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In Vitro Rooting and Acclimatization of Plantlets of Banana (Musa paradisiaca Linn) 'Ambon Kuning'

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Abstract. Ambon Kuning is a popular cultivar of banana in Indonesia because of its good taste and flavor. One way to increase its production is by providing high quality planting materials by use of tissue culture techniques. This study aimed to investigate the effects of plant growth regulators and activated charcoal on in vitro rooting of banana shoots and the effects of media and fertilization on the survival of plantlets of banana 'Ambon Kuning' during acclimatization. In the first experiment microshoots were aseptically cultured in six treatment media, namely MS 0, MS+2 g/l activated charcoal, MS+1 mg/l NAA, MS+2 mg/l NAA, MS+1 mg/l IBA, and MS+2 mg /l IBA for six weeks. The experiment was carried out in a completely randomized design with three replications. Each replication consisted of three bottles, one microshoots per bottle. In the second experiment, plantlets were subjected to factorial treatment. The first factor is three types of mixed media, namely M1 (husk charcoal: compost: sand: cocopeat), M2 (husk charcoal: compost: sand) and M3 (husk charcoal: compost). The second factor was fertilization, namely P0 (without fertilization) and P1 (with fertilization). The experiment was conducted in a completely randomized design with three replications, eight pots per replication, one plantlet per pot. All of the data were subjected to analysis of variance and the mean separation was done using the least significant difference test at 5% level. The first experiment showed that all of the microshoots produced roots. The addition of IBA had no effects on rooting, while the addition of NAA significantly induce rooting. In the second experiment, all the treatments resulted in 100% survival rate. The best plantlet growth was acchieved by M1 (husk charcoal: compost: sand: cocopeat) media. Fertilization led to better growth of plantlets. Results of this research confirmed the previous results that generally auxins induced rooting and that fertilization promoted growth of plants.

INTRODUCTION

Banana (Musa paradisiaca Linn.) is one of the tropical plants that grow well in Indonesia. This plants are very popular to Indonesian people because its fruits taste good and rich in nutrients. Ambon Kuning is one of the popular cultivars in Indonesia. Unfortunately, this cultivar has been mostly cultivated by use of planting materials derived from suckers, which is difficult to be obtained in a large number and uniform in size. Plant tissue culture could be an alternative solution to the problem. By tissue culture techniques, one could clonally propagate large quantities of uniform planting materials in a relatively short time.

Propagation by tissue culture is carried out through four stages, namely: stage 0 (selection and handling of parent plants as explants), stage 1 (culture establishment), stage 2 (shoot multiplication), stage 3 (shoot elongation and rooting) and stage 4 (plantlet acclimatization) [1]. In stage 3, to induce roots, the type of auxin in optimal concentrations is needed [2]. [3] stated that IBA, NAA, or IAA were often added to rooting media at a concentration of 1-5 mg/l to initiate root. [4] Santos (2003) stated that auxin NAA and IBA were commonly used for root induction in vitro. The addition of activated charcoal to the media can also improve rooting .of shoots. [5] reported that the addition of 2 g/l of activated charcoal to rooting media could increase the number and length of roots.

Planting media greatly affects the success of plantlet acclimatization. Planting media used for acclimatization could be a mixture of several components such as compost, sand, cocopeat, husk charcoal, perlite, vermiculite, and

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so on as long as they show properties such as loose, having good aeration and drainage, having no soil-borne pathogen and no weed seeds, and containing sufficient nutrients [3]. The use of rice husk charcoal: compost (1:1) media resulted in the highest number of secondary roots and the longest and heviest roots after plantlet acclimatization in tissue culture of Sansevieria trifasciata [1]. [6] showed that the best acclimatization medium for Ambon Kuning banana plantlets was a mixture of sand: compost (1:1). However, the use of other good materials such as husk charcoal and cocopeat as media components for acclimatization of banana plantlets 'Ambon Kuning' has not been reported yet.

This research aimed to inversitagate the effects of IBA, NAA, and activated charcoal on in vitro rooting of banana shoots and the effects of growing media and fertilization on the survival of banana plantlets 'Ambon Kuning' during acclimatization.

MATERIAL AND METHODS

This study was done from July 2018 to February 2019 at the Plant Science Laboratory, University of Lampung, Indonesia. The plant materials were banana microshoots 'Ambon Kuning' derived from the multiplication stage using media containing benzyladenine (5 mg/l). In the first expeiment, the microshoots (2 cm in length) were aseptically cultured in pre-conditioned media consisting of MS salts [12], 100 mg/l myo-inositol, 0.1 mg/l thiamine-HCl, 0.5 mg/l pyridoxine-HCl, 0.5 mg/l nicotinic acid, 2 mg/l glycine, 30 g/l sukrose, and 1 mg/l BA for 4 weeks. The microshoots were then subcultured to treatment rooting media for 6 weeks. The treatment media were MS 0, MS+AC (activated charcoal) 2 g/l, MS+NAA 1 mg/l, MS+ NAA 2 mg/l, MS+IBA 1 mg/l, and MS+ IBA 2 mg/l. The experiment was arranged in a completely randomized design with three replicates. Each experimental unit consisted of 3 culture bottles (250 ml), one microshoot per bottle. The percentage of rooted shoots, number of leaves, plant height, number of primary roots, and length of primary roots were recorded. All cultures were incubated in a culture room. The culture room was set up at a temperature of 26oC ±2oC and continuously suplied with light of 1,000-2,000 lux from a cool white flourescent lamp.

The plantlets were then used in the the second experiment. The plantlets 13-16 cm in length, with 3-4 leaves and 7-10 primary roots were washed thoroughly under running tap water, then immersed in a fungicide solution of 2 g/l for 10-15 minutes. The plantlets were planted in plastic glass pots (8.2 cm in diameter) containing various media mixtures, namely M1 (rice husk charcoal, compost, sand, and cocopeat, 1:1:1:1), M2 (rice husk charcoal, compost, and sand, 1:1:1), and M3 (rice husk charcoal and compost, 1:1), and covered with transparent plastic bag. This experiment was conducted in a greenhouse equipped with paranet. The floor was covered with husks that had been washed and soaked in a fungicide solution. The plastic cover was opened when the plantlets looked strong and sturdy after 2 weeks after planting. The application of NPK fertilizer (20:20:20), 10 ml/plant with concentration of 2 g/l, was carried out 4 weeks after planting. Fertilization is conducted until the 8th week, once a week together with watering the plants. At 8 weeks after planting, plantlet survival, plant height, number of leaves, leaf greenness level, number of primary roots, and length of primary roots were recorded. The experiment was arranged in a completely randomized design with three replicates. Each experimental unit consisted of 8 plantlets. one microshoot per bottle. The variables recorded were the percentage of plantlet survival, plant height, number of leaves, leaf greenness level, number of primary roots and length of primary roots.

All of the data from the first and second experiment were subjected to analysis of variance.The mean separation was done using the least significant difference test at 5% level.

RESULTS AND DISCUSSION

In the first experiment, all treatments resulted in 100% of survival and rooting of microshoots (Table 1, Figure 1). The height of plantlet and number of roots were not significantly different. Plantlet height produced in all treatments ranged from 13.17□16.39 cm and leaf number of 3.11□4.00. NAA was found to be more effective than IBA and activated charcoal (AC) for rooting induction. The average number of primary roots in MS 0, MS + AC, and MS + IBA media ranged from 6□6.78 roots. The highest number of roots (13.11 roots) were produced by MS + 1 mg/l NAA media treatment. As for root length, the addition of NAA, IBA, or AC, resulted in an increase in root length. The average of the longest plantlet roots was obtained in the treatment of MS + NAA 2 mg/l and IBA 1 mg/l (12.51□12.86 cm) (Table 1).

TABLE 1. Effects of activated charcoal (AC), NAA, or IBA on in vitro rooting of 'Ambon Kuning' banana shoots after 6 weeks of culture

Media	Percentage of rooted shoots	Plant height (cm)*)	Number of leaves*)	Number of primary roots*)	Length of primary roots (cm)*)
MS 0	100	14,14 a	3,11 a	6,00 c	6,64 d
MS + AC 2 gr/l	100	15,40 a	3,33 a	6,78 c	11,55 bc
MS + NAA 1 mg/l	100	14,87 a	3,56 a	13,11 a	10,58 c
MS + NAA 2 mg/l	100	16,39 a	4,00 a	10,56 b	12,51 ab
MS + IBA 1 mg/l	100	13,17 a	3,56 a	6,11 c	12,86 a
MS + IBA 2 mg/l	100	14,88 a	3,67 a	6,78 c	11,53 bc
LSD 0.05		ns	Ns	1,99	1,99

*) Means in the same column followed by different letters differ significantly at $P \leq 0.05$ according to LSD multiple range test.

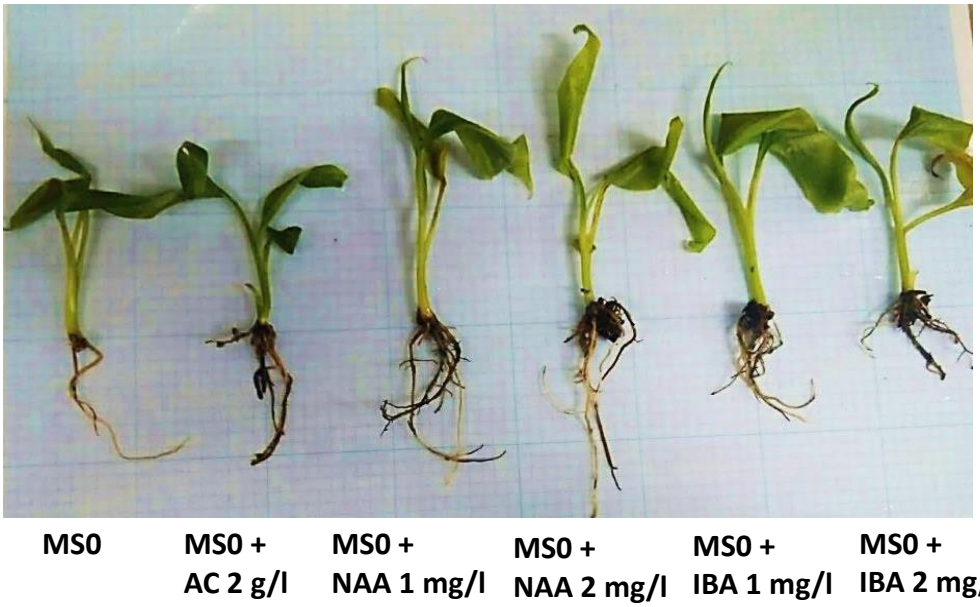

FIGURE 1. The effects of AC (activated charcoal), NAA (naphtaleneacetic acid), and IBA (indolebutyric acid) on in vitro rooting of banana microshoots 'Ambon Kuning' after 6 weeks in culture. MS0= MS salts and other components without plant growth regulators as described in Materials and Methods.

The first experiment showed that NAA 1 mg/l produced the highest number of roots. [7] reported that the combination of 1.2 mg/l NAA and 0.1 mg/l BA is the best treatment combination for the number of banana roots 'Kepok', while the combination of 0.9 mg/l NAA and BA 0.3 mg/l plus 50 mg activated charcoal was the best treatment combination for promoting shoot height. [8] showed that NAA was the most effective auxin for primary root formation on Jamaican guava cuttings compared to IBA or a combination of both. [9] showed that the administration of IAA resulted in the highest number of roots, while the administration of IBA resulted in the highest percentage of survival and administration of NAA resulted in the longest root induction of fig roots through micro cuttings. In olive cuttings, IBA was the best treatment to produce the percentage of rooted cuttings, number of roots, and shoot length [10]. According to [11], the effectiveness of plant plant growth regulator (PGR) in inducing rooting is strongly influenced by plant species and type and concentration of PGR. [5] said that activated charcoal at a certain concentration was able to condition the media to darken, resulting in changes in the synthesis of

endogenous auxin. Based on [5], the addition of 2 g/l activated charcoal could increase root length and number of roots.

TABLE 2. Effects of media mixture and NPK fertilizer on growth of banana plantlets 'Ambon Kuning' after 8 weeks during acclimatization

Media mixture	Plant height (cm)*)	Number of leaves*)	Number of primary roots*)	Length of primary roots (cm)*)	Leaf greenness*)
M1=Rice Husk Charcoal:Compost: Sand: Cocopeat (1:1:1:1,v/v)	19,04 a	4,67 a	6,73 a	9,95 a	37,66 a
M2= Rice Husk Charcoal:Compost: Sand (1:1:1,v/v)	16,18 b	4,17 ab	5,72 b	8,39 b	36,00 b
M3= Rice Husk Charcoal:Compost (1:1,v/v)	13,90 c	3,82 b	5,02 c	7,23 c	32,62 c
LSD 0.05	1,09	0.51	0,29	0,31	1,43
Fertilizer NPK (20:20:20) 2 g L^{-1}					
With fertilizer	17,19 a	4,22 a	6,38 a	8,99 a	36,70 a
Without fertilizer	15,55 b	4,20 a	5,27 b	8,06 b	34,15 b
LSD 0.05	0,89	ns	0,24	0,25	1,17

*) Means in the same column followed by different letters differ significantly at $P \leq 0.05$ according to LSD multiple range test. ns= non-significant.

The second experiment (Table 2) showed that the three different acclimatization media had a significant effect on all observed variables, namely plant height, number of leaves, number of primary roots, primary root length, and leaf greenness, the best being shown by M1 (rice husk charcoal, compost, sand, and cocopeat, 1:1:1:1 v/v) (Figure 2 and 3). Fertilization with 2 g/l NPK (20:20:20) solution also significantly promoted plantlet growth as indicated by all of the variables except the number of leaves (Table 2 and Figure 2). There was no significant interaction between acclimatization media and NPK fertilization (20:20:20) in affecting percent of survival and plant growth.

FIGURE 2. Eight-week-old banana plants 'Ambon Kuning' grown on M1 (rice husk charcoal, compost, sand, and cocopeat, 1:1:1:1 v/v), on M2 (rice husk charcoal, compost, and sand, 1:1:1 v/v), and on M3 (rice husk charcoal and compost, 1:1 v/v)

The second experiment showed that 100% of the plantlets were successfully acclimatized, suggesting that the three types of mixed media fulfill the requirements as good media for plantlet growth, i.e. having loose properties, good aeration and drainage, and containing sufficient nutrients. The mixture of media M1 (husk charcoal: compost: sand: cocopeat) produced the best plant growth based on all observed variables. The second best media was M2 (husk charcoal: compost: sand). The one that produced the lowest plant growth was M3 (husk charcoal:sand). Each media component has its advantages. Husk charcoal has a high water absorption capacity. Compost contains high soil organic matter. Sand is porous so that it can increase soil aeration. Cocopeat has the ability to bind and store

water strongly, and contains essential nutrients, such as calcium (Ca), magnesium (Mg), potassium (K), sodium (N), and phosphorus (P) [11] (Muliawan, 2009). Application of 2 g/l NPK fertilizer (20:20:20) could increase plantlet growth during acclimatization as indicated by increased plant height, number of primary roots, primary root length, and leaf greenness.

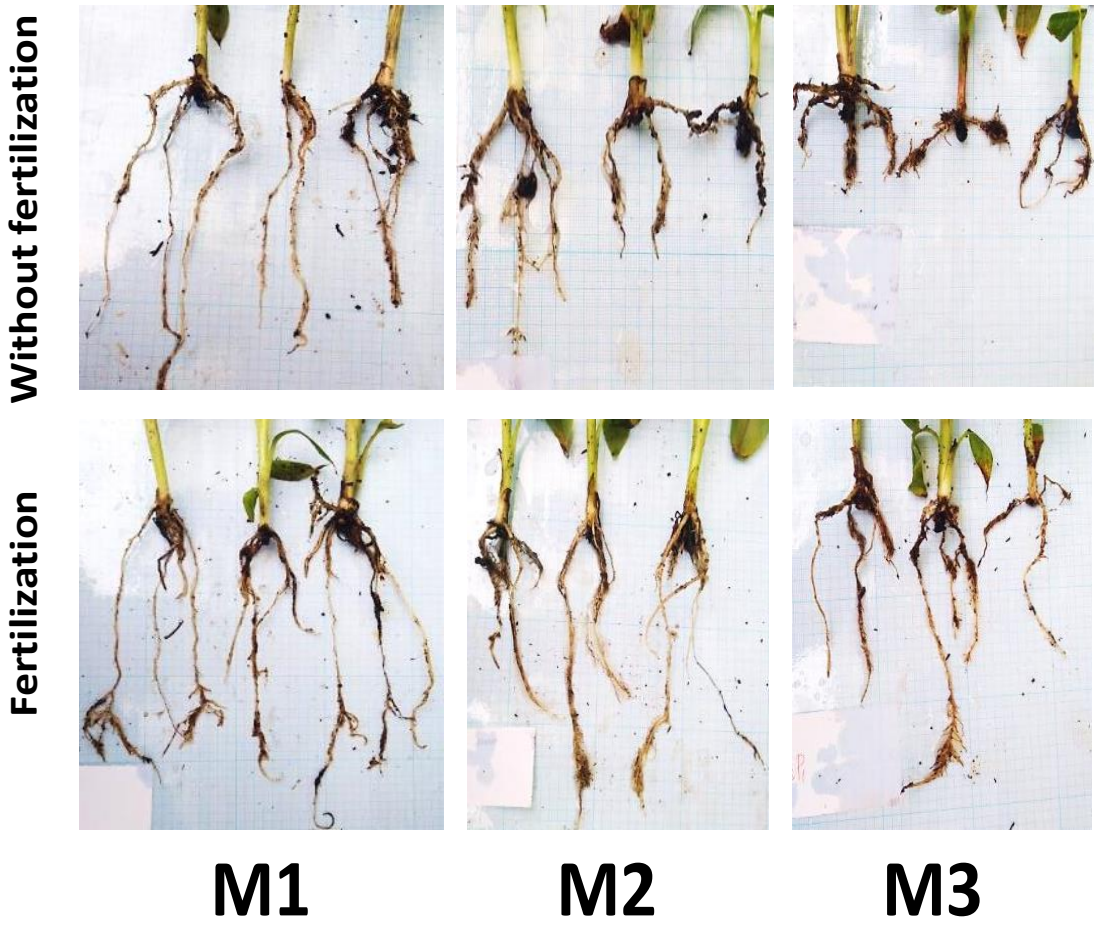

FIGURE 3. Root appearance of eight week-old banana plantlets 'Ambon Kuning' during acclimatization on media M1 (rice husk charcoal, compost, sand, and cocopeat, 1:1:1:1 v/v), M2 (rice husk charcoal, compost, and sand, 1:1:1 v/v), and M3 (rice husk charcoal and compost, 1:1 v/v), without and with NPK (20:20:20) fertilization.

Conclusions

Addition of 1 mg/l or 2 mg/l NAA to MS media significantly induced rooting of banana microshoots 'Ambon Kuning', while the addition of 1 mg/l or 2 mg/l IBA and 2 g/l activated charcoal had no significant effects. During acclimatization, all media mixture i.e. M1 (rice husk charcoal, compost, sand, and cocopeat, 1:1:1:1 v/v), M2 (rice husk charcoal, compost, and sand, 1:1:1 v/v), and M3 (rice husk charcoal and compost, 1:1 v/v) resulted in 100% of survival rate, the best being shown by M1 media mixture as indicated by the growth variables. Fertilization using 2 g/l NPK (20:20:20) solution significantly increased the growth of banana plantlets 'Ambon Kuning' during acclimatization. This results confirmed that auxins generally induce rooting and differet types of auxins may have different effect on rooting. Since responses to hormonal treatment are generally genotype-dependent, this report is expectedly useful for those who micropropagate banana 'Ambon Kuning`.

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