2. EJSR-Arbuscular My-Vol.127-No.3 tahun 2014

by Paper UNIPA

Submission date: 29-Apr-2019 07:26AM (UTC+0700)
Submission ID: 1120844294
File name: 2._EJSR-Arbuscular_My-Vol.127-No.3_tahun_2014.doc (2.11M)
Word count: 3511
Character count: 20787

Arbuscular Mycorrhizal Fungi Increase Growth of Cocoa Seedlings Applied with Papuan Crandallite Phosphate Rock

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Abstract

The mycorrhizal inoculation, consisting of none mycorrhizal, indigenous mycorrhizal from cocoa rhizosphere in Manokwari, and Mycofer from Bogor Agriculture Institute. Papuan Crandallite Phospahte Rock was five consisting of 0, 0.5, 1.0, 1.5 and 2.0 g P_2O_5.seedling^{-1}. Cocoa seeds of F-1 Upper Amazone Hibrid were collected from the Indonesian Coffee and Cocoa Research Institute. The seedlings were grown in polybags with Ultisol acid soil obtained from Jasinga, Bogor. The seedlings were grown for four months under 60 % shading net.

The results showed that increasing phosphate rock dosages in the mycorrhizal inoculated seedlings resulted in linear increase of shoot dry weight and P uptake by 50.14% and 64.88%, respectively; and this was lower than the indigenous mycorrhizal inoculation which increased shoot dry-weight by 66.30% and P uptake by 65.45%. Whereas 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 shoot dry-weight by 127.55% and P uptake by 45.16% than that of the indigenous mycorrhizal, which increased by 96.97% and 21.29% in shoot dry-

weight and P uptake, respectively at phosphate rock dosage of 2.0 g P_2O_5.seedling^{-1} as compared to the non-inoculated seedlings.

Keywords: cocoa, growth, mycorrhiza, phosphate rock, seedling

Introduction

Ivory Coast covers 40% of the world's cocoa seed market and becomes the leading cocoa seed production in the world. Whereas Indonesia is the third biggest country in the world after Ivory Coast and Ghana (Hartemink, 2005). The cocoa plantation reached 1,191,742 ha with the production of 779,474 tones in 2006 (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 development of the cocoa plantation is directed to the marginal lands located outside Java island such as Sumatera, Sulawesi, Maluku, and Papua because the amount of fertile land has declined todays. Those lands are dry lands with low fertility level which require high fertilizer amount. This condition may inhibit the development of cocoa plantation as vast amount of liming and fertilizer are needed.

To achieve good grow and yield of cocoa, it needs good quality seedlings. To have good quality seedling, a good treatment needed in the very beginning by inoculating bio-agent such as beneficial Arbuscular Mycorrhizal Fungi (AMF) which and also by fertilizing.

Compared to industrial fertilizer, phosphate rock as the direct source of P has been widely used and becoming an economic alternative (Kimiti and Smithson, 2002). Papuan Crandallite Phosphate Rock (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 ($CaAl_3(PO4)_2(OH)_5 H_2O$). In Indonesia, this kind of phosphate rock deposit is only found in Ayamaru District, Papua.

It is known that cocoa plant symbioses and depend highly 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 P uptake (Rice and Greenberg, 2000; Smith, 2002). This relate to the increasing P uptake by the spreading of mycorrhyza hyphal which is more significant in low fertile soil (Garcia-Garrido *et al*., 2000).

This research was carried out with the aim to observe the effectiveness of Manokwari indigenous AMF inoculum and Mycofer AMF in increasing the potency of PCPR on the growth of cocoa seedlings. The development of cocoa plantation depend on the availability of good quality seeds. Thus when the seeds are planted, it can produce good growth and high yield.

Materials and Methods

The experiment was carried out during 2009 at Cikabayan University Farm, IPB Bogor, Indonesia. The plant material was Hybrid cocoa seedling F-1 Upper Amazone Hibrid (UAH), collected from the Indonesian Coffee and Cocoa Research Institute (ICCRI) in Jember, East Java. Growing media was the acid soil with Al_{dd} 17.03 cmol.kg^{-1}. PCPR which was obtained from Ayamaru District, West Papua-Indonesia.

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, consisted of none AMF (mo), indigenous AMF from cocoa rhizosphere Manokwari (m_1), and Mycofer from IPB Bogor (m_2); the second factor was five dosages of PCPR 0, 0.5, 1.0, 1.5, and 2.0 g P_2O_5.seedling^{-1}, with 2.0 g P_2O_5 SP-36.seedling^{-1} was as a comparison. The experiment was conducted three times by using 20 cm x 30 cm sized polybags which was filled with 3 kg soil. Each seedling was grown in each polybag with three polybags for each experimental unit.

The inoculum production was done by *Sorghum bicolor* band zeolit as a growing media. The inoculum consists of propagule which is a mixture of spore, infected root, hyphal and growing media. After Most Probable Number test, each plant was inoculated with 10 grams inoculum.

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

Root colonization is measured by the following formula:

$$Root\ colonization\ (\%) = \frac{number\ of\ infected\ roots}{number\ of\ observed\ roots} x100\%$$

The effectiveness of AMF inoculum is measured by formula:

$$The\ AMF\ effectiveness\ (\%) = \frac{Mycorrhyza\ Plant - Non\ Mycorrhyza\ Plant}{Mycorrhyza\ Plant} x100\%$$

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

$$Shoot\ P\ uptake = Shoot\ P\ content\ x\ shoot\ dry\ weight$$

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

Results and Discussion

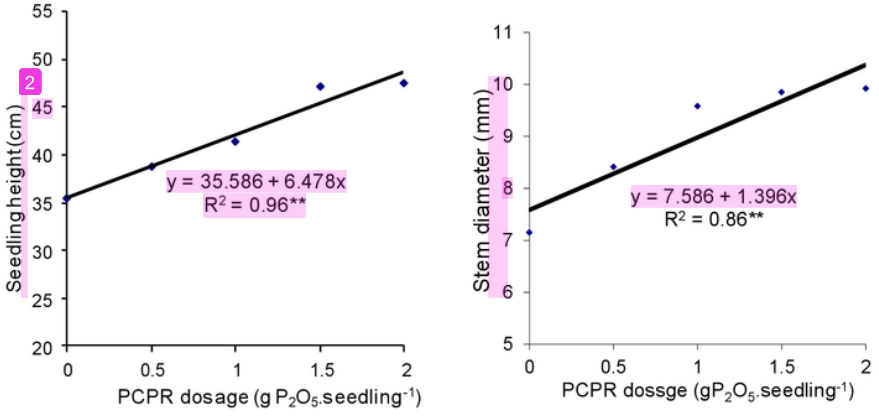
The use of PCPR in several dosages and AMF inoculation influenced significantly the growth of cocoa seedling, but there was no significant interaction between PCPR dosages and type of inoculums. Seedling height, stem diameter, and root dry-weight in 2.0 g PCPR and 2.0 g SP-36 dosage did not show a significant difference (Table 1). PCPR 2.0 g produced the highest seedlings, stem diameter, and root dry-weight. The cocoa seedlings which were inoculated with Mycofer AMF (m_2) produced the tallest seedlings, highest stem diameter and root dry-weight which account for 43.15cm, 7.26 mm, and 2.01 g respectively, and these were significantly different from the seedling inoculated with indigenous AMF (m_1) and non AMF inoculation (m_o).

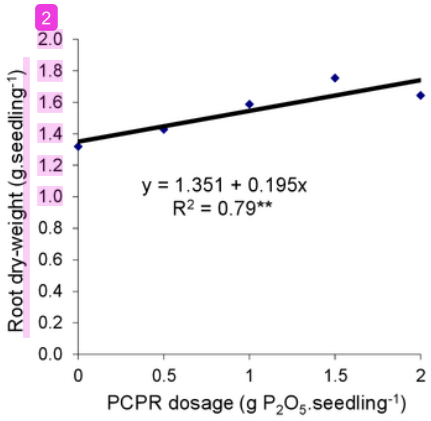
Table 1: Seedling height, stem diameter, roots dry-weight, and shoot-root ratio of cocoa seedling which were resulted from AMF inoculation in several levels of PCPR dosages

Treatment	Seedling Height (cm)	Stem Diameter (mm)	Root Dry-Weight ($g.seedling^{-1}$)	Shoot-root Ratio
PCPR dosage ($g\ P_2O_5.seedling^{-1}$):				
Control: 0	32.48 b	6.15 c	1.32 c	2.57 d
0.5	34.83 ab	6.41 bc	1.43 bc	3.05 bc
1.0	36.39 a	6.58 ab	1.59 abc	3.01 c
1.5	37.00 a	6.85 a	1.75 a	3.07 bc
2.0	38.56 a	6.92 a	1.64 ab	3.47 b
SP-36: 2.0 $g\ P_2O_5.seedling^{-1}$	38.06 a	6.97 a	1.68 ab	3.90 a
AMF Inoculum :				
m_o	27.21 c	5.69 b	1.01 c	3.01 a
m_1	38.00 b	6.93 b	1.69 b	3.21 a
m_2	43.15 a	7.26 a	2.01 a	3.31 a
Effectiveness (%) :				
m_1 vs m_0	39.65 b	21.79 b	67.33b	6.64 b
m_2 vs m_1	13.55 c	4.76 c	18.93c	3.12 c
m_2 vs m_0	58.58 a	27.59 a	99.01a	9.96 a

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

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





The Mycofer AMF inoculum is more effective than the indigenous AMF and non AMF. Compared to non AMF cocoa seedling, Mycofer AMF inoculation increased height, stem diameter, root dry-weight, and shoot-root ratio of cocoa seedling by 58.58%, 27.59%, 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 between both of treatments (Figure 1).

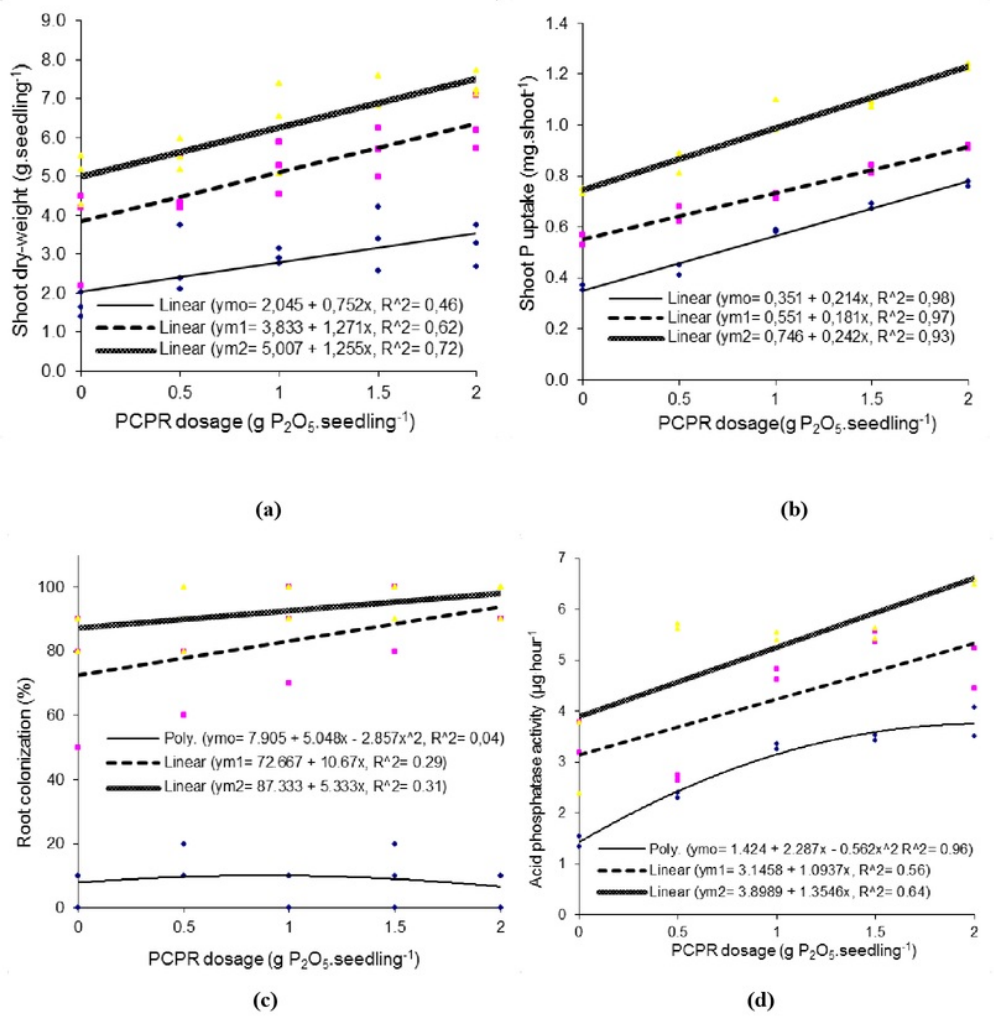
Based on regression analysis, increasing the dosage of PCPR produced a linear increase in shoot dry-weight, shoot P uptake, root colonization, and acid phosphatase activity of cocoa seedling both with and without AMF inoculation (Table 2, Figure 2).

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

Treatment	PCPR dosage (g P_2O_5.seedling^{-1})					SP-36	Curve Response
	0	0.5	1.0	1.5	2.0		
	..... Shoot dry-weight (g.seedling^{-1})						
mo	1.68	2.74	2.93	3.40	3.23	3.88	Linear
m_1	3.62	4.70	5.24	4.70	6.33	7.82	Linear
m_2	4.98	5.55	6.33	7.09	7.35	7.85	Linear
Effectiveness (%):							
m_1 vs mo	115.48	71.53	78.84	38.23	95.97	101.55	
m_2 vs mo	196.43	102.55	116.04	108.53	127.55	102.32	
m_2 vs m_1	37.57	18.09	20.80	50.85	16.11	0.0030	
	..... P uptake (mg.shoot^{-1}).....						
mo	3.50	4.30	5.85	6.85	7.75	8.20	Linear
m_1	5.50	6.50	7.05	8.35	9.40	13.60	Linear
m_2	7.60	8.20	9.90	10.85	11.25	13.70	Linear
Effectiveness (%):							
m_1 vs mo	57.14	51.16	20.51	21.90	21.29	65.85	
m_2 vs mo	117.14	90.70	69.23	58.39	45.16	67.07	
m_2 vs m_1	38.18	26.15	40.43	29.94	19.68	0.007	
	.... Root colonization (%)						
mo	10.00	13.33	6.67	10.00	6.67	3.33	Quadratic
m_1	73.33	73.33	86.67	93.33	96.67	13.33	Linear
m_2	86.67	90.00	93.33	100.00	100.00	16.67	Linear
Effectiveness (%):							
m_1 vs mo	633.30	450.11	1199.40	833.30	1349.33	300.30	
m_2 vs mo	766.70	575.17	1299.25	900.00	1399.25	400.60	
m_2 vs m_1	18.19	22.73	7.68	7.15	3.44	25.06	
	..... Acid phosphatase activity (µg.g^{-1}.hour^{-1}).....						
Mo	1.44	3.47	4.12	3.30	3.34	2.33	Quadratic
m_1	3.49	5.45	4.84	5.45	3.69	3.56	Linear
m_2	3.59	5.52	6.54	5.46	5.68	5.16	Linear
Effectiveness (%):							
m_1 vs mo	142.36	57.06	17.48	65.15	10.48	52.79	
m_2 vs mo	149.31	59.08	58.73	65.45	70.06	121.46	
m_2 vs m_1	2.87	1.28	35.12	0.18	53.93	44.94	

Note: *mo*: non AMF; m_1: Manokwari indigenous AMF; m_2 : Mycofer AMF; vs : versus

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, m_1= Manokwari indigenous AMF, m_2= Mycofer AMF.



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

The Mycofer AMF was better inoculated with cocoa seedling than Manokwari indigenous AMF in all observed variables. This was due to higher root colonization with many varieties of AMF species (Jansa *et al*., 2004), as well as the effectiveness of each AMF

species in mixed inoculums. In general, root colonization by Mycorrhyzal was 40%, and this shows the highest level of P mobility, microbe diversity, the use and the efficiency of P source (Mader *et al*., 2003).

AMF Mycofer inoculation in cocoa seedling was more effective in nutrition supply, 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* (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 & Smith, *Acaulospora scrobiculata* Trappe, and *Acaulospora tuberculata* Janos & Trappe.

Acid phosphatase released by plant root is an external mechanism in responding P deficiency and that will make up P in soil and plant (Chen *et al*., 2002). The increase of acid phosphatase 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 showed that Manokwari indigenous AMF increased the acid phosphatase activity of cocoa seedling root by 17.47% - 142.12% without non AMF inoculation, while inoculation with Mycofer AMF increased by 58.64% – 79.39%. Acid phosphatase can hydrolize 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 Claaessen, 2001; Wasaki *et al*., 2003; Lambers *et al*., 2006).

The most advantage association between AMF and plant root is when P level in soil or growing media is low but sufficient. 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). 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 (Morgan *et al*., 2005). However, the advantage of symbiosis will decrease if fertilizer is given more than an optimal dosage, because nutrient is already available sufficiently for the plant. In this case, the fertilizer is still needed in low dosage, and but it will not inhibit the seed to grow because AMF is still functioning to help the seed in P uptake. According to Bolan *et al*. (1984), the arbuscular formation is sensitive to P supply, therefore 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 an 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 avalability of mycorriza hyphal in roots (Muchovej, 2002). Swift (2004) asserted that one of the positive colonization that effects AMF on host plant is the ability of mycorriza in absorbing P from soil and transferring to the host plant roots. On the other hand, according to Smith *et al*. (2001) P translocation is higher in plant that has mycorriza. Therefore, cocoa seedling with mycorriza has better growth than the seedling without mycorriza, 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 which 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 phothosynthesis, respiration, and regulation of some enzymes which influences growth and metabolism.

Conclusion

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 .seedling^{-1}, if comparing with the control, Mycofer AMF inoculation increased the growth of cocoa seedling by increasing the shoot dry-weight 127.55% and Manokwari indigenous AMF 95.97%. Whereas 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 from inoculation with Mycofer AMF (43.15 cm).

The growth of shoot dry-weight of cocoa seedling increased linearly by increasing PCPR dosage up to 2.0 g of P_2O_5.seedling^{-1}. Without AMF inoculation, the application of PCPR dosage increased shoot dry-weight of cocoa seedling 93.32%, while inoculated seedling with the indigenous AMF and Mycofer AMF increased by 66.30% and 50.14% respectively.

Acknowledgments

We are gratefull to the Agroclimate Laboratory, Agriculture Faculty of The State University of Papua Manokwari-Indonesia, Laboratory of Biotechnology on Forest and Environment, Research Center of Natural Resource and Biotechnology IPB, Bogor-Indonesia, and everyone involved in this research.

Coauthor contributions:

S. Yahya, Sudradjat, Y. Setiadi, K. Idris developed the concept and designed experiments. Y. Setiadi and Sudrajat performed arbuscular mycorrhizal fungi identifications, seedling inoculations, roots colonization and acid phosphatase analysis. P uptake of cocoa seedling was performed by K. Idris.

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