EFFECT OF ASCORBIC ACID, ACTIVATED CHARCOAL AND LIGHT DURATION ON SHOOT REGENERATION OF BANANA CULTIVAR BARANGAN (*Musa acuminata* L.) IN VITRO CULTURE

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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 in 16 hours light) on shoot regeneration. 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. After 4 weeks, degree of explant browning was evaluated. Explants were then cut vertically into two pieces and planted on shoot regeneration media. After 4 weeks in shoot regeneration media, number of shoots were evaluated. MS media supplemented with 1.6 mgl^{-1} IAA and 4.0 mgl^{-1} BAP without ascorbic acid and activated charcoal in darkness for 4 weeks was the most suitable media for shoot regeneration. The shoot regeneration gave average of 10,4 shoots per explant.

Keywords: *explants browning; light duration; phenolic compounds*.

1. INTRODUCTION

Problem often encountered in mass micropropagation of banana cultivar Barangan is the explant browning. Explant browning caused by oxidation of phenolic compounds resulting from injuries the isolation of explant[1]. It leads to the death of explant and failure of shoot regeneration[2]. This is due to quinones produced by oxidation of phenolic compounds[1] are toxic[3] and it diffuses into culture media causing tissue necrosis and death of explant[4].

Phenolic browning of explant, media composition and culture conditions greatly affect shoot regeneration[5]. The concentration and combination of auxin and cytokininin in culture media is a key factor which determines successful shoot regeneration[1]. Besides growth hormone, other compounds such as ascorbic acid and activated carbon can be added to culture media. Both compounds are able to reduce the oxidation of phenolic compounds that can prevent death due to explant browning and increase shoot regeneration[6]. Ascorbic acid is an antioxidant that are able to prevent or inhibit oxidation process[7]. Besides its role as an antioxidant, ascorbic acid is involved in cell division and elongation[8]. Research by[9] on culture of banana cultivar Cavendish showed that ascorbic acid not only prevent death due to explant browning, but also can increase the number of shoots growing on explant. Activated charcoal is an essential component of plant tissue culture media. It is a strong adsorbent that can adsorb toxic subtances[10].

Effect of light duration on regeneration shoots is also evaluated. According to[11] light plays an important role in the regeneration shoots. 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 shoot regeneration on banana 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 0.5 gl^{-1} (K-1), 1 gl^{-1} (K-2), 2 gl^{-1} (K-3). pH media was 5.9-6.1.

2.3. 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 condition have room temperature 27± 2^{o} C and lighting using Phillips lamps of 20 watt which were placed in 20 cm above bottles. Total sample of each treatment was twenty.

After 4 weeks on initiation media, explant browning was evaluated using degree of explants browning (Figure 1). Then explants were cut vertically into two pieces and planted on shoot regeneration media which its concentration is same as initiation media. Cutted explants were incubated at the same condition as mentioned (c).

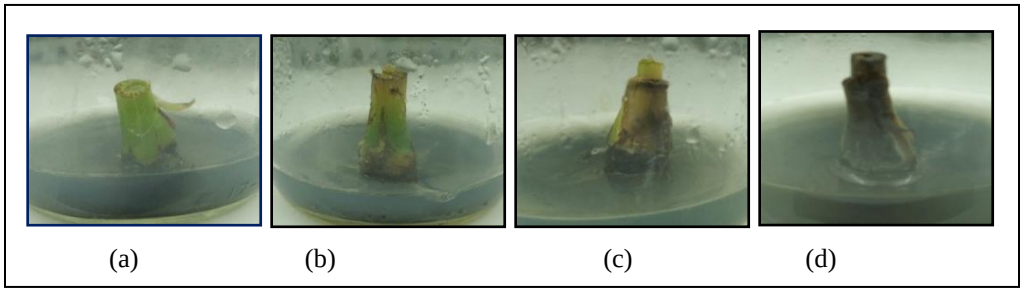


Figure 1. Degree of explant browning: (a) level 1 = no browning; (b) level 2 = a bit browning; (c) level 3 = much browning; (d) level 4 = browning at all explant surface

3. RESULT AND DISCUSSION

3.1. Effect of ascorbic acid and light duration on regeneration shoot

Explants on ascorbic acid free media (A-0) produced a higher number of shoots than those on media with addition of ascorbic acid in concentration of 50 mgl^{-1} (A-1), 100 mgl^{-1} (A-2) and 200 mgl^{-1} (A-3) (Figure 2). Ascorbic acid is known to decay rapidly in plant tissue culture media. Ascorbic acid is oxidized by reactions catalysed by Cu (II) and Fe (III), both of which are component of Murashige and Skoog media[12] . Light and pH accelerated the decay[12]. In darkness, ascorbic acid might be preserve. Ascorbic acid was most stable at pH 4.5[12]. In this research, media culture used at pH 5.9-6.1. Since ascorbic acid in an ephemeral component of culture media, it is quite possible that none exist when explant is planted.
The result showed explants of banana cultivar Barangan produced higher number of shoots at dark condition for 4 weeks (D) than at 16 hours light for 4 weeks (L). Even at dark for 2 weeks followed by light 16 hours for 2 weeks (DL) number of shoots produced higher than at 16 hours light for 4 weeks (L). Similar findings were also reported by[11] in banana cultivars Gros Michel, Bwara and Sukalindizi. Dark conditions enhanced higher number of shoots than light conditions suggesting that banana in vitro culture is a photomorphogenically process. Although light may be essential for plant development, darkness is also beneficial for plant morphogenesis.

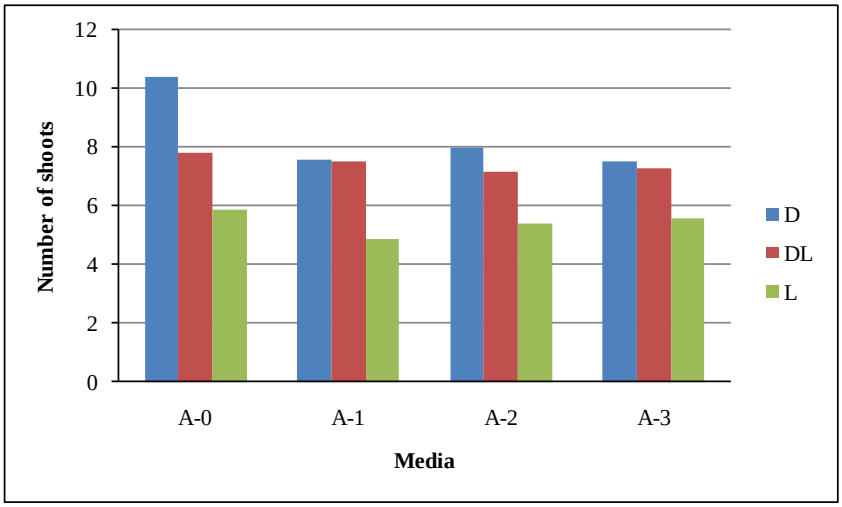


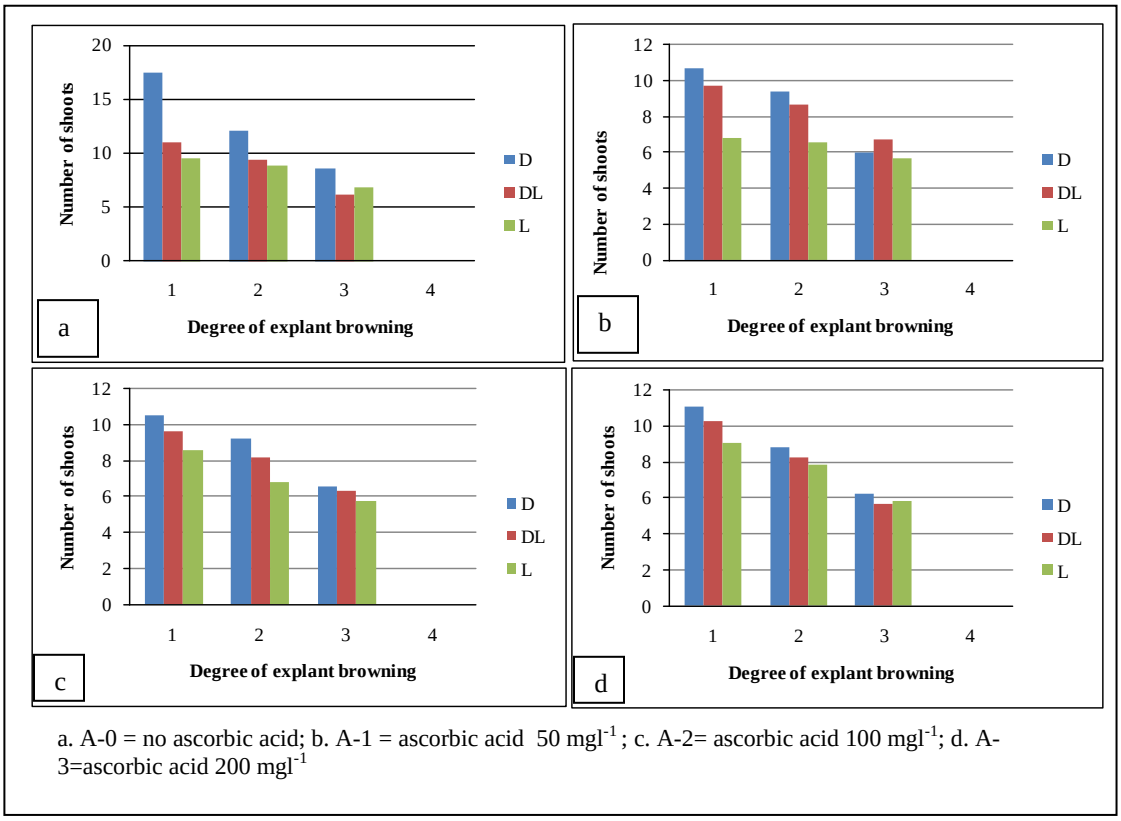
Figure 2. Effect of ascorbic acid and light duration on number of shoots

According to[9] , addition of ascorbic acid to the surface of culture media not only prevented the development of lethal browning but also greatly increased the number of shoot produced. The result showed increasing of ascorbic acid concentration in media did not increase the number of shoot produced (Figure 2). The effectiveness of ascorbic acid to induce regeneration shoots suggests that adding ascorbic acid onto the surface of the media should be tried.

3.2. Effect of light duration and degree of explant browning on number of shoots produced by explant on media with addition of ascorbic acid

Explants with degree of browning level 1, 2 and 3 on ascorbic acid free media and media with addition of ascorbic acid produced shoots. No shoots produced by explant with degree of explant browning level 4 (Figure 3). The explant became necrotic and die. Number of shoots produced depends on degree of explant browning. The higher degree of explant browning, the lesser number of shoots produced.

At all degree of explant browning, increasing of ascorbic acid concentration could not induced more shoots. In ascorbic acid free media, more shoots produced at all degree of explant browning, especially in darkness for 4 weeks (D). It showed that darkness condition is beneficial for phenolics contents of explant incorporated with the use of an unstable ascorbic acid.



a. A-0 = no ascorbic acid; b. A-1 = ascorbic acid 50 mgl^{-1} ; c. A-2= ascorbic acid 100 mgl^{-1}; d. A-3=ascorbic acid 200 mgl^{-1}

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

3. EFFECT OF ACTIVATED CHARCOAL AND LIGHT DURATION ON REGENERATION SHOOT

The result showed that explants on activated charcoal-free media (K-0) produced a higher number of shoots than those on media with addition of activated charcoal in concentration of 0.5 gl^{-1} (K-1), 1 gl^{-1} (K-2) and 2 gl^{-1} (K-3) (Figure 4). Activated charcoal is a strong adsorbent[10]. It adsorbs not only toxic subtances, but also nutrients in media. Use of activated charcoal as an adsorbent was not an appropriate option. The development of a activated charcoal-free media is an alternative.

Figure 4 showed that explant on media with addition of activated charcoal in concentration of 2 gl^{-1} (K-3) produced more shoots than activated charcoal in concentration 0.5 gl^{-1} and 1 gl^{-1}. Similar observations have also been reported by[5] with *Aloe vera* L. Activated charcoal stimulate growth and differentiation during culture

regeneration[5]. However its addition to regeneration media may have adverse effects on growth and development as activated charcoal is able to adsorb high concentration of growth regulators[13]. The non-selective adsorption of growth regulators may result in inhibitory effects of growth in vitro[14]. It will reduce their effectivess in tissue culture.

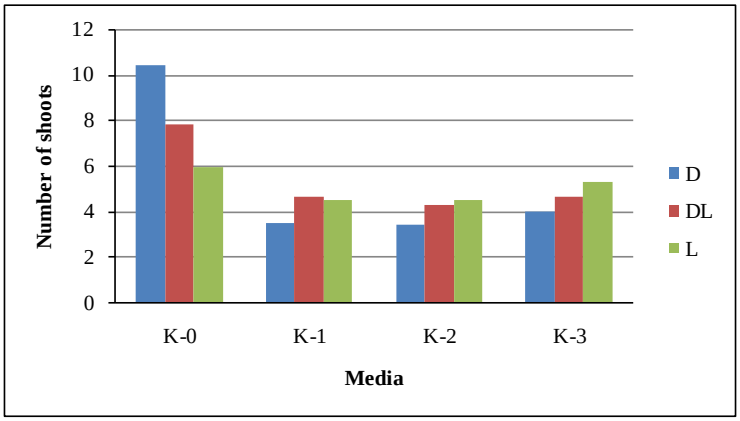


Figure 4. Effect of activated charcoal and light duration on number of shoots

4. Effect of light duration and degree of explant browning on number of shoots produced by explant on addition of activated charcoal media

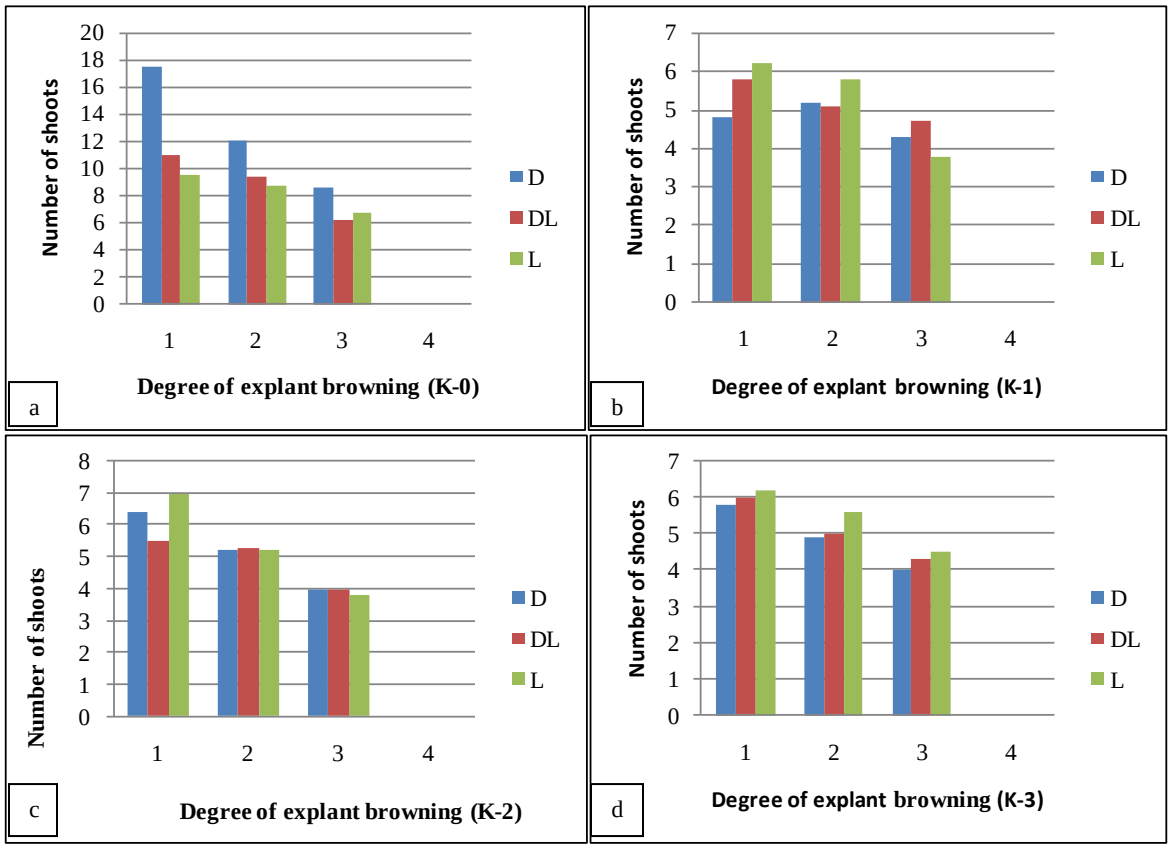


Figure 5. Effect of light duration and degree of explant browning on number of shoots produced by explant on addition of activated charcoal

Explants with degree of browning level 1, 2 and 3 on media with addition of activated charcoal produced shoots. Even on free activated charcoal media. No shoots produced by explant with degree of browning level 4 (Figure 5).

The explant became necrotic and die. Number of shoots produced depends on degree of explant browning. The higher degree of explant browning, the lesser number of shoots produced.
In producing number of shoot, there is a big difference between explant on activated charcoal free media and media with addition of activated charcoal. In all degree of explant browning, increasing of activated charcoal concentration could not induced more shoots. Combination of BAP and IAA was crucial for shoot regeneration. It showed that degree of explant browning incorporated with adsorption properties of activated charcoal result in variations in plant growth regulator levels in media.

4. CONCLUSION

The research described an efficient protocol for shoot regeneration of banana cultivar Barangan in vitro culture. Higher number of shoot produced on media without addition of ascorbic acid and activated charcoal. Dark conditions enhanced higher regeneration shoot than light condition. The optimal collaboration of a dark incubation period together with growth regulator hormone increased regeneration shoot. Although light may be essential for plant development, darkness is also beneficial for plant morphogenesis, mainly at its initial stage of development in vitro. Degree of explant browning greatly affects shoot regeneration. The higher degree of explant browning the lesser number of shoots produced.

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