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EFFECTS OF MEDIA COMBINATION WITH CONCENTRATION OF AB-MIX NUTRIENT ON GROWTH OF BANANA SHOOTS ON *IN VITRO*

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Abstract

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This study aims to examine the effect of MS (Murashige and Skoog) media combination, coconut water (CW) and AB-Mix nutrition on the growth of banana shoots and to review growth differences in banana cultivars. The study was conducted from April-October 2017 at the Tissue Culture Laboratory of Balai Benih Hortikultura, Salaman, Magelang. The experimental design was designed using Completely Randomized Design (CRD) with two treatment factors. The first factor is media consisting of 5 levels and the second factor is cultivar consisting of 4 levels. Each combination is repeated 3 times as a sample. The results showed that the treatment of media and cultivars significantly affected the growth of banana shoots in tissue culture for 42 days after planting (DAP). MS medium treatment showed the highest result on root number, root length, and plant vigor variables. The treatment of Mas Kirana banana cultivars showed the best results on the root number, root length, shoot length, and plant vigor variables.

Key words: tissue culture; banana; coconut water; AB-Mix nutrition; phenol

Introduction

Banana (*Musa paradisiaca* L.) is a horticultural crop that belongs to a group of fruits. This plant is thought to have originated from South Asia and Southeast Asia and is now widespread to tropical and subtropical countries (Satuhu and Supriyadi, 2008). Indonesia is known as a world banana producer. Indonesia has produced 6.20% of the world's total production, 50% of Asia's banana production comes from Indonesia (Satuhu and Supriyadi, 2008).

Tissue culture is a technique to grow a part of the plant in the form of cells, tissues or organs in aseptic conditions. Seeds generated from tissue culture have several advantages, such as having identical properties with the parent, can be reproduced in large quantities so it does not need a large space, able to produce seeds in large quantities in a short time, health and quality of seedlings more guaranteed, and faster seedling speed compared to conventional propagation (Widianti, 2003). In addition, the resulting seeds are free of pests and diseases, and the cost of transport is relatively cheaper and easier (Meynarti, 2010).

Nutritional requirements for growth in vitro vary depending on the type of plant used. Culture growing media is one of the requirements for good culture (George et al., 2008). Nutrients needed include macro and micro nutrients. Nutrients are given in the form of mineral salts. Macro elements are needed in large enough quantities, generally given in the form of compounds. Revised MS's basic media (Murashige and Skoog, 1962) is the most widely used medium with other basic media. George and Sherrington (1984) mention some common macronutrient compounds used in tissue culture media, among others KNO_3; NH_4NO_3; $Ca(NO_3).4H_2O$; $NaNO_3$; $CaCl_2.2H_2O$; $MgSO_2.7H_2O$; KCl; KH_2PO_4; $NH_4H_2PO_4$; $NaH_2PO_4.2H_2O$; Na_2SO_4; $(NH_4)2SO_4$; NH_4Cl; K_2SO_4.

Nutrition AB-Mix contains macro and micro nutrients. Nutrition A consist of NO_3^-, NH_4^+, Ca^{2+}, and Fe. Nutrition B consist of $H_2PO_4^-$, SO_4^-, K^{2-}, Mn, Zn, B, Cu and Mo. Both

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types of nutrition should not be mixed in concentrated conditions. Nutrition A contains elements of Ca while nutrition B containing phosphate and sulfate anions. When Ca is mixed with sulfate it will form $CuSO_4$ or gypsum which is a precipitate because of its low solubility so it cannot be absorbed by plant roots (Suhardiyanto, 2011).

Coconut water contains auxin and cytokinin hormones, both of which are used to support embryonic cell division (Lawalata, 2011). Auxins play a role spur the callus formation, forming chlorophyll in the callus, encouraging the callus morphogenesis process, root formation and embryogenesis process. The role of cytokinin plays a role in splitting cell division, ending meristem proliferation, inhibition of root formation and encourages the formation of chlorophyll in callus (Surachman, 2011).

Bananas are divided into two types based on how to consume them, namely *banana* and *plantain*. This type of *banana* is consumed freshly and commonly called "*banana table*", among others Ambon Kuning (AAA), Ambon Hijau (AAA), Ambon Putih (AAA), Barangan (AAA), Berlin (AA), Lampung (AA), Mas (AA), Raja Bulu (AAB), Raja Sereh (AAB). While type of "*plantain*" are consumed after cooking, among others Pisang Tanduk (AAB), Uli (AAB), Kepok (BBB) and Siam (ABB) (Valmayor et al., 2000).

Materials and Methods

Experimental Site

This research was conducted at Tissue Culture Laboratory, Balai Benih Hortikultura, Salaman, Magelang from April-October 2017.

Planting Materials

Materials planting for the subculture use the banana plantlet of Mas Kirana, Cavendish, Raja Bulu, and Kepok with uniform size and age.

Planting Media Material

Materials for MS media include stock solvent of macro nutrients consisting of KNO_3, $CaCl_2$, NH_4NO_3, KH_2PO_4 and $MgSO_4$; micro nutrients comprising $CuSO_4$, H_3BO_3, $ZnSO_4$, Na_2MoO_4, KI, $CoCl_2$ and $MnSO_4$; a vitamin solvent consisting of *Myo-inositol*, *Pyridoxine* HCl, *Thiamine* HCl, and *Nicotinic acid*; as well as Na_2EDTA and $FeSO_4$ buffer solvent. Nutrition of coconut water is taken from young coconut fruit that still have soft flesh. Nutrition is "AB-Mix for leaf". As a media hardener is used "agar powder" materials; sugar as a carbon source; and HCl or NaOH as regulator of pH (5.8-6.2).

Phenol Test Materials

The total phenol analysis (Senter et al., 1989) used an agarose sample of planting medium with the age of 42 DAP; reagents *Follin Ciocalteu*; Na_2CO_3 solution; and aquadest.

Tools Sterilization Method

Sterilization tools such as culture bottles, dissection equipment consisting of tweezers, scalpels and petri dishes using autoclave at 121°C for 60 minutes at 17.5 psi pressure and then drying for 15 minutes.

Total Phenol Method

The analyzed material was weighed 1 gram and diluted to 100 ml. The result of dilution was taken 1 ml and added 5 ml of Na_2CO_3 alkalis 2% and left at room temperature for 10 min. Then plus 0.5 ml of the *Follin Ciocalteu* reagent is then shaken out and kept at room temperature in dark conditions for 30 minutes. After that the absorbance is set at $\lambda = 750$ nm. The total content of phenol was calculated based on the standard curve obtained from pure phenol solution (10-50 ppm).

$$\text{Total phenol (\%)} = \frac{\text{x.dilution factor}}{\text{mg sampel}} \text{ x } 100\%$$

Plant Vigor Method

Observations were made at 42 DAP by scoring method. The vigor method is performed by looking at the visually healthy look of the plantlet's visibility criteria. Vigor plants set with scores values include:

Score 1: long and many roots; uniform; wide leaves; long and large stems;

Score 2: long and little roots; uniform; leaves rather wide; medium bars;

Score 3: the roots are short and few; not uniform; small leaves; medium bars;

Score 4: has no roots, is not uniform; small leaves; stunted stem;

Score 5: does not grow or die.

Experimental Design

The experimental design was designed using *Completely Randomized Design* (CRD) with two treatment factors. The first factor is media consisting of 5 levels and the second factor is banana cultivars consisting of 4 levels. The first factor is P0 = MS media; P1 = medium ½ MS + 100 ml/L coconut water + 100 ppm AB-Mix; P2 = medium ½ MS + 100 ml/L coconut water + 200 ppm AB-Mix; P3 = medium ½ MS + 100 ml/L coconut water + 300 ppm AB-Mix; P4 = medium ½ MS + 100 ml/L coconut water + 400 ppm AB-Mix. The second factor is K1 = Mas Kirana (AA); K2 = Cavendish (AAB); K3 = Raja Bulu (ABB); K4 = Kepok (BBB) (Figure 1). Based on the two

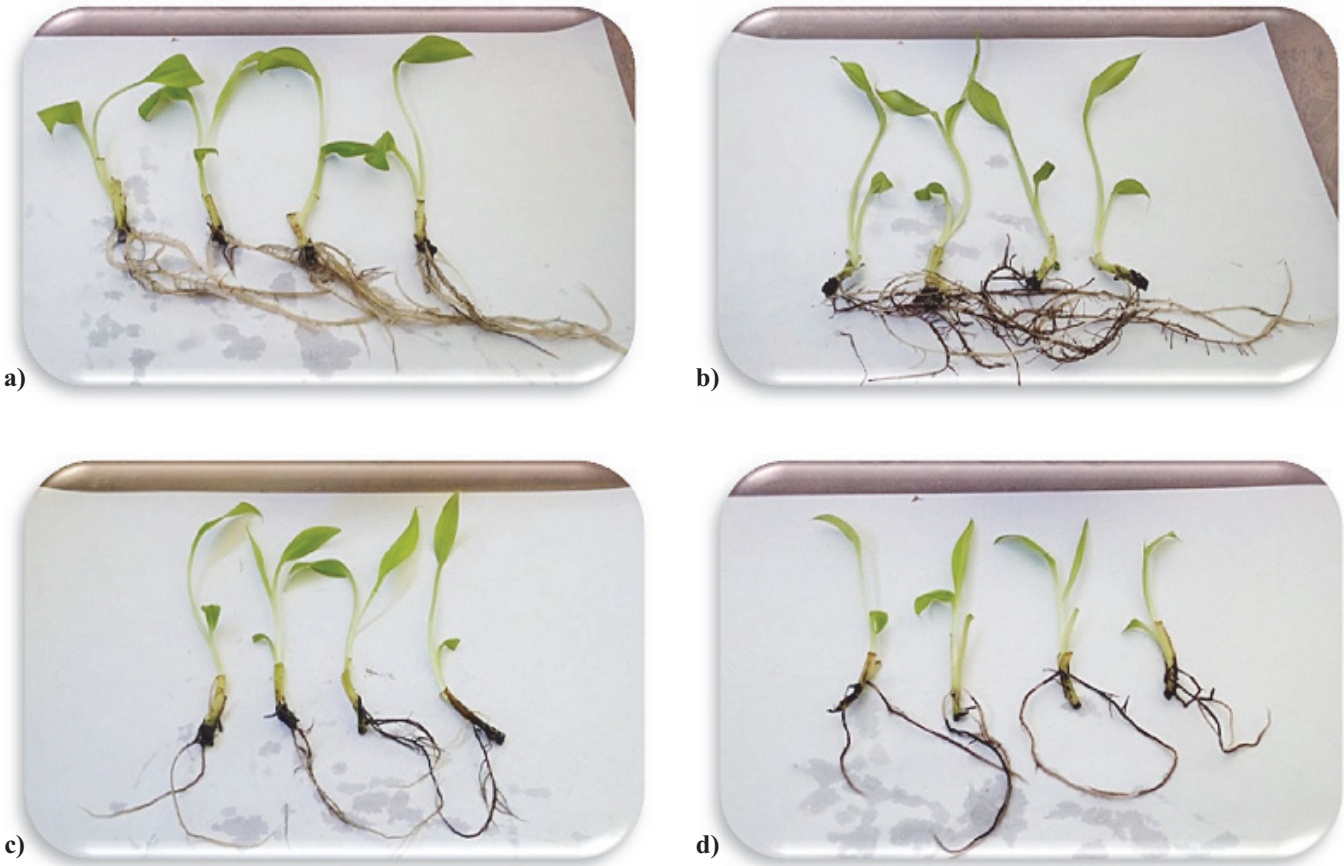

Fig. 1. Vigor plants of (a) Mas Kirana; (b) Cavendish; (c) Raja Bulu; (d) Kepok on MS medium

treatment factors, 20 treatment combinations were repeated three times so that there were 60 experimental units.

Statistic Analysis

The observed data were analyzed using SPSS version 16 (SPSS Inc., Chicago) SPSS analysis for "Windows" and if there was a real difference followed by Duncan's Multiple Range Test (DMRT) at 5% level.

Results and Discussions

The purpose of media modification is to see the effect of media on the growth of banana plantlet in the hope that media modification is good for plant growth. Wetherell (1982) states that for certain purposes the composition of the media can be further modified. Planting materials have similarities in planting age, media composition, culture conditions, and plant parts. It turned out that between the cultivars showed different results on each treatment.

The use of various banana cultivars aims to determine the response of each cultivar to the use of various media. If a cultivar shows a good response from a medium and other cultivars show the same response, then the medium is appropriate for the plant and is good for development. In addition, the use of a variety of banana cultivars to determine the total content of phenols and see the growth of plants based on differences in genomes.

Based on the multiple factor F test, the media treatment as the first factor significantly influences the root number, root length, and plant vigor variables. But no significant effect on shoot length. The cultivar treatment as the second factor had significant effect on root number, root length, shoot length and plant vigor.

Number of Roots

Root serves to absorb nutrients in the media, so the number and length of roots support the growth of plants. Latifah et al. (2017) explains that in tissue culture, the number of

roots indicates that the plantlet is healthy and able to absorb nutrients in the medium optimally. The number of roots signifies the reach of the plantlet in absorbing nutrients. The more the number of roots the wider the reach of the plantlet in absorbing nutrients.

Based on Table 1, the effect of media treatment significantly on the root number variables. MS medium gives the best result in forming roots with an average of 3.67 root pieces per plantlet. This is because MS medium has a complex nutrient elements, both macro and micro nutrients are complete and balanced for growth plantlet. In his research, Zulkarnain (2009) says that the MS medium has a higher salt content than other media, in addition to its high nitrate content.

The addition of coconut water aims to give the hormone auxin and cytokinin on ½ MS medium. The results of the analysis of young coconut water showed that plant growth regulator kinetin (cytokinin) was 273.62 mg/L and 290.47 mg/L zeatin. While the auxin content is 198.55 mg/L. In old coconut, the content of cytokinin and auxin is lower, 202.75 mg/L kinetin, 184.69 mg/L zeatin, and 97.60 mg/L auxin (Syahid et al., 2009). Auxin and cytokines in coconut water function in the formation of roots. In his research, Yong et al. (2009) explains that the cytokines contained in coconut water have the ability to promote cell division and tissue differentiation especially in shoot and root formation. Bey et al. (2005) adds that radicles will change shape into roots with the help of auxin processed by the leaves after the leaves are formed. Auxin processed at the top of the leaf will then be sent through the phloem to the root of the plant.

Table 1
Effect of media on the number of roots, root length, and plant vigor at 42 DAP

Media	Number of roots	Root length (cm)	Plant vigor
P0	3.67 c	7.70 b	1.50 a
P1	3.33 bc	5.00 a	1.75 ab
P2	2.92 abc	4.60 a	1.75 ab
P3	2.67 ab	4.27 a	2.17 bc
P4	2.17 a	3.15 a	2.42 c

The numbers followed by different letters show a real difference based on the Duncan's Multiple Range Test(DMRT) at the 5% level
P0: MS Media; P1: ½ MS + 100 ml/L coconut water + 100 ppm AB-Mix, P2: ½ MS + 100 ml/L coconut water + 200 ppm AB-Mix; P3: ½ MS + 100 ml/L coconut water + 300 ppm AB-Mix; P4: ½ MS + 100 ml/L coconut water + 400 ppm AB-Mix

In Table 2, the effect of cultivar treatment was very significant on the number of roots. Cultivar Kepok shows the number of roots that differ significantly with other cultivars. Kepok has the least number of roots with an average of 1.07 pieces per plantlet. This is like in research Radian (1992) which explains that until 8 weeks after the subculture of banana root Kepok and Candi did not grow. In addition, according to Su et al. (2011), the medium without the addition of cytokinins is better when compared with cytokinin-containing media for root formation, this is because cytokines can inhibit endogenous auxin biosynthesis in root formation. In this study, natural hormone from coconut water was the same for all banana cultivars. So based on the explanation is allegedly on cultivar Kepok have a low endogenous auxin than other bananas.

Root Length

The longer the roots the more optimal absorption of nutrients. Based on Table 1 it is known that the media treatment has an effect on the root length variable. MS media showed very different results than other media. MS medium is able to form a root with an average length of 7.7 cm. Suspected use of AB-Mix nutrients result in inhibition of plant cell division. This is because the higher the concentration of AB-Mix then the nutritional solution is more concentrated and can not be absorbed properly by plant roots. Wijayanti and Widodo (2005) explain that concentrated solutions can not be absorbed by the roots as a maximum due to the cell osmotic pressure being smaller than the osmotic pressure outside the cell, so there is likely to be a return flow of liquid plant cells (plasmolysis).

Based on Table 2, Mas Kirana cultivars have the highest root length of 10.43 cm and are very different from Cavendish, Raja Bulu and Kepok. In the cultivar of Raja Bulu and Kepok have a low root length and not significantly different,

Table 2
Effect of cultivars on the number of roots, root length, shoot length, and plant vigor at 42 DAP

Banana cultivars	Number of roots	Root length (cm)	Shoot length (cm)	Plant vigor
K1	4.60 d	10.43 c	14.53 d	1.00 a
K2	3.73 c	5.460b	12.54 c	1.40 a
K3	2.40 b	2.710 a	9.753 b	2.20 b
K4	1.07 a	1.180 a	8.013 a	3.07 c

The numbers followed by different letters show a real difference based on the Duncan's Multiple Range Test (DMRT) at the 5% level
K1: Mas Kirana (AA), K2: Cavendish (AAB); K3: Raja Bulu (ABB); K4: Kepok (BBB)

respectively 2.71 cm and 1.18 cm. This is like the study of Avivi et al. (2013) which explains that the average length of the roots of bananas Raja Nangka and Kepok not significantly different that is 2.2 cm. Kepok banana has a low root length suspected due to low endogenous hormone. According to Su et al. (2011), the medium without the addition of cytokinins is better when compared to cytokinin-containing medium for root formation, this is because cytokines can inhibit endogenous auxin biosynthesis in forming roots.

Banana Raja Bulu and Kepok have a low root length allegedly related to the "B" genome on the banana. In his research, Resmi and Nair (2011) reported that bananas with the AAA genome had a higher shoot multiplication factor than those with AAB, ABB, BB, and BBB. This is because of the high phenol compound in bananas with the "B" genome. Bananas with genome "B" tend to be difficult to initiate and inhibit shoot regeneration. The phenol compound is formed as a result of the opening when the planting process. The phenol may inhibit the absorption of nutrients from the media. Sprout growth is positively correlated with root growth. So if the shoots grow well, then the roots also grow well. According to Pierik (1987) when the growth of roots is also influenced by the growth of shoots, shoots grow well spur root growth, if the growth of shoots inhibited the growth of the roots were inhibited.

Shoot Length

Cultivar treatment showed significant effect on shoot length variables. Based on Table 2, the shoot length of Mas Kirana showed the highest yield with an average of 14.53 cm and the Cavendish banana had a lower shoot length with an average of 12.54 cm.This is not in accordance with the research of Avivi et al. (2013) which states that the length of the banana shoots of Raja Nangka has the highest length with an average shoot length of 13.37 cm and the lowest is the Mas Kirana with an average length of 6.88 cm. It is suspected because of differences in endogenous hormones in stimulating shoot growth.

The addition of plant growth regulator is able to manipulate plant growth to improve. The absence of plant growth regulator in the media aims to determine actual growth ability, so that the role of endogenous hormone will be known. According to Weaver (1972), plant growth regulator plays a very important role in plant cells and commonly used in tissue culture is derived from cytokines and auxins. Naturally the plant has an endogenous auxin called IAA (*Indole Acetic Acid*) produced in shoot meristems in canopy and root shoot meristem (Salisbury and Ross, 1992).

Shoot extension will increase if the number of shoots grows less. In his research, Ramesh and Ramassamy (2014) stated that plant height is thought to be influenced by the number of shoots that appear, so that fewer shoots appear, the higher the plant, and on the contrary, this is because the energy required for shoot extension is used for the formation of young other shoots, so high shoods may be inhibited. This is in accordance with the research undertaken, where shoots do not occur multiplication. No multiplication due to the absence of plant growth regulator in the media used. Plant growth regulator is available only from coconut water and endogenous hormones from the plantlet.

Vigor Plant

Plant vigor is a heftiness plants visually. Based on Table 1, the treatment media of MS; ½ MS + 100 ml/L coconut water + 100 ppm AB-Mix; and ½ MS + 100 ml/L coconut water + 200 ppm AB-Mix based on the analysis of variance showed no significant different results. All three media have vigor with Score 1. In medium ½ MS + 100 ml/L coconut water + 300 ppm AB-Mix and ½ MS + 100 ml/L coconut water + 400 ppm AB-Mix have vigor with Score 2. Based on analysis of variance, MS media is very different with ½ MS + 100 ml/L coconut water + 300 ppm AB-Mix and ½ MS + 100 ml/L coconut water + 400 ppm AB-Mix. Suspected AB-Mix nutrition with a composition of 300 ppm and 400 ppm is too thick so it is difficult to be absorbed by plant roots. Root is a plant organ that is very important because it plays a role in the absorption of nutrients from planting media for growth of plants (Prasetyo, 2005).

In Table 2, cultivars Mas Kirana (AA) and Cavendish (AAB) have the best vigor with Score 1. Raja Bulu (ABB) and Kepok (BBB) sequentially have vigor with Score 2 and 3. Based on the table, bananas with genome "B" are more and more the lower the quality of plant vigor.This occurs because of browning on the banana plantlet with the genome "B" as a result of phenol compounds that close the wound from the incision caused when the subculture is done. According Purwanto (1991) the existence of a number of genom "B" affect the level of *phenol* content and *polyphenoloksidase* activity, the more the number of genom "B" the higher the activity of *polyphenoloksidase* enzyme.

Cultivars Raja Bulu has a less than optimal vigor like Mas Kirana and Cavendish. In his research, Kasutjianingati et al. (2011) explains that Raja Bulu is not optimal in yielding plantlet with perfect vigor (with perfect root, stem and leaves) and entering the criteria of big shoot (>3 cm) to be successfully acclimatized to produce ready-to-plant seedlings. Vigor effect on the success during acclimatization. The use of less vigor culture seeds causes many plants to die (Pardal et al., 2005). This is because plants with good vigor have complete organs such

as roots, stems and leaves, so in this study the plant was successfully done 100% acclimatization.In his research, Slamet et al. (2005) explained that the quantitative vigor of soybean culture seeds successfully acclimatized is the height of seed 5-6 cm, the number of shoots 2-3 pieces, and the number of roots 2-4 pieces.

Conclusions

Based on the results of research can be concluded that AB-Mix nutrition has a poor influence as a nutritional enhancer for tissue culture medium. The higher the given nutrients show a poor effect on plant growth.The more concentrated the AB-Mix solution the more difficult it is to be absorbed by plant roots.Banana cultivars with genome "A" show better results than genome "B" in various variables. The more "B" genomes in banana cultivars affect the decreasing quality of vigor. Banana cultivars with genome B have high phenol content.

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