EFFECT OF ASCORBIC ACID, ACTIVATED CARBON AND LIGHT DURATION ON EXPLANT BROWNING OF BANANA CULTIVAR BARANGAN (*Musa acuminata* L.) IN VITRO CULTURE

Kusuma Kariyana & Nisyawati
Department of Biology, Faculty of Mathematic and Natural Science,
Universitas Indonesia, Depok 16424, Indonesia

ABSTRACT

The research was conducted to determine the effect of ascorbic acid (50 mgl^{-1}, 100 mgl^{-1}, 200 mgl^{-1}) and activated charcoal (0.5 gl^{-1}, 1 gl^{-1}, 2 gl^{-1}) independently with different light duration (darkness for 4 weeks, 16 hours light for 4 weeks and 2 weeks in darkness followed by 2 weeks in16 hours light) on explant browning. Explants of banana cultivar Barangan (*Musa acuminata* L.) were planted on MS basal media supplemented with 1.6 mgl^{-1} IAA, 4.0 mgl^{-1} BAP and cultured for 4 weeks. At the end of the experiment, degree of explant browning and height of explant were evaluated. Ascorbic acid (50 mgl^{-1}, 100 mgl^{-1}, 200 mgl^{-1}) as well as activated charcoal (0.5 gl^{-1}, 1 gl^{-1}, 2 gl^{-1}) could be used in reducing explant browning but the most suitable media is activated charcoal in concentration of 2 gl^{-1} cultured in darkness for 4 weeks. Height of explant on media with ascorbic acid as well as activated charcoal with all light duration was not influenced by explant browning except in browning level 4.

Keywords: *light duration; phenolic compounds; quinines.*

1. INTRODUCTION

Banana is one of the most important fruit crops grown all over the world. In Indonesia, Barangan (*Musa acuminata* L.) is a high yielding commercial cultivar. Presently, a great demand of seedlings is needed to fulfill the banana plantation. One alternative to assure that the demand is met would be a large scale of production through in vitro techniques. Banana are propagated vegetatively using corms and suckers. In vitro propagation of banana provides advantages over traditional propagation such as high multiplication rate, uniformity of shoots, the available of disease-free material all year round and short harvest interval in comparison with conventional plants[1].

Mass micropropagation of banana cultivar Barangan has a problem of high mortality due to lethal browning of explant which leads to the death of explants. When explants were cut, the wounded tissues release phenolic compounds which result in enzymatic oxidation by polyphenol oxidase in the presence of oxygen[2] . Banana tissues are known to contain large amount of phenolic compounds[3]. To reduce the lethal browning of banana explant, ascorbic acid and activated charcoal is added to culture media independently. Ascorbic acid is an antioxidant used to control oxidation of phenols and has been added to culture media to reduce the lethal browning assoiated with poor growth[4]. Besides its role as an antioxidant, ascorbic acid is involved in cell division and elongation[5]. Activated charcoal is an essential component of plant tissue culture media. It prevents browning of cultured tissues and media by adsorption of toxic compounds such as polyphenols released by wounded tissues[6].

Effect of light duration on explant browning is also evaluated. Light is considered to be one of the most important due to the decisive influence on plant development, but it is known that phenolic oxidation products are formed under illumination[4]. Obtaining optimal techniques in reducing oxidative browning of banana in vitro requires a research into light duration during incubation period in conjunction with the use of an antioxidant or adsorbent in media. The main objective of this research was to investigate the effect of ascorbic acid and activated charcoal in culture media independently and light duration on explant browning on banana cultivar Barangan (*Musa acuminata* L.) in vitro culture.

2. MATERIALS AND METHODS

2.1. Sterilization procedure

Decapitated suckers of banana cultivar Barangan (10 x 10 x 15 mm^3) were surface sterilized as follow: explants were sterile aquadest plus 3 drops of Tween for 5 minutes. Then rinsed by sterile aquadest 3 times each for 3 minutes. Use fungicide (0.1 g/l) plus bactericide (0.1 g/l) for 10 minutes, then rinse explants by sterile aquadest 3 times each for 3 minutes. Explants were sterilized using alcohol 70% for 1 minutes then rinsed by sterile aquadest. Finally explants were sterilized by 20% NaClO solution for 15 minutes then rinsed 3 times by sterile aquadest 3 times each for 5 minutes. Explants were then planted on initiation media.

2.2. Initiation media

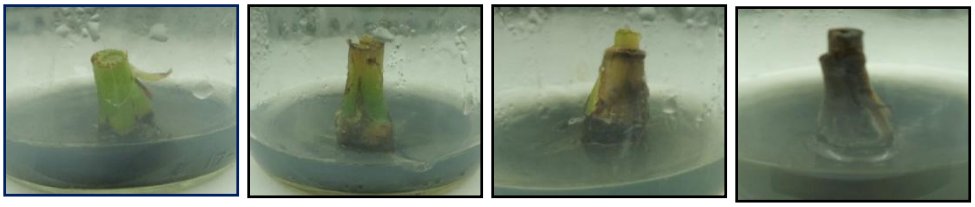
Surface sterilized explants were planted on Murashige and Skoog (1962) basal media supplemented with growth regulator hormone BAP 4 mgl^{-1} and IAA 1.6 mgl^{-1}. Ascorbic acid and activated charcoal were added into media independently. The concentration of ascorbic acid was 50 mgl^{-1} (A-1), 100 mgl^{-1} (A-2), 200 mgl^{-1} (A-3) and activated charcoal was 0.5 gl^{-1} (K-1), 1 gl^{-1} (K-2), 2 gl^{-1} (K-3). pH media was 5.9-6.1.

3. CULTURE CONDITION

Explants were incubated at:

1). Darkness for 4 weeks (D).
2). Light 16 hours for 4 weeks (L).
3). Darkness for 2 weeks followed by light 16 hours for 2 weeks (DL).

All culture conditions have room temperature 27± 2^{o} C and lighting using Phillips lamps of 20 watt which were placed on 20 cm above the bottles. Total sample of each treatment was twenty. After 4 weeks explants were incubated at different condition of light duration, explant browning was evaluated using degree of explant browning (Figure 1).



(a) (b) (c) (d)

Figure 1. Degree of explant browning: (a) level 1 = no browning; (b) level 2 = a bit browning; (c) level 3= much browning; (d) level 4 = browning at all explant surface

3. RESULT AND DISCUSSION

3.1. Effect of ascorbic acid and light duration on degree of explant browning

Explants on ascorbic acid free media (A-0) have higher degree of explant browning than those on media with addition of ascorbic acid 50 mgl^{-1} (A-1), 100 mgl^{-1} (A-2), and 200 mgl^{-1} (A-3). According to[7] addition of ascorbic acid in media could reduce quinones that form caused by oxidation reaction of phenolic compounds by PPO enzyme. Ascorbic acid will react with quinones and turned it back to phenolic compounds.

The result showed increasing of ascorbic acid concentration did not reduce degree of explant browning (Figure 2). Ascorbic acid is known to decay rapidly in plant tissue culture media[8]. It is oxidized by reactions catalysed by Cu (II) and Fe (III), both of which are component of Murashige and Skoog media[8]. Adding ascorbic acid onto the surface of the media might be useful for preventing the decay. Light and pH affect the stability of ascorbic acid[8]. In darkness, ascorbic acid might be preserve. Ascorbic acid was most stable at pH 4.5[8]. In this research, culture media used at pH 5.9-6.1.

Phenolic compounds are most commonly distributed in plant tissues. No tissues lacks of phenolic compounds and high concentrations can be found in actively growing cells[9]. Tissues containing relatively high concentrations of phenolic compounds are difficult to culture since when phenol becomes oxidized, they form compound called quinones which are toxic to plant tissue and caused explants browning[10].

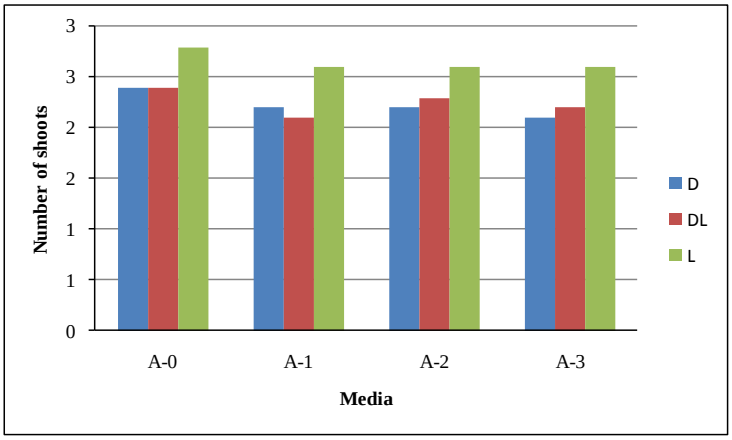


Figure 2. Effect of ascorbic acid and light duration on explant browning

The effectiveness of ascorbic acid in reducing lethal browning is depend on the light duration. In light 16 hours condition for 4 weeks (L) degree of explant browning is higher than in dark condition for 4 weeks (D) and in dark for 2 weeks followed by light 16 hours for 2 weeks (DL). Generally, there is a rise in total phenolics content in plants grown in the sunny situations relative to the shady ones[11].

3.2. Effect of light duration and degree of explant browning on height of explant on media with addition of ascorbic acid

Height of explant on ascorbic acid free media and media with addition of ascorbic acid at all light duration was not influenced by explant browning except in browning level 4 (Figure 3). Degree of explants browning 1, 2 and 3 gave average explant height varied between 5.7 and 7.1 cm. Explants with browning level 4 exhibited severe browning. They showed only a little growth. Explant growth showed by an increasing of explant height 0.1-1.6 cm. When explant browning occurred, the explant should be immediately transferred to fresh media every 14 days until browning gradually diminished[12]. Without those treatment, explant would become necrotic and die.

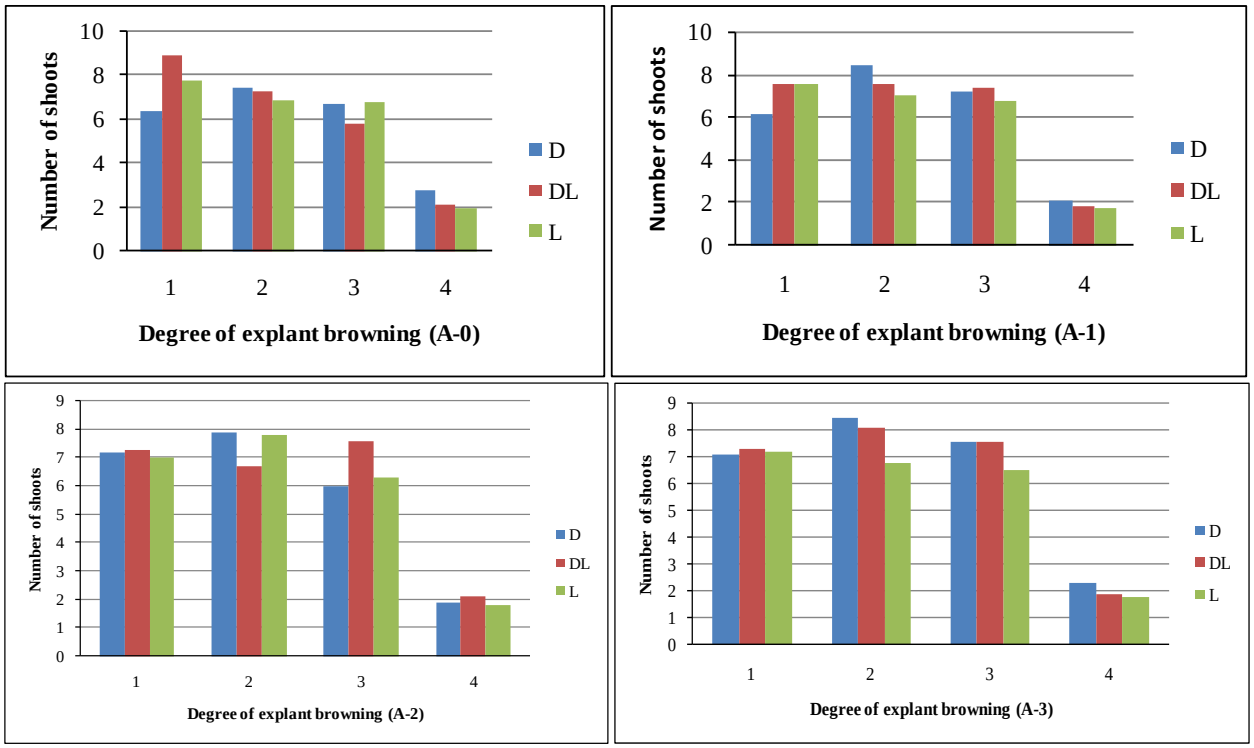


Figure 3. Effect of light duration and degree of explant browning on height of explant on media with addition of ascorbic acid

3.3. Effect of activated charcoal and light duration on degree of explant browning

Activated charcoal free media caused higher degree of explant browing than media with addition of activated charcoal in concentration of 0.5 gl^{-1} (C-1), 1 g/l^{-1} (C-2) and 2 gl^{-1} (C-3) (Figure 4). This finding confirms those of [13] with faba bean that addition of activated charcoal to media greatly reduces the lethal browning caused by the release of phenolic compound. Addition of activated charcoal to culture media was necessary to counteract browing for the initial survival of the explant. Activated charcoal in concentration of 0.5 gl^{-1} and 1 gl^{-1} was insufficient to counteract browning effects. Media with activated charcoal 2 gl^{-1} was found to be an optimal concentration to reduce explant browning.

The highest degree of explant browning is on activated charcoal free media (C-0) in light 16 hours for 4 weeks (L). Because there is a rise in total phenolics content in light conditions relative to the dark ones[11] and no phenolic adsorption resulted in highest degree of explant browning.

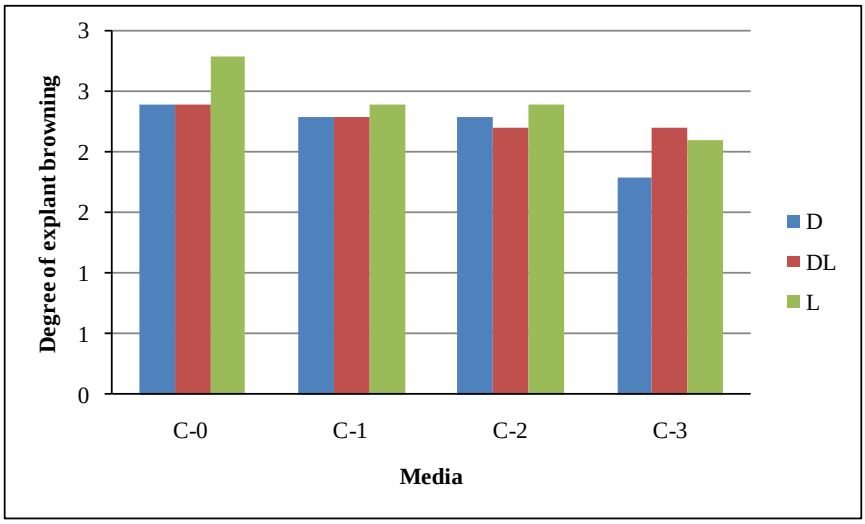


Figure 4. Effect of activated charcoal and light duration on degree of explant browning

3.4. Effect of light duration and degree of explant browning on height of explant in media with addition of activated charcoal.

Explants with browning level 4 exhibited severe browning. According to[14] in addition to suppressing phenolics and thus browning, adding activated charcoal to the culture media enhanced the elongation of explant. This supports our findings. Degree of explants browning 1, 2 and 3 gave average explant height varied between 5.7 and 6.6 cm. Explant with degree of browning level 4 only showed a little growth. Explant growth showed by an increasing of explant height 0.1-0.9 cm.

Phenolics perform a broad range of physiological roles in plant, which may be growth inhibitory and promotory[11]. Upon being oxidized, phenolic compounds turned into highly toxic quinones and polymerized causing discoloration od the media and death of the explants[9]. At the same time, they play a role in enhancing plant growth and development[11]. This phenomenon clearly explained the height of explant was not influlenced by degree of explant browning except in level 4 (Figure 5).

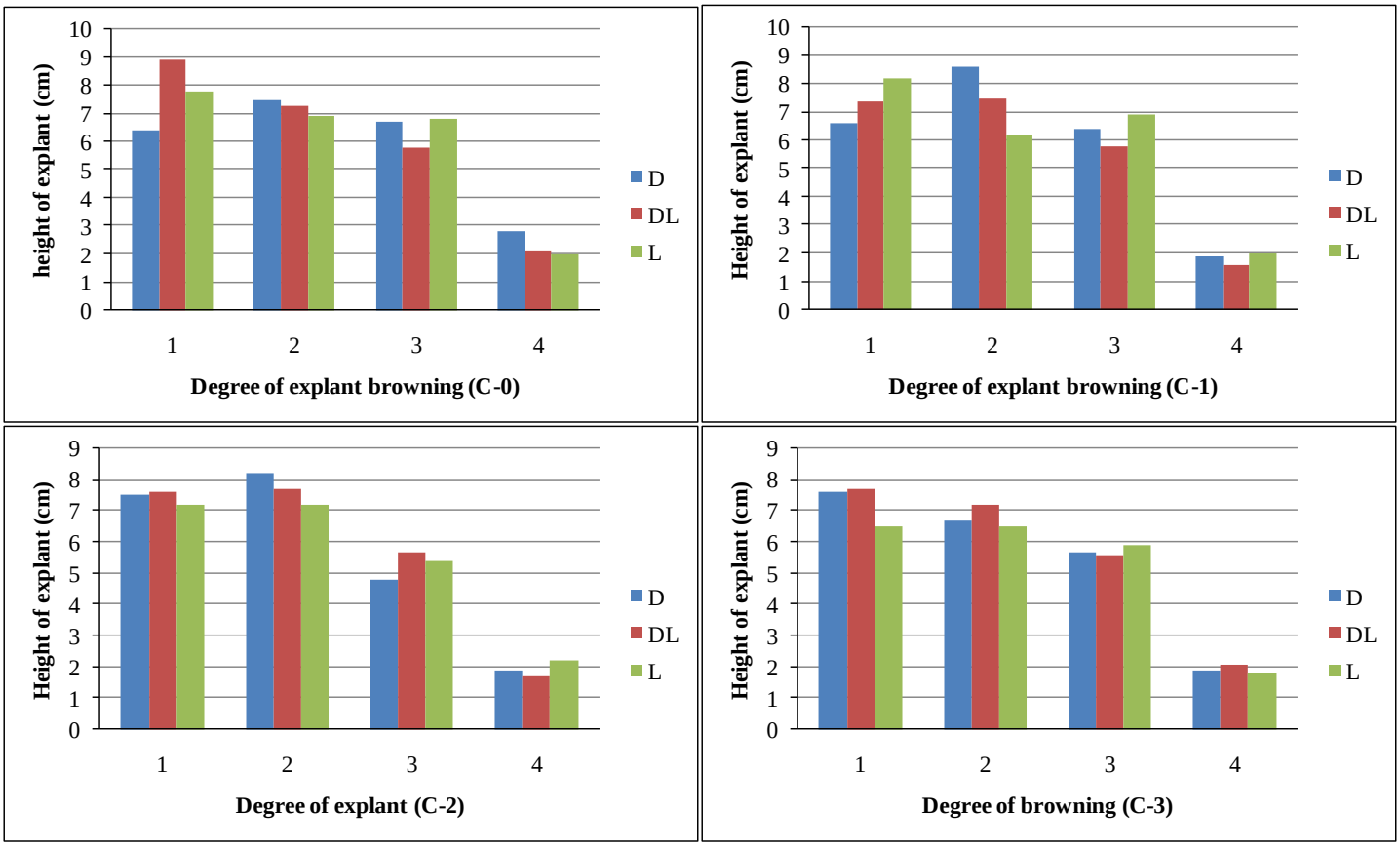


Figure 5. Effect of light duration and degree of explant browning on height of explant on media with addition of activated charcoal

4. CONCLUSION

Addition of antioxidant or adsorbent in culture media can be used in reducing explants browning. Its concentration as well as culture condition is important. Media with ascorbic acid in concentration of 50-200 mgl^{-1} was not sufficient enough in reducing lethal browning especially in light condition. The most suitable media is activated charcoal in concentration of 2 gl^{-1} incubated in darkness for 4 weeks. Incubation of cultures in darkness is effective in reducing browning.

Growth of explant on media without and with addition of ascorbic acid as well as activated charcoal with all light duration was not influenced by degree of explant browning except in browning level 4. Explants with browning level 4 exhibited severe browning.

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