

The Effectiveness of AMF Inoculum to Enhance the Potency of Papuan Crandallite Phosphate Rock and the Growth of Cocoa Seedling

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ABSTRACT

The Effectiveness of AMF Inoculum to Enhance the Potency of Papuan Crandallite Phosphate Rock and the Growth of Cocoa Seedling (A. Suparno): Phosphate rock fertilizer has slow solubility; therefore, it is suitable and more effective to be applied on annual crop plantation. To increase the solubility and the effectiveness of phosphate rock application, inoculation of the Arbuscular Mycorrhizal Fungi (AMF) is required. Experiments were conducted at the Cikabayan Farm, Bogor Agriculture Institute, West Java. The objective was to study the effectiveness of AMF inoculation in increasing the potency of Papuan Crandallite Phosphate Rock to the growth of cocoa seedlings. A factorial experiment was set up in a complete randomized design. The first factor was AMF inoculation, consisting of none AMF, Manokwari indigenous AMF and Mycofer AMF, and the second factor was five dosages of the Papuan Crandallite Phosphate Rock (PCPR), consisting of 0, 0.5, 1.0, 1.5 and 2.0 g P₂O₅ per seedling. The dosage of 2.0 g P₂O₅ SP-36 per seedling was used as a comparison. Cocoa seeds of F-1 Upper Amazon Hybrid (UAH) were collected from the Coffee and Cacao Research Center, Jember, East Java. The seedlings were grown in polybags of 20 cm x 30 cm size, with ultisol acid soil obtained from Jasinga (West Java) as the growing medium under 60% of shading net for four months. The media contained 17.03 cmol kg⁻¹ of exchangeable Al. The results showed that increasing the dosages of PCPR at the AMF inoculated seedlings resulted to the linear increase of shoot dry weight and P uptake by 50.14% and 64.88%, respectively; and this was lower than the inoculation of Manokwari indigenous AMF which increased shoot dry-weight by 66.30% and P uptake by 65.45%. Whereas the shoot dry-weight and P uptake of the non-mycorrhizal seedlings increased by 73.56% and 121.94%, respectively. Mycofer inoculants were found to be much more effective in increasing the shoot dry-weight by 127.55% and P uptake by 45.16% than that of the Manokwari indigenous AMF, which increased by 95.97% and 21.29% in shoot dry-weight and P uptake, respectively at the PCPR dosage of 2.0 g P₂O₅ per seedling as compared to the non-inoculated seedlings.

Keywords: *Acaulospora tuberculata*, *A. scrobiculata*, Crandallite Phosphate-rock, Cocoa, *Gigaspora margarita*, *Glomus aggregatum*, *G. etunicatum*, *G. manihotis*

INTRODUCTION

Indonesia is the third biggest country in the world after Ivory Coast and Ghana for the cocoa seed production; 40% of the world's cocoa seed market is dominated by Ivory Coast (Hartemink, 2005). The cocoa plantation in 2006 reached 1,191,742 ha with the production of 779,474 Mg (Dirjen Perkebunan, 2007). The development of cocoa plantation development is very essential because this sector plays an important role in providing job opportunity, income source for farmers and foreign exchange income.

The amount of fertile land is becoming somewhat limited today, therefore development of the cocoa plantation is going to the other directions to the marginal lands located outside Java island such as Sumatera, Sulawesi, Maluku, and Papua. Those lands are dry lands with low fertility level which require high amount of fertilizer. This condition may inhibit the development of cocoa plantation as high inputs of liming and fertilizer are needed.

The successful of cocoa plantation area development was started by the availability of good quality seeds, thus when they were planted, the seeds

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would have both high capability to grow and to produce optimally. To have good quality seedling, it needs a good treatment in the very beginning by inoculating bio agent such as Arbuscular Mycorrhiza Fungi (AMF) which is beneficial and also by fertilizing, so when the seeds are planted and having good care of, they will be able to adapt, grow and produce optimally.

Phosphate rock as the direct source of P has been widely used and becoming an economic alternative compared to industrial fertilizer (Kimiti and Smithson, 2002). PCPR deposit is the source of potential phosphate rock, however it has not been exploited yet. This high level of the phosphate rock has first been documented on Shroo's research in 1958 (Schroo, 1963). Different from the phosphate rock in other locations which is in the form of rock, the phosphate rock in Papua (Ayamaru) is in the form of soil. Subsequent research showed that the phosphate rock is composed of Crandallite mineral ($\text{CaAl}_3(\text{PO}_4)_2(\text{OH})_5 \cdot \text{H}_2\text{O}$). In Indonesia, this kind of phosphate rock deposit is only found in Ayamaru District, Papua.

It is known that cocoa plant has symbioses and a high dependency on AMF (Miyakasa and Habte, 2001). The AMF association with the plant's root plays an important role and becomes an efficient way to increase the uptake of P (Rice and Greenberg, 2000; Smith, 2002). This has a relation to the increasing uptake of P by the spreading of hyphal of Mycorrhiza which is more significant in low fertile soil (Garcia-Garrido *et al.*, 2000).

This research was carried out with the aim of funding the effectiveness of Manokwari indigenous AMF inoculum and Mycofer AMF in increasing the potency of Papuan Crandallite Phosphate Rock and the growth of cocoa seedling. The development of cocoa plantation depend on the availability of good quality seeds. Thus when the seeds are planted, it should produce good growth and high yield.

MATERIALS AND METHODS

The experiment was carried out at Cikabayan University Farm, IPB Bogor, Indonesia in 2008. The plant material used was Hybrid cocoa seedling F1 UAH collected from the Coffee and Cocoa Research Center in Jember, East Java. Growing media is the acid soil with Al_{dd} 17,03 cmol kg^{-1} . Papuan Crandallite Phosphate Rock (PCPR) which was obtained from Ayamaru District, West Papua.

The seedlings were grown for four months under 60% shading net. The experiment was set up in a Completely Randomized Design. The first factor was AMF inoculation, consisting of none AMF, Manokwari indigenous AMF and Mycofer; the second factor was five dosages of Papuan Crandallite Phosphate Rock (PCPR): 0, 0.5, 1.0, 1.5, and 2.0 g P_2O_5 per seedling, with 2,0 g P_2O_5 SP-36 per seedling used as a comparison. The experiment was conducted three times by using 20 cm x 30 cm sized poly bag which was filled by 3 kg soil. Each seedling was grown in each polybag with three polybags for each experimental unit.

The inoculum production was done by sorgum and zeolit as a growing media. The inoculum consists of propagule which is a mixture of spore, infected roots, hyphal and growing media. After Most Probable Number test, each plant was inoculated for 10 gram inoculum.

The observed variables were the growth of seedlings, P uptake, root colonization, acid phosphatase activity and the effectiveness of AMF inoculum.

Root colonization is measured by the following formula:

$$\text{Root colonization (\%)} = \frac{\text{number of the infected roots}}{\text{number of the observed roots}} \times 100\%$$

The effectiveness of AMF inoculum is measured by formula:

The effectiveness (%) =

$$\frac{\text{Mycorrhiza Plant} - \text{Non Mycorrhiza Plant}}{\text{Mycorrhiza Plant}} \times 100\%$$

The activity of acid phosphatase was measured by using Alef *et al.* (1998) method.

Data of the experimental result was analysed using Analysis of Variance and if the treatment showed a significant effect, it would be followed by LSD test. The effect of PCPR dosages to the growth of cocoa seedling was analysed by using regression correlation analysis. All the data were analyse using SAS v 9.0 software.

RESULTS AND DISCUSSIONS

Results

The research result showed that the use of PCPR in several dosages and AMF inoculation definitely influenced the growth of cocoa seedling, but there was no significant interaction between PCPR dosage

and type of inoculums. Seedling height, stem diameter, and root dry-weight in PCPR 2.0 g and 2.0 g SP-36 dosage did not show a significant difference (Table 1). PCPR 2.0 g dosage produce the greatest height of seedlings, stem diameter, and root dry-weight. The cocoa seedlings which were inoculated by Mycofer AMF (m_2) produced the highest height of seedlings, stem diameter and root dry-weight which account for 43.15cm, 7.26 mm, and 2.01 g respectively, and these were different from the seedling inoculated by indigenous AMF (m_1) and non AMF inoculation (m_0).

The Mycofer AMF inoculum is more effective than the indigenous AMF and non AMF. Comparing to non AMF cocoa seedling, Mycofer AMF inoculation increased the height, stem diameter, root dry-weight, and shoot-root ratio of cocoa seedling by 58.58%, 2.59%, 99.01%, and 9.96% respectively.

PCPR dosage and AMF inoculation significantly increased the growth of height, stem diameter, and root dry-weight in linear, but there was no interactive effect (Figure 1).

Based on the regression analysis, increasing of the dosage of PCPR produced a linear increase in

Table 1. Seedling height, stem diameter, roots dry-weight, and shoot-root ratio of cocoa seedling which were resulted by AMF inoculation in several levels of PCPR dosages.

Note: The number in one column followed by the same letter is not significantly different in LSD Test for 95%.
 m_0 : non AMF; m_1 : Manokwari indigenous AMF; m_2 : Mycofer AMF.

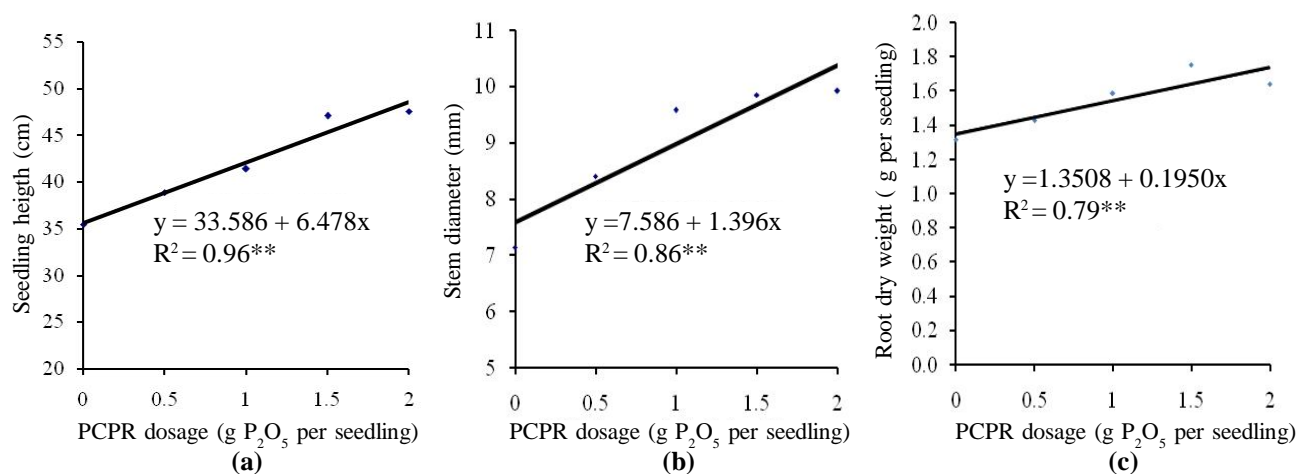


Figure 1. Seedling height (a), stem diameter (b), and root dry-weight (c) AMF inoculation resulted from several levels of PCPR dosage. **: very significant, *: significant.

Table 2. Shoot dry-weight response, shoot P uptake, root colonization, and acid phosphates activity of cocoa seedling to PCPR dosages at different AMF inoculums.

Note. *m₀*: non AMF, *m₁*: Manokwari indigenous AMF, *m₂*: Mycofer AMF.

shoot dry-weight, shoot P uptake, root colonization, and acid phosphates activity of cocoa seedling both with and without AMF inoculation (Table 2, Figure 2).

Comparing to Manokwari indigenous AMF, in all levels of PCPR dosage, Mycofer AMF inoculation always gave higher response in shoot dry-weight, shoot P uptake, root colonization and acid phosphate activity.

Discussion

The Mycofer AMF inoculated cocoa seedling's production were better than Manokwari indigenous AMF in all observed variables. This was due to the

root colonization that was high with many varieties of AMF species (Jansa *et al.*, 2004). Besides, the effectiveness of each AMF species in mixed inoculums determined the effectiveness as well. In general, the root colonization by Mycorrhizal was 40%, and therefore P mobility, microbe diversity, the use and the efficiency of P source were in the highest level (Mader *et al.*, 2003).

AMF Mycofer inoculation in cocoa seedling was more effective in nutrition supplying, especially P, compared to indigenous AMF. This is because there are many varieties of Mycofer inoculums and these species have been tested on other species. AMF Mycofer inoculum consists of *Glomus manihotis* (INDO-1), *Gigaspora margarita*, *Glomus etunicatum*

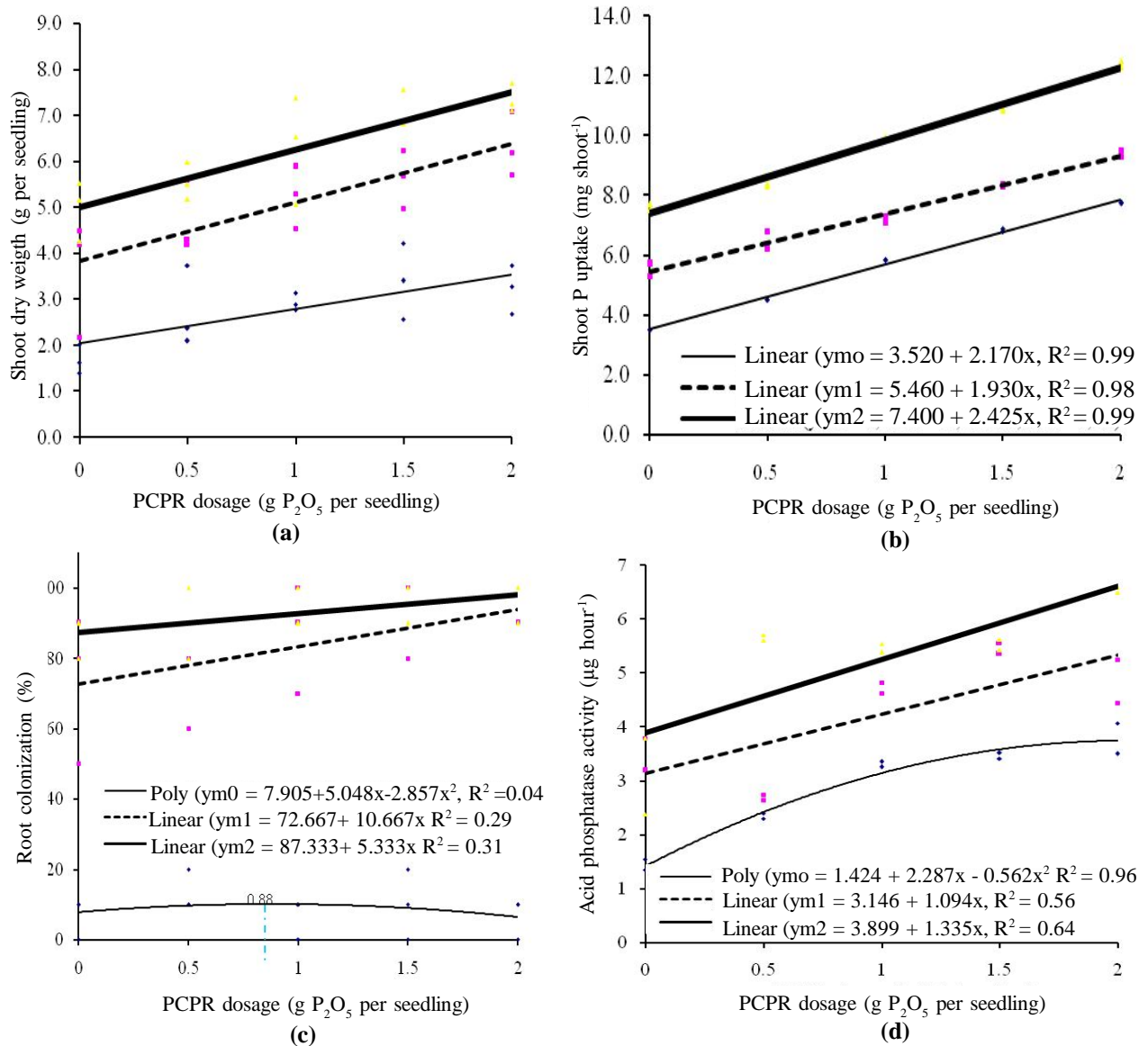


Figure 2. Shoot dry-weight (a), shoot P uptake (b), root colonization (c), and acid phosphatase activity (d) AMF inoculation result in various PCPR dosages; mo= non AMF, m₁= Manokwari indigenous AMF, M₂= Mycofer AMF.

(NPI-126) and *Acaulospora tuberculata* (INDO-2), while Manokwari indigenous AMF from the result of morphological spore identification in LIPI, Cibinong consists of three species: *Glomus aggregatum* Schenck and Smith, *Acaulospora scrobiculata* Trappe, and *Acaulospora tuberculata* Janos & Trappe

Acid phosphatase released by the root is an external mechanism in responding P deficiency that will make up P in soil and plant (Chen *et al.*, 2002). The increase of phosphatase acid activity in mycelium intra radical will also increase the transfer of P from

fungi to the plant (Ingrid *et al.*, 2002). George *et al.* (2006) stated that acid phosphatase secretion is a mechanism to increase P uptake of the plant. This research shows that Manokwari indigenous AMF is increasing the acid phosphatase activity of cocoa seedling root for 17.47% - 142.12% without non AMF inoculation, while inoculation with Mycofer AMF 58.64% - 79.39%. Acid phosphatase can hydrolyze P organic compound. This enzyme can be found in rhizosphere when the plant lack of P, thus it can increase the availability of P in rhizosphere (Tarafdar and Claassen, 2001; Wasaki *et al.*, 2003; Lambers *et al.*, 2006).

The most advantage association between AMF and root is when P level in soil or growing media is low but sufficient enough. If the root's P is very low, AMF will become parasitic, whereas if the P level is high, the plant will have sufficient P without AMF (Miyakasa *et al.*, 2003). Morgan *et al.* (2005) stated that when plants lack of some essential nutrients such as P, the symbiosis relationship between plants and Mycoriza will be mutuality and will promote the plants to grow. However, the advantage of symbiosis will decrease if the fertilizer is given more than an optimal dosage, because nutrient as available for the plant and the symbiotic function is decreased. Therefore, the fertilizer is still needed in low dosage but it does not inhibit the seed to grow and AMF is still functioning to help the seed in P uptake. According to Bolan *et al.* (1984), the arbuscular formation is sensitive to the P supply so an appropriate dosage of P supply is needed. Nagahashi *et al.* (1996) added that available P in high or low concentration will directly inhibit the development of AMF. Thus, P uptake in a nearly optimal dosage can improve the arbuscular formation.

During symbiosis, mycorrhiza obtain carbohydrate and other growing factors from host plant as the source of energy for its growth and development, while the plant itself can increase P and other nutrients uptake by the availability of hyphal in root that has mycorrhiza (Muchovej, 2002). Swift (2004) asserted that one of the positive colonization that effects of AMF on host plant is the ability of mycorrhiza in absorbing P from the soil and transferring to the host plant root. Smith *et al.* (2001) argued that P translocation is higher in the plant that has mycorrhiza, therefore cocoa seedling with mycorrhiza has better growth than the seedling without mycorrhiza. This is because P is one of some important nutrients in saving energy and structural integrity of the plant (Taiz and Zeiger, 2002).

Phosphorus (as phosphate, PO_4^{3-}) is an integral component of important compounds of plant cells, including the sugar-phosphates intermediates of respiration and photosynthesis, and the phospholipids that make up plant membranes. It is also a component of nucleotides used in plant energy metabolism (such as ATP) and in DNA, RNA, nucleic acids, coenzymes, phosphoprotein, dan phytic acid (Taiz and Zeiger, 2002). Therefore phosphorus is a critical element for growth, development, and reproduction of plant. Phosphorus is a key substrate in energy metabolism and nucleic acid biosynthesis, gene coding, and

membrane construction. Phosphorus also plays an important role in photosynthesis, respiration, and regulation of some enzymes in which it influences the growth and metabolism.

CONCLUSIONS

Mycofer inoculum is more effective in increasing PCPR potency and cocoa seedling growth than Manokwari indigenous inoculum. In AMF dosage of 2.0 g P_2O_5 per seedling, if comparing with the control, Mycofer AMF inoculation increased the growth of cocoa seedling by increasing the shoot dry-weight by 127.55% and Manokwari indigenous AMF 95.97%, while shoot P uptake increased by 45.16% and 21.29% in Mycofer AMF inoculation and Manokwari indigenous AMF respectively. The highest cocoa seedling is resulted by inoculation with Mycofer AMF (43.15 cm).

The growth of shoot dry-weight of cocoa seedling increased linearly by the increasing of PCPR dosage until 2.0 g of P_2O_5 per seedling. Without AMF inoculation, the application of PCPR dosage increased shoot dry-weight of cocoa seedling by 93.32%, while inoculated seedling by the indigenous AMF and Mycofer AMF increased by 66.30% and 50.14% respectively.

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