Combination of MS medium and Gandasil D on banana shoots growth in vitro

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Abstract. Cavendish banana has a high economic for food export commodities. High export opportunities need to be balanced with increased productivity by selecting superior seedlings through in vitro seed propagation. The tissue fruitfulness depends on the media used. The media used substitution between MS media and Gandasil D leaf fertilizer with different concentrations. The researcher also used a completely randomized design (CRD) of one factor with five treatment combinations and six repetition. The treatment used is MS 100% as a control; 75% MS + 25% Gandasil D; 50% MS + 50% Gandasil D; 25% MS + 75% Gandasil D; 100% D. The variables observed include time of buds, number of shoots, plantlet height, time of leaves, number of leaves, time of roots, number of roots, and root length. The data analysis used is the physical analysis method based on the F test of 5% level. The results of the research showed that 100% Gandasil D and 25% MS + 75% Gandasil D treatment had the highest influence on the number of leaves, number of shoots, plant height, and number of roots. The mixture of MS media and leaf fertilizer can be used as an alternative medium for multiplication of Cavendish bananas.

1. Introduction

Banana is one of the popular agricultural commodities of the community. Banana plants have been known since BC and the name Musa was taken from a doctor in Roman times (63 BC–14 AD) [1]. Indonesian banana production in 2017 amounted to 7,162,678 tons. This figure shows an increase in production compared to 2016 with 7,007,117 tons with a harvest area of 89,615 ha, one of which is Cavendish banana. Cavendish bananas are consumed by many people directly or become food preparations. Another advantage of cavendish bananas is the larger fruit size and has a bunch of about 10 combs.

Quality banana production is currently constrained by the availability of quality seeds. Propagation of bananas is usually by using tillers that grow from the weevils or by dividing the weevils according to the buds, but the number of tillers obtained is also unproductive. Another effort to improve seed quality in
tissue culture. The banana propagation technique in vitro is an effort to meet the demand for quality banana seeds and uniform growth.

Tissue culture is a method of propagation using plant parts and growing them in aseptic media. Parts of the plant can later develop and regenerate into complete plants [2]. Media is a major success factor in tissue culture. The composition of the media used in tissue culture can be different types and concentrations. Murashige and Skoog (MS) contain high macro and micronutrients which can affect plant tissue growth. Alternative media can also be used for in vitro culture. Alternative media compositions that are often used are the addition of leaf fertilizers that contain macro and micronutrients needed by plants. Besides, the use of alternative media tends to be cheaper compared to Murashige and Skoog (MS) growing media. The application of leaf fertilizer itself aims to meet the needs of macro and micronutrients needed by plants and to reduce the tendency to use relatively expensive MS media [3].

The application of leaf fertilizer in banana growth media in vitro has been widely carried out. The combination of MS media treatment with leaf fertilizer gives the average appearance of leaves faster than the treatment of full leaf fertilizer media. The provision of this treatment also aims to determine the optimal dose for the growth of the banana shoots [4].

2. Materials and methods
This research was carried out in May–December 2019 at the Plant Physiology and Biotechnology Laboratory, Faculty of Agriculture, Sebelas Maret University Surakarta. The research method used was an experimental method with a completely randomized design (CRD) of one factor with 5 treatment combinations that were repeated 6 times so that there were 30 experimental units. The combination of treatments given include G1: 100% MS (control); G2: 75% MS + 25% Gandasil D; G3 = 50% MS + 50% Gandasil D; G4 = 25% MS + 75% Gandasil D; and G5 = 100% D Success. Variables observed include the time the bud appeared, when the roots appeared, the time the leaves appeared, the number of leaves, number of shoots, plantlet height, root length, and number of roots. The data obtained were analyzed with an F test of 5% level and if it was significantly different it was followed by a DMRT of 5% level.

3. Results and discussion
3.1. When the buds appear
One of the success factors in multiplication is the emergence of shoots. The appearance of the shoots is marked by the appearance of a green protrusion on the side of the explant. Shoots generally appear at week 1 of observation or 7 days after planting.

Table 1. Effect of treatment of MS media and Gandasil D leaf fertilizer on shoot emergence time

| Treatment | When the buds appear (day) |
|-----------|-----------------------------|
| G1        | 4.50a                       |
| G2        | 5.00a                       |
| G3        | 4.50a                       |
| G4        | 5.67a                       |
| G5        | 3.67a                       |

Based on the analysis results of various treatments of MS and Gandasil D media, it did not significantly affect the time of the emergence of cavendish banana shoots in units of days after planting (Table 1). All treatment combinations give results that appear almost the same bud. The use of different planting media treatments that are used does not spur acceleration of the appearance of cavendish banana shoots because the average time of emergence of banana explant shoots tends to be uniform. The shoots that appear in explants show the success of the multiplication stage in tissue culture. Explant growth in
vitro culture was influenced by the media used [5]. The type of culture media and the concentration of nutrients influence the speed of in vitro growth, elongation, and morphogenesis quality [6].

3.2. When the roots appear
That success in tissue culture is characterized by the emergence of roots [7]. The appearance of the roots is marked by the appearance of white lumps in the explant bottom explodes which are submerged by planting media. Good rooting condition in terms of the number of roots and root length because it can affect the success of explant life when grown outside the culture bottle during the acclimatization process [8].

Table 2. Effect of treatment of MS media and Gandasil D leaf fertilizer on root emergence time.

| Treatment | When the roots appear (day) |
|-----------|----------------------------|
| G1        | 4.00a                      |
| G2        | 4.67a                      |
| G3        | 4.33a                      |
| G4        | 5.83a                      |
| G5        | 4.50a                      |

Based on the results (Table 2) of a variety of analysis that there is no real effect on the substitution of MS media with Gandasil D leaf fertilizer on the growth of Musa Acuminata L. The number of roots formed is quite low. This is thought to be due to the lack of auxin and cytokinin requirements. The provision of growth influence substances (PGR) as an alternative to meet the needs of explant growth hormone. The initiation of root primordia requires a combination of auxin and cytokinin [9]. The use of MS media substituted with other alternative media does not always show good results, because alternative media are not able to support sustainable growth [4].

3.3. When the leaf appear
The appearance of leaves in vitro culture shows that explants can live and develop in the treatment given. The leaves undergo growth following the explant growth cycle that grows in vitro. The observed leaves are leaves that have been fully opened.

Table 3. Effect of treatment of MS media and Gandasil D leaf fertilizer on leaf emergence time

| Treatment | When the leaf appear (day) |
|-----------|----------------------------|
| G1        | 5.83a                      |
| G2        | 8.17a                      |
| G3        | 8.33a                      |
| G4        | 10.00a                     |
| G5        | 9.83a                      |

Different treatments of MS and Gandasil D media composition based on the results of the analysis of variance did not have a significant effect on the time of leaf appearance (Table 3). The highest average leaf emergence time was in the G4 treatment. The addition of leaf fertilizer can be done as an alternative medium in the multiplication of bananas in tissue culture because it has macro and micronutrients that are useful for plant growth and development [10].

3.4. Number of leaves
The number of leaves is calculated from the first week of observation to the last observation for 12 weeks. Leaves that are counted are leaves that have been opened from new leaf rolls. Based on the results of the
analysis, the treatment of MS media with leaf fertilizer had a significant effect on the number of cavendish banana leaves (Table 4, Figure 1).

Based on the analysis results that can be seen in Table 4, the highest number of leaves was found in the G5 treatment with an average of 5.17 strands and significantly different from the G1 treatment which had an average number of leaves of 1.67 strands. In the vegetative growth phase, it is necessary to provide a high N content, because these elements are the main ingredients for making proteins needed in cell division. The application of Gandasil fertilizer with a concentration of 2 g/L in orchid plants gave good results for plant height, number of leaves, and width of leaves compared to Hyponex [11].

**Table 4.** Effect of treatment of MS media and Gandasil D leaf fertilizer on number of leaves at 12 weeks after planting.

| Treatment | Number of leaves (sheet) |
|-----------|--------------------------|
| G1        | 1.67<sup>a</sup>         |
| G2        | 3.33<sup>ab</sup>        |
| G3        | 4.67<sup>b</sup>         |
| G4        | 5.00<sup>b</sup>         |
| G5        | 5.17<sup>b</sup>         |

**Figure 1.** Banana explants when observing the number of leaves of 10 week after planting.

### 3.5. Number of shoots

The formation of shoots in vitro culture largely determines the success of the production of large, uniform, and relatively short seedlings. The more shoots that form, the more seeds produced through tissue culture. The number of shoots was observed starting from week 1 to 12 weeks after planting.

**Table 5.** Effect of treatment of MS media and Gandasil D leaf fertilizer on the number of shots at 12 weeks after planting.

| Treatment | Number of roots |
|-----------|----------------|
| G1        | 0.50<sup>a</sup> |
| G2        | 1.67<sup>ab</sup> |
| G3        | 1.83<sup>b</sup>  |
| G4        | 2.00<sup>b</sup>  |
| G5        | 2.33<sup>b</sup>  |
The treatment of G1 based on the results of the analysis of variance was significantly different from the treatment of G4 and G5 (Table 5, Figure 2). The highest number of shoots resulted from G5 treatment with an average of 2.33. The Gandasil D leaf fertilizer treatment tended to increase the average number of shots compared to the control treatment [12]. The concentration of leaf fertilizer when used appropriately can spur the growth of shoots. The nutrients available in Gandasil D leaf fertilizer are at a balanced concentration so that plant cells are stimulated to continue to differentiate and spur the number of shoots. The Gandasil D fertilizer contains macro and microelements that stimulate cells will continue to differentiate to spur a rapid increase in the number of nodes and leaves. Element N is one of the essential elements that act as an extension of the nodes and leaves in the vegetative phase in plant growth [13].

![Figure 2. Explants of bananas when observing the number of shoots of 10 week after planting.](image)

3.6. Plant height

Plant height is the most easily observed growth measure. Plant height is the parameter most often used to determine the growth and effect of a treatment on crop yields. The multiplication of banana plantlets was then measured height at the end of observation.

**Table 6.** Effect of treatment of MS media and Gandasil D leaf fertilizer on plant height at 12 weeks after planting.

| Treatment | Plant height (cm) |
|-----------|-------------------|
| G1        | 5.16<sup>a</sup>  |
| G2        | 32.5<sup>ab</sup> |
| G3        | 38.33<sup>ab</sup>|
| G4        | 83.67<sup>b</sup> |
| G5        | 96.16<sup>b</sup> |

Based on the results of the analysis of the different treatments G1 significantly lower compared to other treatments (Table 6). The G5 treatment gave the highest yield with a plant height of 96.16 cm, but it was not significantly different from the G4 treatment. The application of Gandasil D leaf fertilizer showed an average height increase of orchid plantlets which tended to increase compared to control treatments [10]. Adding N, P, and K nutrients in the Gandasil D media can provide increased growth. The nutrients available in Gandasil D leaf fertilizer are at a balanced concentration so that plant cells are stimulated to continue to differentiate and spur the number of shoots [14].
3.7. Number of roots

The calculation of the number of roots is done every week of observation until the end of the observation. The more the number of roots produced, the more nutrients will be absorbed by plants. The number of roots is calculated since the first root appeared in the bottle.

The results of the analysis of variance showed that the G5 treatment gave significantly different results with the G1 treatment but not significantly different from the G3 and G4 treatments (Table 7, Figure 3). The highest average number of roots in the G5 treatment was 18.83. The number of roots is important for the growth of explants or plants in tissue culture in vitro [15]. The increasing number of roots is good for the absorption of nutrients from the media. The average increase in the number of roots in the Gandasil D fertilizer media without nano-silica increased the number of roots [4].

Table 7. Effect of treatment of MS media and Gandasil D leaf fertilizer on the number of roots at 12 weeks after planting.

| Treatment | Number of roots |
|-----------|----------------|
| G1        | 6.83<sup>a</sup> |
| G2        | 13.17<sup>ab</sup> |
| G3        | 16.67<sup>b</sup> |
| G4        | 18.67<sup>b</sup> |
| G5        | 18.83<sup>b</sup> |

Figure 3. Roots of cavendish banana explants 10 week after planting.

3.8. Root length

The root is the bottom of the axis of plants and generally grows in the soil with the direction to grow to the center of the earth and away from light. In in vitro culture, the roots usually grow in the growing media which contains nutrients. Observation of root length was observed at the end of the observation or the 12th week.

Table 8. Effect of MS media treatment and Gandasil D leaf fertilizer on root length at 12 weeks after planting.

| Treatment | Root length (cm) |
|-----------|-----------------|
| G1        | 5.33<sup>a</sup> |
| G2        | 12.67<sup>b</sup> |
| G3        | 14.00<sup>b</sup> |
| G4        | 1467<sup>b</sup> |
| G5        | 16.17<sup>b</sup> |
Based on the results of the F test analysis the G1 treatment was significantly lower with all treatments. The highest average root length, namely G5 treatment was 16.17 cm (Table 8). The Gandasil D fertilizer affects the number of roots and root length because they contain nicotinic acid such as those contained in cassava 2.2 mg effect on increasing the number and length of roots [16]. Another element, namely phosphorus, also influences root formation. The longer the root the better the absorption of nutrients [11].

4. Conclusion
Based on the results of research and discussion it can be concluded that the addition of Gandasil D to MS media has a significant effect on the number of leaves, many shoots, plant height, root length, and some roots. MS and Gandasil D media substitution can be used as alternative media to reduce MS media dependency and save on the cost of making media.

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