Kepok banana explants on MS media riched fruit extract (%)

| Fruit Extract         | Direct (Plantlets) | Indirect (Callus) |
|-----------------------|--------------------|-------------------|
| Without Extract       | 84                 | 16                |
| Vitamin Synthesis      | 82                 | 18                |
| Melon Extract          | 86                 | 14                |
| Banana Extract         | 40                 | 60                |
| Papaya Extract         | 84                 | 16                |

4.2. Effect of temperature on Kepok banana explant growth

Microclimate, like temperature, can affect plant biochemistry and physiology. Thus, higher temperatures can spur plant metabolisms such as respiration, transpiration, and photosynthesis. The performance of metabolism can be optimized by balancing the availability of raw materials in the form of nutrients, vitamins, hormones, CO₂, H₂O, and O₂ that work synergistically and optimum.

The treatment of differences in the temperature of growing room for morphogenesis of Kepok banana explants had not had an effect that can increase plant growth and development. However, treatment at room temperature without air conditioning and outdoor can be a choice to streamline seed production costs by saving electricity from the use of air conditioners and lights. Additionally, it can save maintenance costs and it can reduce contamination.

5. Conclusion

The proliferation of Kepok banana explants gave a direct result in the form of explant multiplication speed in responding to the environment influenced by the genetic and physiology factors of explants. Meanwhile, it also gave indirect results in the form of callus. The growth of Kepok banana explants was effective in media MS enriched with papaya extract (150 g.L⁻¹) and coconut water (100 ml.L⁻¹) gave equal effectivity outcome with the addition of synthesized vitamin to explants speed and morphogenesis results using the parameters of the number of shoots, number of leaves, number of roots, and root length as well as showing the highest results in parameter height of plantlets and grown at room temperature (±28°C).

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