The effect of Murashige and Skoog (MS) and Growmore fertilizer media composition on growth of Ambon banana plants in vitro

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Abstract. Efforts to propagate banana seeds with good quality on a large scale can be pursued through tissue culture techniques. In tissue culture often uses Murashige and Skoog (MS) media but these media are difficult to obtain. Growmore is a leaf fertilizer that has the potential to replace MS media because it has a complete nutrient content. This study aims to obtain the most effective composition of MS media and Growmore fertilizer for the growth of ambon banana shoots in vitro. The study was conducted at the Plant Physiology and Biotechnology Laboratory, Sebelas Maret University using a completely randomized design pattern (CRD) with a single factor divided into 5 levels with 6 replications. Variables observed include the time of buds, number of shoots, plant height, time of leaves, number of leaves, time of roots, number of roots, root length. The results showed the composition of 25% MS + 75% Growmore gave the highest results on the number of shoots, the composition of 50% MS + 50% gave the best results on plantlet height, number of leaves, and number of roots.

1. Introduction
Bananas are high economic value commodities in Indonesia. Ambon Banana is one of the most popular bananas by the community. National demand for bananas has increased from year to year. This increase in demand for bananas should be offset by increased production. The limited availability of quality banana seeds in Indonesia is an obstacle to increasing banana production [1]. Procurement of banana seeds on a large scale with good quality can be done with tissue culture techniques. Tissue culture (tissue culture) is a technique of isolating parts that are grown separately, driven to multiply themselves, eventually regenerated back into complete plants. Through tissue culture, it is possible to produce banana seeds in a relatively short time and are free from plant pathogens.

Growing media is one of the keys that determine success in tissue culture [2]. Murashige and Skoog (MS) media are very widely used because of their complex nutrient content and are ideal for a variety of plant cultures. MS media generally use materials with a high level of purity (pro-analysis) [3], so that it requires expensive costs, long ordering time, and difficult to obtain. Substitution material is needed to replace the use of MS media. Leaf fertilizer has the potential to be a banana tissue culture
media but needs further study to find out the results provided. Growmore is a leaf fertilizer that contains complete macro-micro nutrients, including the content of N, P, K, S, Mg, Fe, Zn, Ca, Co, Mn, Mo, B, and Cu. These elements are almost the same as MS media.

2. Materials and methods

This research was conducted in June-December 2019 at the Plant Physiology and Biotechnology Laboratory, Faculty of Agriculture, Sebelas Maret University, Surakarta. The tools used include Laminar Air Flow Cabinet (LAFC), autoclaves, magnetic stirrers, pH meters, petri dish, analytical scales, culture bottles, sprayer bottles, scalpel knives, tweezers, bunsen lamps, hot plates, goblets, plastic wrap, paper. Planting material in the form of ambon banana explants aged 2 weeks after the second subculture. The chemicals used in this study included MS stock solutions, Growmore fertilizer, white agar, aquades, methylated spirits, 1N NaOH, 1N HCl, granulated sugar, 70% alcohol.

The research method used was an experimental method with a Completely Randomized Design (CRD) with a single factor divided into 5 levels and 6 replications. These treatments include: 100% MS (M1), 75% MS + 25% Growmore (M2), 50% MS + 50% Growmore (M3), 25% MS + 75% Growmore (M4), 100% Growmore + compactor (M5). This proportion is a percentage of the normal dose in both MS and Growmore media. The normal dose used on Growmore fertilizer is 2 g / l. Variables observed include the time of buds, number of shoots, plantlet height, time of leaves, number of leaves, time of roots, number of roots, length of roots. Data were processed by ANCOVA test if the covariate variable did not have a real effect, an analysis was carried out by ANOVA test and if it had a significant effect, it was continued by DMRT (Duncan Multiple Range Test) continued to test with α level of 5%.

3. Results and discussion

3.1. Time of buds appear

The emergence of shoots in plants inoculated in vitro is one indicator of success in tissue culture activities.

Table 1. Analysis of correlations between response variables

| Time of buds | Number of buds | Plantlet height | Time of leaves | Number of leaves | Time of root | Number of roots | Root length |
|--------------|----------------|-----------------|----------------|-----------------|--------------|----------------|-------------|
| Time          |                |                 |                |                 |              |                |             |
| of buds      |                |                 |                |                 |              |                |             |
| buns         | .006           | .225            | .705           | .573            | .187         | .871           | .753        |
| Number of     | .226           | -.076           | .384*          | .107            | 1            | .247           | -.031       |
| leaves        |                |                 |                |                 |              |                |             |
| Time          |                |                 |                |                 |              |                |             |
| of leaves     | .637**         | -.123           | -.034          | .247            | -.101        | 1              | .195        |
| Number of     | .516           | .859            | .187           | .594            | .301         | .940           |             |
| roots         |                |                 |                |                 |              |                |             |
| Time          |                |                 |                |                 |              |                |             |
| of root       | - .283         | .454*           | -.031          | .396*           | .195         | 1              | .454*       |
| Number of     | .420           | .130            | .871           | .030            | .301         | .012           |             |
| roots         |                |                 |                |                 |              |                |             |
| Root          | .085           | -.360           | .378*          | .060            | .292         | .014           | .454*       |
| length        | .655           | .051            | .039           | .753            | .117         | .940           | .012        |

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

Time of buds appear is important to observe the time when shoots emerge to know the response due to injury when planting an explant and know the response to the treatment given [4]. Media composition had no significant effect on the time of emergence of shoots on Ambon banana explants.
When the first shoots appear, all treatments occur 2 to 5 days after planting (DAP). In explants of banana plants takes between 1-15 days to form buds [5].

The emergence of early shoots in explants was influenced by the speed of the emergence of roots. In tissue culture, the emergence of the first organ in explants is not always preceded by the emergence of shoots, but sometimes roots emerge faster than shoots. These two components of growth influence one another, as can be seen from the magnitude of the correlation value 0.637 (Table 1). The faster the appearance of roots will spur the emergence of shoots and vice versa. The emergence of roots before the emergence of shoots, because these roots are the main organs of cytokinin synthesis which will translate cytokinins into shoot areas through the xylem and subsequently shoots, cytokinins stimulate cell division to produce shoots [6].

3.2. Number of buds

The growth of explants is seen from the change in color, swelling of the explants, and finally the formation of shoots [7]. The response to color changes that occur in explants, due to the response to the stimulation of light given and the development of chlorophyll. Explants began to differentiate by slipping off their shawls one by one. This sheath will open up to the innermost layer and buds will begin to form. The shoots that appear are usually marked by the appearance of a whitish-green part of the explant growing spots.

The number of shoots becomes the most important factor in the in vitro method because the more shoots that are formed the new shoots will appear in large numbers [8]. The development of Ambon banana plantlets as a whole has increased in new shoots that are formed. The addition of newly formed buds generally occurs in plantlets aged 7–28 (DAP). The percentage of sprouts showed the accumulation of growth of a plant [9]. The results of covariance analysis of plantlet height on the number of shoots showed that the observation variable of shoot number did not show any real difference due to the treatment of media composition, but rather the influence of other response variables namely plantlet height. The relationship between the two response variables is shown by the correlation value with the coefficient of -0.701 (Table 1), which means that the more shoots produced the higher the plantlet will be low. The majority of failures in tissue culture were caused by a lack of appropriate techniques and lack of knowledge about nutrient media [10]. A good explant cutting will not cause a cut point to grow on the explant.

3.3. Plantlet height

Ambon banana plantlet height was observed at the end of the observation. The composition of the media did not affect the shoot height but was influenced by other variables, namely the number of shoots. The higher the number of shoots, the lower the number of shoots. These two aspects of growth influence one another. The high shoots and vigor showed healthy shoots and food reserves contained in stems more than in stems that were not high [11]. Plant height can increase photosynthetic yield. Photosynthetic results will be transplanted to other organs during vegetative growth. Because vegetative growth will determine the generative phase and crop production [12].

The relationship between the variable number of shoots and shoot height is shown by the negative correlation value, which is 0.701 (Table 1). A simple correlation is used to measure the relationship between yields and other characters [13], while the coefficient analysis measures the interrelationships between various plant characters. So that in this relationship if the shoots grow high, the number of shoots produced will be low. The fewer shoots produced, the higher the plantlet tends to increase [14]. This is because the energy needed to form prospective shoots has been used to extend cells.

3.4. Time of leaves appear

The leaves of the banana plant cover each other so that they form like stems. Along with the growth of buds, other plantlet organs will be formed. Observation of the time the leaves appear is done every day until the appearance of the first leaf on the plantlet. The difference in composition in each treatment media did not have a significant effect on the results obtained but rather due to the time variable that
The close relationship between the two variables is indicated by the value of the results of simple correlation analysis with a value of 0.488 (Table 1). Simple correlations are used to measure the relationship between results and other characters that can theoretically or predictably influence the results [15].

The faster emergence of shoots in explants causes the leaves to form faster shrubs. Called positive correlation because if the emergence of buds will be followed faster with the emergence of leaves that are also faster. Where if a variable is increased, it will increase the other variables. Therefore improvement of one trait is sometimes followed by enhancement of another trait. The leaves will appear when the physiological condition of the shoots is good and the leaves will start appearing by themselves. Leaves will form and develop naturally after the shoots have formed [16].

### 3.5. Number of leaves

The number of leaves is related to photosynthesis, plant metabolism, and nutrient absorption because leaves are the main organ where photosynthesis takes place. The greater the number of leaves, the more plants produce photosynthates for food reserves [11]. The number of leaves counted is the leaves that have opened perfectly on each plantlet.

**Table 2.** Effect of composition of MS Media and Growmore Fertilizer on the number of Ambon banana plantlet leaves.

| Composition of MS and Growmore Media | Number of leaves |
|--------------------------------------|------------------|
| M1 (100% MS)                         | 3.84bc            |
| M2 (75% MS + 25% Growmore)           | 3.43ab            |
| M3 (50% MS + 50% Growmore)           | 4.19c             |
| M4 (25% MS + 75% Growmore)           | 2.39a             |
| M5 (100% Growmore)                   | 2.61ab            |

Note: Figures followed by the same letter in each treatment show no significant difference in the DMRT test level of 5%.

The composition of the media significantly affected the variable number of leaves (Table 2). Some research results show that the use of media with lower nutrient content gives better results for the development of culture. In the somatic embryogenesis of the orchid plant *Coelogyne cristata*, 50% MS media gave better results compared to full MS media [17]. The M3 treatment produced the number of leaves with the highest mean of 4.19 and was not significantly different from the M1 treatment.

Macro elements are needed for vegetative growth including leaf formation. The macronutrients include N, P, K, Ca, Mg, S. The macronutrient content in M3 treatment media are: 614.6 ppm N-total, 40.9 ppm P, 495.1 ppm K, 72.4 ppm Ca, 6.3 ppm Mg, 423.05 ppm S (Table 3). The media with M1 treatment had 868.2 ppm N-total macronutrients, 38.8 ppm P, 782.2 ppm K, 119.8 ppm Ca, 3.6 ppm Mg, 714.5 ppm S (Table 3). The content of each element between the two treatment media tends to be more in the M1 treatment.

This difference does not cause a difference in response to the variable number of leaves formed in the treatment of M3 and M1. This is presumably due to some higher amount of elements in the M3 treatment media, for example, Mg content. Magnesium plays an important role in the formation of leaf green matter. Media formulations of 50% MS and 50% Growmore were able to give the best results on the number of leaves [19]. The more the number of leaves formed, the more light is absorbed for the process of photosynthesis so that carbohydrates for growth are also more numerous [20].

In the vegetative growth phase, plants need a balanced supply of macronutrients, especially N, P, K to increase the growth component [21]. The low K content in M5 formulation causes micronutrient deficiency. The adequacy of the K element in the vegetative phase can produce the highest number of leaves [22]. The element K can increase photosynthesis by increasing the rate of photophosphorylation, which produces ATP and NADPH which play an important role in the process of photosynthesis and plant metabolism to increase the number of leaves [23].
Table 3. Nutrient content in each treatment media

| Element (ppm) | Media composition |
|---------------|------------------|
|               | M1   | M2   | M3   | M4   | M5   |
| N total       | 868.2 | 741.4 | 614.6 | 487.8 | 361  |
| P             | 38.8  | 39.85 | 40.9  | 41.95 | 43   |
| K             | 782.2 | 638.65| 495.1 | 351.55| 208  |
| Ca            | 119.8 | 96.1  | 72.4  | 48.7  | 25   |
| Mg            | 3.6   | 4.95  | 6.3   | 7.65  | 9    |
| Na            | 9.2   | 7.5   | 5.8   | 4.1   | 2.4  |
| S             | 714.5 | 568.775| 423.05| 277.325| 131.6|
| Fe            | 5.5   | 4.3595| 3.219 | 2.0785| 0.938|
| Mn            | 7.24  | 5.6085| 3.977 | 2.3455| 0.714|
| Cu            | 0.01304| 0.05628| 0.099 | 0.14276| 0.186|
| Zn            | 6.52  | 4.9365| 3.353 | 1.7695| 0.186|
| B             | 1.08  | 0.881 | 0.682 | 0.483 | 0.284|
| Co            | 0.006 | 0.005 | 0.003 | 0.002 | 0    |
| Mo            | 0.091 | 0.073 | 0.055 | 0.037 | 0.019|
| Glycine       | 2     | 1.5   | 1     | 0.5   | -    |
| Nicotine acid | 0.5   | 0.375 | 0.25  | 0.125 | -    |
| Pyrodoxin HCl | 0.5   | 0.375 | 0.25  | 0.125 | -    |
| Thyamine HCl  | 0.1   | 0.075 | 0.05  | 0.025 | -    |
| Myo-inositol  | 100   | 0     | 75    | 50    | 25   |
| Sukrosa       | 8     | 8     | 8     | 8     | 8    |

Note: in 1 liter of media [18]

3.6. Time of roots appear

Roots can grow when plantlets have produced new shoots and leaves. The new shoots and leaves are expected to produce endogenous auxin and translocate it to the basal part and induce root formation [24]. Media composition does not affect the speed at which roots appear. The time of emergence of the root is influenced by the speed of the emergence of shoots in explants. The relationship between these two variables is shown by the positive correlation value of 0.637 (Table 1). The correlation coefficient measures the reciprocal relationship between two characters and the degree of closeness and the direction of the relationship between the two [25]. The direction of the relationship between the two positive variables means that the faster the bud appears, the faster the roots appear. Carbohydrate content in explants is a major factor for the development of primordial shoots and roots, with sufficient food reserves then explants will be able to bring up the shoots [26].

The relationship between root growth patterns and shoots is further explained [27] that early shoot growth, its activity depends on the accumulation of carbohydrates in explants, then these carbohydrates move towards meristematic tissue so that the root growth rate has not yet occurred. After active shoots, photosynthesis and physiological activity will increase which causes the mobilization of assimilates to the aerial area (plant tissue that respires) and one of them is the root. So that after the emergence of shoots is followed by the appearance of roots. Banana tissue contains phenolic enzymes, especially the enzyme polyphenol oxidase which is naturally an important phyto-auxin in bananas to initiate the emergence of roots [28].

3.7. Number of roots

Observation of the number of roots was done at the end of the observation when the plantlet was 12 MST. The number of roots showed significant differences between treatments due to the composition of MS media and Growmore fertilizer given (Table 4). The highest yield on the root number variable was in the M3 treatment with an average of 6.83 and it was not significantly different from the M1 and M2 treatments. One element that plays an important role in root growth is phosphorus (P). The M3 treatment media contained 40.9 ppm of P element (Table 3). This concentration can give the highest
yield on the variable root length.

**Table 4.** Effect of composition of MS Media and Growmore fertilizer on the number of Ambon banana explant roots

| Composition of MS and Growmore Media | Number of roots |
|-------------------------------------|-----------------|
| M1 (100% MS)                        | 6.10b           |
| M2 (75% MS + 25% Growmore)          | 6.12b           |
| M3 (50% MS + 50% Growmore)          | 6.83b           |
| M4 (25% MS + 75% Growmore)          | 3.26a           |
| M5 (100% Growmore)                  | 3.96a           |

Note: Figures followed by the same letter in each treatment show no significant difference in the DMRT test level of 5%

Element P plays an important role in the process of energy transfer and the process of re-forming carbohydrates into sugar as well as improving the efficiency of the performance of chloroplasts [29]. The P element that has been well absorbed by the plantlet will be translocated to all parts of the plant vegetative component, especially in the roots. The total N-content (614.6 ppm) and P (40.9 ppm) in the composition of 50% MS and 50% Growmore are suspected to be proportional so that they are ideal for root development. Meanwhile, a medium with a single composition of Growmore fertilizer, the number of roots produced is less due to an unbalanced nitrogen composition so that nitrogen accumulation occurs in the area where the root candidates are growing [3].

3.8. Roots length

Root lengthening is a phase that occurs after the appearance of roots in the plantlet. Roots must be of good quality so that the acclimation process can run well [30].

**Table 5.** Effect of composition of MS Media and Growmore Fertilizer on Ambon Banana plantlet root length

| Composition of MS and Growmore Media | Root length (cm) |
|-------------------------------------|------------------|
| M1 (100% MS)                        | 22.75c           |
| M2 (75% MS + 25% Growmore)          | 11.92ab          |
| M3 (50% MS + 50% Growmore)          | 19.77bc          |
| M4 (25% MS + 75% Growmore)          | 11.98ab          |
| M5 (100% Growmore)                  | 7.67a            |

Note: Figures followed by the same letter in each treatment show no significant difference in the DMRT test level of 5%

The media composition of MS and Growmore Fertilizer showed a significant effect on Ambon Banana root length. Then proceed with the DMRT test at a level of 5% and the results obtained that there are significant differences between treatments (Table 5). The M1 treatment had the longest root with an average of 22.75 cm and was not significantly different from the M3 treatment which had an average root length of 19.77 cm. The M5 treatment is the treatment with the shortest root length, which has an average of 7.67 cm. Based on [19] the 50% MS + 50% Growmore formulation was able to give the best results on root length compared to using a single formulation of Growmore fertilizer. This relates to the availability of nutrients used to stimulate root growth.

The elements P and N are very important to stimulate the vegetative growth of roots. Phosphorus functions as a component of nucleic acids and coenzymes [31]. The macro element needed by plants to stimulate cell elongation during the vegetative period is the element nitrogen (N) [32]. The N and P elements contained in the M3 treatment media were 614.6 ppm and 40.9 ppm (Table 3) and whereas the M1 media contained N and P respectively 868.2 ppm and 38.8 ppm (Table 3). The content of element P in the M3 treatment media was higher than that of the M1 treatment media, even though the total N content in the M3 treatment media was lower. This is thought to be the cause of the
composition of the media 50% MS + 50% Growmore can show better results than media with a single composition of Growmore fertilizer. The rate of root lengthening was also influenced by internal factors including the supply of photosynthate (sucrose) from leaves [33].

4. Conclusion
Based on the results of research and discussion it can be concluded that the composition of MS media and Growmore fertilizer significantly affected the number of leaves, the number of roots, and the length of the Ambon banana plantlet roots in vitro culture. Media composition of 50% MS + 50% Growmore can replace 50% of MS media requirements so that it gives the best results on the number of leaves, a number of roots, and root length of Ambon banana plantlets in vitro culture.

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