THE SUCCESS OF BANANA PLANLETS ACCLIMATIZATION BY THE APPLICATION OF *TRICHODERMA*-BASED COMPOST AND ARBUSCULAR MYCORRHIZAE FUNGI IN GROWING MEDIA

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Abstract. Acclimatization of *in vitro*-regenerated plantlets often requires modifications to growing media because the root architecture of regenerated plantlets have not yet well-developed. Therefore, the objective of our study was to find the effective dose of *Trichoderma*-based compost and arbuscular mycorrhizae isolates in growing media at plantlet acclimatization. The trial was conducted for approximately 4 months at the Plant Biotechnology Laboratory, Faculty of Agriculture, University of Jambi, Indonesia. Plant materials were *in vitro*-regenerated plantlets of Banana cv. Barangan Kuning, transplanted on acclimatization media consisted of soil and *Trichoderma*-based compost (1:1, 1:2 and 2:1) and arbuscular mycorrhizae fungi (0, 10, and 20 g per plantlet). The trial used a factorial randomized block design with 3 replications. Each experimental unit consisted of 10 polyethylene bags with one individual plant in it. Data were recorded on survival rate, plantlet height, pseudostem diameter, leaf number, as well as length and width of fully developed leaves. Analysis of Variance and Least Significant Difference tests were employed to see the effect of the *Trichoderma*-based compost and mycorrhizae applications on plantlet growth. Results showed that all plantlets succeeded acclimatization (100% survival rate). Statistical analysis indicated that the combination of *Trichoderma*-based compost + mycorrhizae or the application of mycorrhizae alone did not show significant effect on all variables. *Trichoderma*-based compost, however, was found to significantly affect plantlet height, leaf number, and leaf length, but not pseudostem diameter and leaf width. Though there was no significant effect of mycorrhizae, microscopic study proved that mycorrhizal infection on plantlet roots had been occurred. Our investigation confirmed the benefit of *Trichoderma*-based compost in growing media during acclimatization, in which the ratio of 2:1 of soil and *Trichoderma*-based compost resulted in the best plantlet growth.

Key words: *in vitro* propagation; plant tissue culture; plantain; *Musa* spp.

INTRODUCTION

Banana is one of tropical plants having high economic value as source of vitamins and minerals. Banana fruits can be served fresh as table fruits or cooked in a variety of dishes. In the world banana trade, Indonesia is the third largest banana producers, following India and China mainland, with total production of 8,182,760 metric tons in 2020 [25].

Bananas are normally propagated vegetatively by rhizomes and suckers (sword suckers and water suckers). This method of propagation takes time, produces limited plants, and opens the opportunity for the spread of plant pests and diseases. In addition, the use of water sucker as propagation material will not produce healthy banana clumps. Therefore, we need a technology that can produce large number of healthy banana plants in a relatively short time. The technology we offer is *in vitro* plant propagation by tissue culture techniques [32].

The success of banana propagation by tissue culture much depend upon the number plantlets which survive acclimatization. Acclimatization is a critical stage, since *in vitro* plantlets have limited cuticle, lack of lignification, underdeveloped vascular tissue, and non-functional stomata [31]. Therefore, acclimatisized plantlets are prone to excessive transpiration, microbial attacks, high temperature and light intensity. As a consequence, modifications of microclimate factors are required, especially temperature, humidity, and light intensity. In addition, modification of growing substrate is also needed since acclimatized plantlets have not yet regenerate good root architecture [2, 15].

Many studies on the role of growing substrate in acclimatization of banana plantlets have been reported, such as the use of cocopeat + husk charcoal + sand [2], sand + vermicompost [1], and sand + husk charcoal [12]. In addition, the application of arbuscular mycorrhizal fungi in acclimatization media of banana plantlets have also been widely reported [7, 18, 28].

It is clear that growing media is crucial for the survival of banana plantlets during acclimatization. The survival rate will increase when the substrate is supplemented with *Trichoderma* and/or mycorrhizae. However, since the success of acclimatization varied among banana cultivars, it is worthy of an in-depth investigation to seek for effective dose of *Trichoderma* and mycorrhizae isolates for use in growing substrate during acclimatization of banana plantlets. Thus, this investigation is a strategic effort to support the mass rapid propagation of Banana cv. Barangan Kuning through tissue culture.

The objective of the study was to find the effective dose of *Trichoderma*-based compost and arbuscular mycorrhizae isolates in growing media at plantlet acclimatization of Banana cv. Barangan Kuning.

MATERIALS AND METHOD

Plant preparation

Plant materials used in this trial were banana cv. Barangan Kuning plantlets from *in vitro* culture that have been pre-acclimatisized for about 4 weeks in plastic tray (Figure 1). The plantlets were pre-acclimatized *in* on potting media containing

Trichoderma-based compost and arbuscular mycorrhyzae.

Figure 1. *In vitro* plantlets of banana cv. Barangan Kuning that have been pre-acclimatisized for about 4 weeks, ready for use as plant materials.

Compost preparation

Trichoderma-based compost was prepared in the Laboratory of Biotechnology and Plant Breeding, University of Jambi according to the procedure of Eliyanti et al. [5]. The materials were cow manure (100 kg), rice husk charcoal (10 kg), *Trichoderma* sp. on rice media (300 g), coconut water (10 L), and clean water. Cow manure, rice husk charcoal, coconut water and rice containing *Trichoderma* sp. were mixed thoroughly and evenly. Then water was added until the mixture becomes moist and incubated in dark condition at temperature of 27 - 30 °C for two weeks. The ready to use *Trichoderma-based* compost are indicated by blackish brown in color and crumb structure.

Mycorrhizae preparation

The mycorrhizal isolates were obtained from ultisol of ex coal mining soils from Tebo Regency, Jambi Province [6] consisted of *Glomus* sp-3, *Glomus* sp-6, *Glomus* sp-15 and *Glomus* sp-16. Mycorrhizae were propagated in the Laboratory of Seed Technology, Faculty of Agriculture, Jambi University based on Kartika's procedure [6]. The materials were soil mixed with mycorrhizae, clean zeolite, *Pueraria javanica* seeds, and liquid fertilizer. Zeolite was put into culture container and then 10 g of mycorrhizal soil was added. Seeds of *P. javanica* were sown on it and covered with zeolite to the brim. This culture was maintained in greenhouse for approximately 4 months with 16-photoperiod, temperature of 28 - 34 °C, and 64 - 80% relative humidity.

Media preparation

Acclimatization was carried out in a greenhouse. Natural light of 70% intensity was used with daily photoperiod of 16-hours.

The acclimatization media tested in this study was topsoil soil from the Research and Teaching Farm, Faculty of Agriculture, University of Jambi. The media were prepared according to the treatment being tested. The soil and compost were mixed in a black plastic bag of 5 kg. Likewise, mycorrhizae were given according to the treatment along with the transfer of the seedlings into the acclimatization media. Mycorrhizae was applied by inserting it into the planting hole before the plantlets were planted.

Experimental design

The experiment was arranged in a factorial Randomized Block Design. Two factors were evaluated: 1) three levels of soil:trichoderma (1:1, 1:2 and 2:1), and 2) three doses of mycorrhizae (0, 10 g and 20 g per seedling). Therefore, there were 9 treatment combinations which was repeated 3 times, resulting in total 27 experimental units. Each experimental unit consisted of 10 individual seedlings, making the overall number of banana seedlings used in this experiment was 270 plants.

Observation and data analysis

The variables observed were survival rate, height of seedlings, diameter of pseudostem, number of leaves, length of leaf length, width of leaf, and mycorrhizal colonization [19]. The Analysis of Variance (ANOVA) using Microsoft Excel Spreadsheet application [13] was employed in data analysis. The difference between treatment means were analyzed using the Least Significant Difference test [3].

RESULTS

Survival rate

Following 8 weeks of transplantation 100% of plantlets successfully survived acclimatization. Regardless of the compositions and dosages, the use of *Trichoderma*-based compost and arbuscular mycorrhizae in the acclimatization media showed a positive effect on the survival rate of banana plantlets during acclimatization. Growth rate, however, varied among plantlets depending on the treatment.

Seedling height

Based on ANOVA, it was found that the interaction between media composition and mycorrhizae did not show significant effect on seedling height (Table 1). Similarly, the dose of mycorrhizae alone also did not significantly affect the height of banana seedlings (Table 3). In the other hand, the media composition showed highly significant effect (*P-value* = 0.001) on seedling height (Table 2). Among the three tested compositions, the 2:1 (2 parts soil + 1 part compost) composition resulted in the highest seedling (79.63 cm on an average) and significantly different from the 1:1 and 1:2 compositions.

Pseudostem diameter

The analysis of variance showed that media composition or mycorrhizae individually or the interaction between media composition and mycorrhizae did not significantly affect pseudostem diameter. Data on the effect of media composition and arbuscular mycorrhizae doses are presented in Table 1, 2 and 3.

Leaf number

There was no significant effect of the interaction between media composition and mycorrhizae on the number of leaves (Table 1). The number of leaves was not also affected by the application of mycorrhizae

alone (Table 3). However, the composition of the media showed highly significant effect (*P-value* = 0.003) on the number of leaves (Table 2). Among the three tested media, the composition of 2 parts soil + 1 part compost (2:1) resulted in the highest number of leaves (7.67 leaves on average) and significantly different from other three compositions.

Leaf length

The ANOVA showed that the combination of media composition and mycorrhizae, or mycorrhizae alone did not significantly affect leaf length (Table 1 and Table 3). In contrast, media composition significantly affects leaf length of banana seedling at the acclimatization stage (*P-value* = 0.008) (Table 2).

Table 1. The effect of different media composition and doses of arbuscular mycorrhyzae on the height, pseudostem diameter, leaf number, leaf length and leaf width of banana cv. Barangan Kuning plants at the end of 8 weeks of acclimatization.

Growth responses	Doses of mycorrhyza (per plant) (g)	Media compositions (soil : compost)		
		1 : 1	1 : 2	2 : 1
Seedling height (cm)	0	50.62 ± 9.36	76.20 ± 15.23	79.97 ± 10.30
	10	58.20 ± 9.66	54.23 ± 14.15	75.12 ± 8.75
	20	36.22 ± 5.25	50.05 ± 8.83	81.50 ± 7.92
Pseudostem diameter (cm)	0	1.33 ± 0.25	1.87 ± 0.38	1.76 ± 0.22
	10	1.41 ± 0.20	1.32 ± 0.32	1.44 ± 0.14
	20	0.98 ± 0.14	1.18 ± 0.20	1.87 ± 0.18
Leaf number	0	6.17 ± 0.65	6.83 ± 0.60	7.83 ± 0.48
	10	7.33 ± 0.33	6.17 ± 0.75	7.33 ± 0.33
	20	5.33 ± 0.37	6.50 ± 0.71	8.00 ± 0.48
Leaf length (cm)	0	24.77 ± 4.82	34.95 ± 7.07	39.28 ± 5.67
	10	27.77 ± 4.65	26.37 ± 6.47	30.63 ± 2.65
	20	17.92 ± 3.08	26.10 ± 4.19	36.43 ± 3.42
Leaf width (cm)	0	10.05 ± 1.96	13.40 ± 2.64	13.47 ± 1.80
	10	11.42 ± 1.81	10.28 ± 2.69	10.85 ± 1.05
	20	7.22 ± 1.34	9.50 ± 1.76	13.83 ± 1.16

± Standard Error (n=6).

Table 2. The effect of media composition on the height, pseudostem diameter, leaf number, leaf length and leaf width of banana cv. Barangan Kuning plants at the end of 8 weeks of acclimatization.

Growth responses	Media composition (soil : compost)	Plantlet height (cm)
Seedling height (cm)	1 : 1	46.69 ± 5.29 a
	1 : 2	58.32 ± 7.77 a
	2 : 1	79.63 ± 4.98 b
Pseudostem diameter (cm)	1 : 1	1.24 ± 0.12
	1 : 2	1.46 ± 0.18
	2 : 1	1.69 ± 0.11
Leaf number	1 : 1	6.17 ± 0.35 a
	1 : 2	6.44 ± 0.38 a
	2 : 1	7.67 ± 0.24 b
Leaf length (cm)	1 : 1	22.94 ± 2.59 a
	1 : 2	28.23 ± 3.48 ab
	2 : 1	36.03 ± 2.42 b
Leaf width (cm)	1 : 1	9.56 ± 1.03
	1 : 2	11.06 ± 1.36
	2 : 1	12.72 ± 0.81

Note: values followed by the same letter(s) are not significant different according to the Least Significant Difference (LSD) test at α = 0,01 (SE = Standard Error, n = 18).

Table 3. The effect of different doses of arbuscular mycorrhyzae on the height, pseudostem diameter, leaf number, leaf length and leaf width of banana cv. Barangan Kuning plants at the end of 8 weeks of acclimatization.

Growth responses	Doses of micorrhyza (per plant) (g)	Plantlet height (cm)
Seedling height (cm)	0	68.93 ± 7.19
	10	62.52 ± 6.41
	20	53.19 ± 6.77
Pseudostem diameter (cm)	0	1.65 ± 0.17
	10	1.39 ± 0.13
	20	1.34 ± 0.13
Leaf number	0	6.94 ± 0.36
	10	6.94 ± 0.31
	20	6.39 ± 0.41
Leaf length (cm)	0	33.00 ± 3.53
	10	28.26 ± 2.66
	20	25.94 ± 2.95
Leaf width (cm)	0	12.31 ± 1.23
	10	10.85 ± 1.07
	20	10.18 ± 1.03

± Standard Error (n=18).

The composition of 2:1 (2 parts soil + 1 part compost) resulted in the greatest increase in leaf length and was significantly different from 1:2 or 1:1 composition.

Leaf width

Analysis of variance of leaf width data did not show any significant effect of interaction media composition and mycorrhizae (Table 1). Likewise, the media composition or the mycorrhizae individually did not show any significant effect on leaf width of the banana seedlings at the acclimatization stage (Table 2 and Table 3).

Mycorrhizal colonization

Observations on root infection did not show any infection in control treatment. In contrast, the application of 10 g and 20 g inoculum per plant showed an average infection of 34.07% to 88.89% (Table 4), with moderate to very high criteria [19].

Table 4. The effect of different doses of arbuscular mycorrhizae on root infection of banana cv. Barangan Kuning seedlings at acclimatization stage.

Doses of mycorrhizae (per plant)	Root infection (%)	Criteria
0 g	0.00 ± 0.00 a	Very low
20 g	34.07 ± 6.33 b	Moderate
10 g	88.89 ± 6.47 c	Very high

Values followed by the same letter(s) are not significant different according to the Least Significant Difference (LSD) test at α = 0,01 (± Standard Error, n=9).

DISCUSSION

Acclimatization is an important last step of *in vitro* procedure for plant propagation. It is an effort to introduce *in vitro*-regenerated plantlets to *in vivo* environment for they can grow and develop as normal plants [31]. Acclimatization is a crucial stage because *in vitro*-regenerated plantlets are susceptible to water loss through transpiration due to underdeveloped root architecture and vascular tissues from root to shoot. As the consequence, special treatments are frequently needed, including substrate modification for normal development of roots [2, 15].

All plantlets transplanted to various compositions of *Trichoderma*-based compost and mycorrhizal treatments succeeded acclimatization. In other words, the survival rate was 100% (Figure 2). This indicates that the growing media meet the requirements for successful root growth of banana seedlings. Our study indicated a positive effect of *Trichoderma*-based compost, which might improve root architecture, increase the length of main and lateral roots so as to increase the effectiveness of nutrient uptake [4, 14, 29, 30].

Positive response of seedlings grown on media with soil + *Trichoderma*-based compost at 1:1, 1:2 or 2:1 compositions indicates good media support. Statistically, the composition of 2:1 was better than others for seedling height, leaf number and leaf length. This means that *Trichoderma*-based compost has taken a part in improving the growing media to support root development and overall seedling growth. Figure 3 shows different growth of seedling on different compositions of soil + *Trichoderma*-based compost.

Figure 2. The performance of plantlets acclimatized on media enriched with *Trichoderma*-based compost and arbuscular mycorrhizae (8 weeks after transplanting).

In contrast, the application of *Trichoderma*-based compost along with arbuscular mycorrhizae did not show any significant effect on all variables. It was presumably due to sufficient water content in the media that can be easily absorbed by root hairs, so the involvement of mycorrhizae in obtaining water was no longer required. Besides, at the time of observation the association of mycorrhizae with banana seedling roots was presumably not yet been established. In onion grass (*Allium vineale*), Ronsheim [20] reported that during the first 6 months of transplanting the total biomass of mycorrhizae-treated plants was not significantly different from those non mycorrhizae-treated. However, 15 months after transplanting, there was significant difference in total biomass of mycorrhizae-treated compared to non mycorrhizae-treated plants.

Figure 3. Growth performance of banana seedlings on different media composition and doses of arbuscular mycorrhizae (8 weeks after transplanting).

Mycorrhizal colonization did not affect the quantity and quality of tomato yields in soilless cultivation as reported by Maboko et al. [11]. In line with that, better water holding capacity in the media equipped with *Trichoderma*-based compost was believed to contribute to the undisturbed water uptake by roots resulting in better growth and development. The colonization of arbuscular mycorrhizae in banana roots did not depend on growing media as shown by the colonization that took place in all media composition.

The application of mycorrhizae at 10 g per plant showed profuse root infection and became moderate when the dose increased to 20 g per plant. The root infection indicates that there has been association

between mycorrhizae and banana roots (Figure 4). Padri et al. [17] suggested that mycorrhizae associate with plant roots through infection by forming structures typical of mycorrhizal colonization, namely hyphae, vesicles and arbuscules. The principle of mycorrhizae work is by infecting roots of host plant, intensively producing mass of hyphae so that mycorrhizal-associated plants are able to increase their capacity for water and nutrient uptake. However, not all types of spores can colonize plant roots. The level of colonizaion is largely determined by the compatibility of mycorrhizal fungi with plant roots [16].

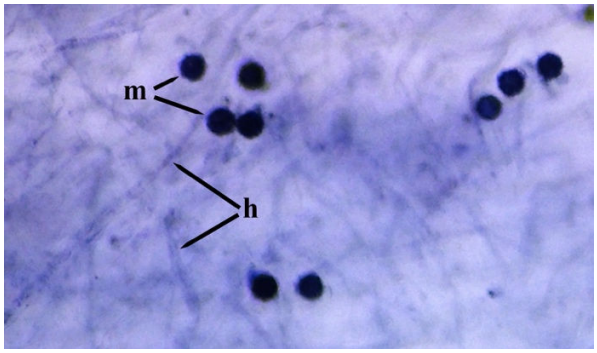

Figure 4. Profuse hyphae development indicating the association between arbuscular mycorrhizae colony and banana roots (m = micorrhizae, h = hyphae).

Sasli and Ruliyansyah [23] suggested that the percentage of root infection by mycorrhizae can be influenced by the nutrient availability in the soil. Plants grown on soils with high nutrient content tend to have less root infection, while plants grown on soils with low nutrient content will have high level of root infections. This because in soils with low nutrient content the role of mycorrhizae is needed to help with water and nutrients uptake. Meanwhile, in soils with high nutrient content, the role of mycorrhizae is not optimal since the nutrients are already available in the rhizosphere. Saputra et al. [22] reported that mycorrhizal infection in roots of banana cv. Nipah grown in ultisols was higher than in alluvial and peat soils. This because the ultisoll type of soils have the lowest nutrient content compared to other soil types. Low nutrient content causes a high level of mycorrhizal root infection.

Table 4 shows that root infection decreased with increasing in mycorrhizal dose. This may be due to higher mycorrhizal doses causing competition for root exudates as the main requirement for mycorrhizae, while the exudate released by plant roots is limited. As a result, the number of mycorrhizae capable of infecting roots decreases. Saidi et al. [21] suggested that exudates released by host plants in the form of carbohydrates, amino acids and other organic acids are the primary requirements for mycorrhizae growth and development. Plants that have sufficient amounts of nutrients release exudates in small amounts, so that the activity of mycorrhizal fungi in these conditions is very low or decreased. The contradictory situation occurs in nutrient-deficient plants that can release a lot of root exudates.

The association of arbuscular mycorrhizae with plant roots depends on soil ecology, light, plant and fungal species and their adaptability to each other [8, 10, 24, 27]. Although there was no significant response under certain conditions, plants associated with mycorrhizae still had better growth than non-mycorrhizal plants due to better nutrition [9, 26].

In conclusion, our study showed that the survival rate of banana cv. Barangan Kuning plantlets during acclimatization could be increased by the application of *Trichoderma*-based compost and arbuscular mycorrhizae fungi in growing substrate, in which the best medium composition was 2:1 (2 parts of soil + 1 part of compost). Though the effect was not significant, the application of arbuscular mycorrhizae might be useful to improve media condition for better growth of banana plantlets during acclimatization stage.

Acknowledgement. The authors wish to thank Rector University of Jambi for financial supports of this research. This research was funded by University of Jambi Internal Research Grant, Contract No.: 1319/UN21.11/PT01.05/SPK/2022, 17 May 2022.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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Received: November 24, 2022
Accepted: May 26, 2023
Published Online: July 1, 2023
Analele Universităţii din Oradea, Fascicula Biologie
https://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House