The Screening of Growing Media for Acclimatization of Banana Plantlets

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Abstract. Media for acclimatization is one of the important factors for the success of plant propagation through tissue culture techniques, because the regenerated plantlets do not yet have a well-developed root system. This situation causes plantlets to be very sensitive to environmental stresses, both abiotic and biotic. Therefore, plantlet acclimatization requires special handling, and modifications to environmental conditions are needed, especially in relation to growing media. This study aimed to obtain the best media composition and dose of arbuscular mycorrhizal fungi (AMF) to support the growth of banana plantlets during the acclimatization stage. This investigation was conducted at the Plant Biotechnology Laboratory Faculty of Agriculture University of Jambi for approximately 4 months. Tissue culture generated plantlets of banana cv. Barangan were acclimatisized on media consisted of s-oil and trichocompost (1:1, 1:2 and 2:1) and AMF (0, 10, and 20 g per plantlet). The trial was arranged in a factorial randomized block design with 3 replications, making the total of 27 experimental units. Each unit consisted of 10 polybags containing one individual plant. Data were recorded on the percentage of live seedlings (survival rate), increase in plant height, increase in number of leaves, increase in stem diameter, length, and width of fully developed leaves. Statistical analysis using Analysis of Variance, followed by the Least Significant Difference test at 5% confidence interval was employed to see the effect of the media compositions and AMF on plantlet growth. Results showed that all transplanted plantlets suceeded acclimatization process (100% survival rate). Statistical analysis revealed that either the combination of media compositions and dosages of AMF or AMF alone did not show any significant effect on all variables observed. The media composition, however, was found to have significant effect on plant height, leaf number, and leaf length, but not on stem diameter and leaf width. This investigation confirmed benefit of the use of trichocompost in acclimatization media, in which the composition of 2:1 of soil and trichocompost gave the best the growth of banana plantlets during acclimatization.

Keywords: Plant propagation · In vitro culture · Organic fertilizer · Musa spp

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1 Introduction

Banana is one of many tropical fruit plants that are highly demanded. Banana fruits can be served fresh as table fruit or in various forms of processed products, cooked or fried.

Apart from its high economic value, conventional propagation of banana takes time, the number of seeds produced is limited, and opens the opportunity for the spread of pathogens due to the use of vegetative propagules. In addition, non-uniform seeds produced can lead to increased production costs. Therefore, we need a technology that can provide quality and healthy banana seeds in large quantities and in a relatively short time. The modern technology to solve these problems is vegetative plant propagation by utilizing plant tissue culture techniques [1].

The success rate of plant propagation by tissue culture is highly dependent on the survival rate of plantlets in the acclimatization stage from *in vitro* to *in vivo* conditions. Acclimatization is a critical stage, because plantlets regenerated from *in vitro* culture have several disadvantages, such as underdeveloped cuticle, lack of stem lignification, underdeveloped vascular tissue, and often not functioning stomata. This situation causes plantlets to be very sensitive to transpiration, fungal and bacterial attacks, high light intensity and high temperature. Therefore, plantlet acclimatization requires special handling, and even modifications to environmental conditions are needed, especially in relation to temperature, humidity, and light intensity. In addition, the growth medium also plays an important role, especially if the acclimatized plantlets have not yet formed a good root architecture.

Reports indicated the important role of media composition on the success of acclimatization of banana plantlets, such as cocopeat + husk charcoal + sand [2], sand + vermicompost [3], and sand + charcoal husks [4]. Besides, the use of mycorrhizae in order to increase the success of acclimatization of banana plantlets had also been widely reported [5, 6, 7].

From the abovementioned description, the role of media is very important in increasing the survival rate of plantlet during acclimatization. The survival rate will be better if the media is enriched with *Trichoderma* and/or arbuscular mycorrhyzae fungi (AMF). However, considering the results obtained are still varied among banana cultivars, in-depth research is needed to obtain an effective dose of *Trichoderma* and indigenous AMF isolates for use in the acclimatization medium of banana plantlets. Therefore, this study is very important to support efforts of the provision of healthy and uniform banana cv. Barangan Kuning seedlings through tissue culture.

The aim of this study was to obtain the best ratio of soil: trichocompost and the dose of AMF isolates to support the survival rate and growth of banana cv. Barangan Kuning plantlets during the acclimatization stage.

2 Materials and Method

2.1 Plant Material

The plant materials used were plantlets of banana cv. Barangan Kuning regenerated from tissue culture. Plantlets were obtained from the Plant Tissue Culture Laboratory of the Department of Food Crops and Horticulture, Tanjung Jabung Barat Regency.

The plantlets had been pre-acclimatized *in vivo* for approximately one month. Trichocompost materials, soil and mycorrhizal isolates were prepared at the Laboratory of Biotechnology and Plant Breeding, Faculty of Agriculture, University of Jambi.

2.2 Experimental Design

The trial used a factorial Randomized Block Design consisted of two factors: (1) the medium composition (soil: Trichokompos) which consisted of 3 ratios, and (2) the dose of AMF per seedling which consisted of 3 doses. Thus, there were 9 treatment combinations tested. Each combination was repeated 3 times (3 blocks), so that in total there were 27 experimental units. Each experimental unit consisted of 10 polybags containing one individual seedling, making the total number of seedlings used in this experiment was 270 plants.

2.3 Observed Variables

The observed variables were survival rate (percentage of live plantlets), seedling height, pseudostem diameter, number of leaves, leaf length, leaf width, and mycorrhizal colonization. Data were analyzed using Analysis of Variance (ANOVA) with Microsoft Excel Spreadsheet application [8]. Furthermore, to see the difference between treatment means, the Least Significant Difference test was carried out [9].

3 Results and Discussion

3.1 Results

3.1.1 Survival Rate

Observations made at 8 weeks after transplantation showed that 100% of the acclimatized plantlets successfully passed the critical period and survived. This indicates that regardless of the compositions and dosages, the involvement of Trich and mycorrhizae in the media has a positive influence on the success of plantlet acclimatization. However, the rate of growth and development of plants varied, depending on the treatment given.

3.1.2 Seedling Height

The ANOVA did not show any effect of the interaction between medium composition and dose of AMF on seedling height. Likewise, the seedling height was not significantly affected by the dose of AMF alone. However, the composition of the tested media showed a highly significant effect (*P-value* = 0.001) on seedling height. Among the three tested media, the 2:1 (2 parts soil + 1 part trichocompost) composition produced the largest seedling height (79.63 cm on average) and significantly different from the 1:1 and 1:2 compositions (Table 1).

Table 1 The effect of media composition on the average height of banana cv. Barangan Kuning seedlings in acclimatization stage

Soil: trichocompost	Seedling Height (cm)
1: 1	46.69 ± 5.29a
1: 2	58.32 ± 7.77a
2: 1	79.63 ± 4.98b

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 2 The effect of media composition on the average leaf number of banana cv. Barangan Kuning seedlings in acclimatization stage

Soil: trichokompos	Leaf number
1: 1	6.17 ± 0.35a
1: 2	6.44 ± 0.38a
2: 1	7.67 ± 0.24b

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)

3.1.3 Pseudostem Diameter

The ANOVA did not show any significant effect of the interaction between media composition and AMF on pseudostem diameter. Likewise, the pseudostem diameter was not significantly affected by either the composition of the media or the dose of mycorrhizae individually.

3.1.4 Leaf Number

There was no significant effect of the interaction between medium composition and AMF on leaf number. The number of leaves was not also affected by the application of AMF alone. However, the composition of the medium alone showed highly significant effect (*P-value* = 0.003) on the number of leaves. Among the three media compositions, the composition of 2:1 (2 parts soil + 1 part trichocompost) resulted in the highest number of leaves (7.67 leaves on average) and significantly different from other three compositions (Table 2).

3.1.5 Leaf Length

The ANOVA shows that the interaction of medium composition and AMF doses, or AMF doses alone did not show significant effect on leaf length. However, the medium composition showed a highly significant effect (*P-value* = 0.008) on leaf number of

Table 3 The effect of media composition on the average leaf length of banana cv. Barangan Kuning seedlings in acclimatization stage (cm)

Soil: trichokompos	Leaf length (cm)
1: 1	22.94 ± 2.59a
1: 2	28.23 ± 3.48a
2: 1	36.03 ± 2.42b

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)

seedling at the acclimatization stage. The composition of 2:1 (2 parts soil + 1 part trichocompost) resulted in the largest increase in leaf length and was significantly different from 1:2 or 1:1 composition (Table 3).

3.1.6 Leaf Width

Analysis of variance on leaf width data did not show any significant interaction between medium composition and the AMF dose. Likewise, the medium composition or the dose of AMF individually did not show any significant effect on the leaf width of the banana seedlings at the acclimatization stage.

3.2 Discussion

Shoots and plantlets regenerated by *in vitro* culture are generally heterotrophs and must turn into autotrophs when they are transferred to *in vivo* environment. Transfer or known as acclimatization is the final step of the procedure for plant propagation by tissue culture. Zulkarnain [1] stated that acclimatization is an effort to condition plantlets from aseptic *in vitro* conditions so that they can grow and develop in septic *in vivo* environment. Acclimatization is an important process in a series of applications of tissue culture techniques for plant propagation.

One of the weaknesses of *in vitro* regenerated plantlets is that the root architecture and vascular tissues from root to shoot are not fully developed, so that *in vitro* shoots are very sensitive to water loss due to transpiration. Therefore, the acclimatization of *in vitro* plantlets requires special handling, even the modification of growing medium is frequently required so that roots can develop perfectly.

The results showed that all plantlets acclimatized to various media compositions and AMF treatments succeeded the critical period. This means that the survival rate of plantlets is 100% in all tested media and AMF doses (Fig. 1). This indicates that apart from conducive acclimatization environment, the growing media were believed to be able to meet the requirements for successful root growth.

As previously mentioned, the media used was a mixture of soil and trichocompost (*Trichoderma*-based compost). The results indicated a positive effect of trichocompost, which might improve root architecture, increase the length of main and lateral roots so as to increase the effectiveness of nutrient uptake by plants. [10, 11, 12, 13].

Fig. 1. Growth performance of plantlets on various ratios of soil and tricompost and inoculated with various doses of AMF at acclimatization stage (8 weeks after transplanting)

Good support of growing medium was shown by the response of the seedling planted on the media with either composition of 1:1, 1:2 or 2:1. Statistically, the composition of 2:1 gave better results compared to others for variables of seedling height, number of leaves and leaf length. This indicates that trichocompost has an important role in improving the conditions of the growing medium to support root development and overall plant growth. Figure 2 shows the differences in seedling growth on various compositions of growing media.

However, the interaction between media composition and AMF application did not significantly affect all variables. It was assumed that this could be due to the condition of the media with sufficient water content that was easily absorbed by the root hairs, so that it no longer required the involvement of mycorrhizal hyphae in obtaining water. Another possibility is that the time of observation was too short for mycorrhizae to associate with roots. In *Allium vineale* (wild garlic or onion grass), Ronsheim [14] found that up to 6 months after planting the total biomass of plants treated with mycorrhizae was not significantly different from that of plants not treated with mycorrhizae. However, when the plants were 15 months old, the total biomass of plants treated with mycorrhizae and not treated with mycorrhiza showed a significant difference. Therefore, the application of AMF in this study did not have a significant effect on the growth and development of banana seedlings at the acclimatization stage, presumably related to observation time which was too early.

This was in accordance with report by Maboko *et al.* [15] on tomato, where mycorrhizal colonization did not affect the quantity and quality of tomato yields in soilless cultivation. In line with that, the better water holding capacity in the medium equipped with trichocompost was believed to contribute to the undisturbed water absorption by plants resulting in good growth and development. The colonization of banana roots by AMF was not dependent on the growing medium; roots colonization by AMF ranging from 10% to 100% in all media composition.

The effect of mycorrhizae on plants depends on soil ecology, light conditions, plant and fungal species and their adaptability to each other [16, 17, 18, 19]. Although it did not

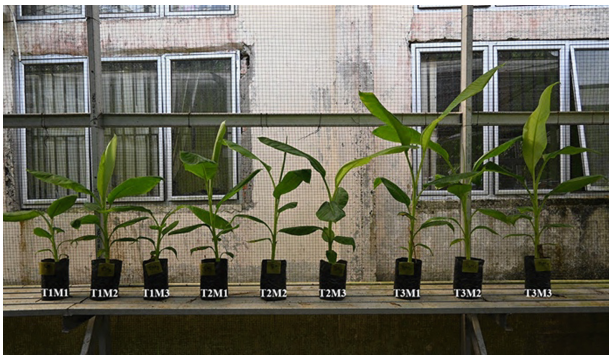

Fig. 2. Different performance of banana cv. Barangan Kuning grown on different media composition and doses of AMF at acclimatization stage (8 weeks after transplanting)

show any positive response under certain conditions, plants associated with mycorrhizae still had better growth than non-mycorrhizal plants due to better nutrition [20, 21].

4 Conclusions

1. Media consisting of a mixture of soil and trichocompost could increase the survival rate of banana cv. Barangan Kuning plantlets during acclimatization.
2. The best media for acclimatization of plantlets of banana cv. Barangan Kuning plantlets was 2 parts of soil + 1 part of trichocompost. This composition is also considered to be more economical than others.

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