3. JAS-Inoculation of-Vol.7-No.5 tahun 2015

by Paper UNIPA

Inoculation of Arbuscular Mycorrhizal Fungi Increase the Growth of Cocoa and Coffee Seedling Applied With Ayamaru Phosphate Rock

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Abstract

Researh was carried out aimed to study the response of cocoa and coffee seedlings inoculated by Arbuscular Mycorrhizal Fungi (AMF) and applied with Ayamaru Phosphate Rock (APR). The research was cunducted at Bogor Agriculture Institute (IPB) and Papua University (Unipa) Manokwari Indonesia. Cocoa seed (Upper Amazone Hybrid F-1) and Arabica coffee seed (*Coffea arabica*) were collected from the Indonesian Coffee and Cocoa Research Institute (ICCI) Jember Indonesia. Cocoa dan coffee seedling were planted in polybag with acid soil medium, under 60% shading net. Cocoa and coffee seedlings were grown for 16 weeks after planting. AMF inoculum used Mycofer that was collected from IPB consisted of four species i.e *Gigaspora margarita* (INVAM-105), *Glomus etunicatum* (NPI-126), (*Acaulospora tuberculata* (INDO-2), *Glomus manihotis* (INDO-1) and indigenous AMF Manokwari consisted of three species i.e *A. tuberculata* Janos & Trappe, *A. scrobiculata* Trappe, and *G. aggregatum* Schenck & Smith. Ayamaru Phosphate Rock (APR) was collected from Ayamaru District, West Papua, Indonesia. The result showed that the growth of cocoa seedling applied with APR up to 4 g P_2O_5.seedling^{-1} and the growth of coffee seedling applied with APR up to 8 g P_2O_5.seedling^{-1} increased linearly. Compared to control (without AMF), inoculation of AMF Mycofer increased the cocoa seedling's height up to 50.58% and shoot dry-weight 127.55%, whereas height of coffee seedling increased by 27.29% and shoot dry-weight by 121.21%. For all APR dosage, the growth of cocoa and coffee seedling inoculated with AMF Mycofer inoculum was better than inoculated by indigenous AMF and without AMF (control).

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

1. Introduction

Arbuscular Mycorrhiza Fungi (AMF) is widely known to have an important role in improving growth, yield, and quality of agriculture plants, horticulture, plantation, forestry, recovering land degradation and stabilizing upland ecosystem (Vosátka & Albrechtová, 2009; Gianinazzi et al., 2010).

Association between AMF and plant root was formed by the obligate fungi system with approximately 80 – 90 % of upland plants (Wang & Qui, 2006) or approximately 73% of flowering plant family (Brundrett, 2009), both those that have root and without root (Read et al., 2000; Pressel et al., 2010), and they also have mutualism characteristic which covering 83% of dicotyledone and 79% of monocotyledone (Swift, 2004).

Swift (2004) states that one of the several significant responses of AMF infection on host plant is the increase of P uptake that mainly due to the capability of AMF to absorb P from soil and transfer it to the host plant roots. When the plant is in deficiency of P or N, this symbiotic association will be advantage and improve plant growth (Morgan et al., 2005).

Cocoa and coffee are perennial plants that are able to symbiotic with AMF to improve their plant growth. Miyakasa & Habte (2001) state that cocoa and coffee are depend highly on symbiotic with AMF. Mutualism symbiotic between AMF and plants is beneficial for plants in enhancing growth and yield, improving plant vitality, and increasing minerals absorbtion area by root plants, due to smaller diameter of AMF hyphae than hair diameter of plant root (Sylvia, 2005).

Generally, great number of high quality seedlings are expected by seedling producer. High quality seedlings produced by breeding or genetic selection, culture technic, planting method, and accurate plant management will determine the success of seedling growth in the field (van den Driessche, 1994; South, 2000; Carneiro et al., 2007), particularly at marginal lands. Marginal lands are generally acid soil which P is bounded by Fe and Al. This causes unavailability of P to plant and reduces plant growth. Cocoa and coffee are perennial plants, therefore the effectiveness of AMF inoculation can be more optimal if the inoculation process was done in the seedling period and as a result when transferred to the field, plants will grow and yield better.

Cacao and coffee are exported commodities which generate high economic income, and these commodities have been broadly cultivated. Therefore, researh was carried out to study the growth of cocoa and coffee seedlings in response to the inoculation of Arbuscular Mycorrhizal Fungi (AMF) and applied with Ayamaru Phosphate Rock (APR). Effective AMF will produce good quality of cacao and coffee seedlings and this will increase fertilizer efficiency.

2. Materials and Methods

The experiment was carried out in 2009 at Cikabayan University Farm, IPB Bogor, Indonesia and at Unipa's Green House in 2012. The plant material was Hybrid cocoa seedling F-1 Upper Amazone Hibrid (UAH) and Arabica coffee 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 $cmol.kg^{-1}$. APR which was obtained from Ayamaru District, West Papua Indonesia.

The plantlets were grown for four months under 60 % shading net. The experiment was set up in a Completely Randomized Design. For cocoa seedling trial, 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 APR 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. For coffee seedling trial, 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 APR 0, 2, 4, 6, and 8 g $P_2O_5.seedling^{-1}$, with 2.0 g P_2O_5 SP-18.$seedling^{-1}$ was as a comparison.

The experiment was conducted three times by using 20 cm x 30 cm sized polybag which was filled with 3 kg soil. Each seed 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 consisted of propagule which was a mixture of spore, infected root, hyphal and growing_media. After Most Probable Number test, each plant was inoculated by 10 grams inoculum.

The observed variables were growth of seedlings, shoot dry-weight, leaf number, seedling height, stem diameter, root dry-weight, shoot-root ratio, shoot P uptake, root colonization, 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:

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

P uptake was measured by:

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

The data of the experimental result was analysed by using Analysis of Variance, and followed by LSD test if the treatment showed a significant effect. LSD test was used to compare the effect of AMF between APR and SP-36 application. The effect of APR dosages levels to the growth of cocoa and coffea seedling was analysed by regression correlation analysis. All the data were analysed using SAS v 9.0 software.

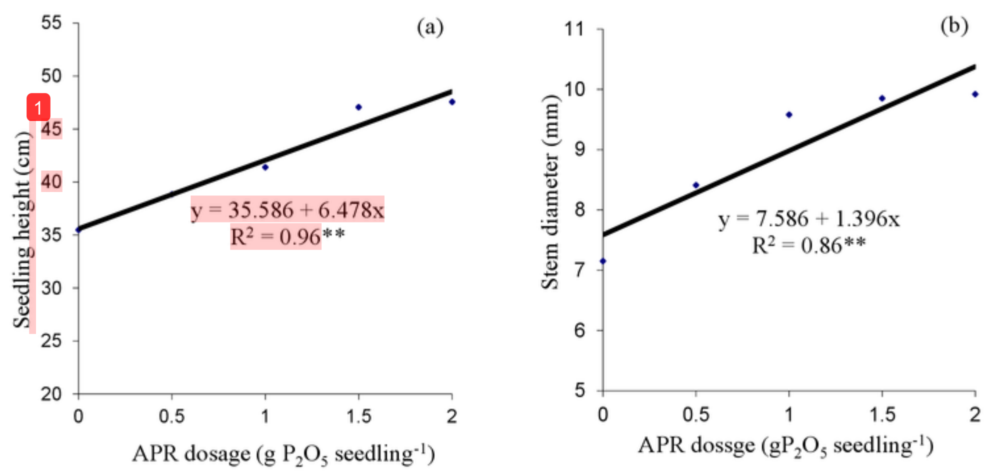
3. Result and Discussion

Cocoa and coffee belong to perennial plant that depend highly on symbiotic with AMF. AMF inoculation and APR application increased growth of both of cocoa and coffee seedlings. As the application level of APR dosage increased the seedling growth also increased at all APR dosage levels. AMF inoculation produced better response on the growth of cocoa and coffee seedling (Table 1,3). AMF Mycofer was more effective to increase the growth of cocoa and coffee seedling if compared to seedling without AMF and seedling inoculated by indigenous AMF (Figure 1, 3).

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

Treatment	Seedling Height (cm)	Stem Diameter (mm)	Root Dry-Weight (g.seedling^{-1})	Shoot-root Ratio
APR 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
LSD Test (95%)	*3.93*	*0.49*	*0.29*	*0.42*
AMF Inoculum :				
mo	27.21 C	5.69 C	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	21.79	67.33	6.64
m_2 vs m_1	13.55	4.76	18.93	3.12
m_2 vs m_0	58.58	27.59	99.01	9.96

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.



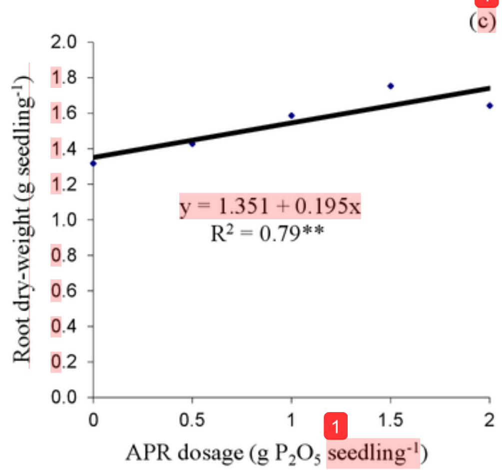


Figure 1. The relationship of seedling height (a), stem diameter (b), and root dry-wight (c) of cocoa seedlings to APR dosage levels. *Note*: **: highly significant, *: significant.

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

Treatment	APR 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	

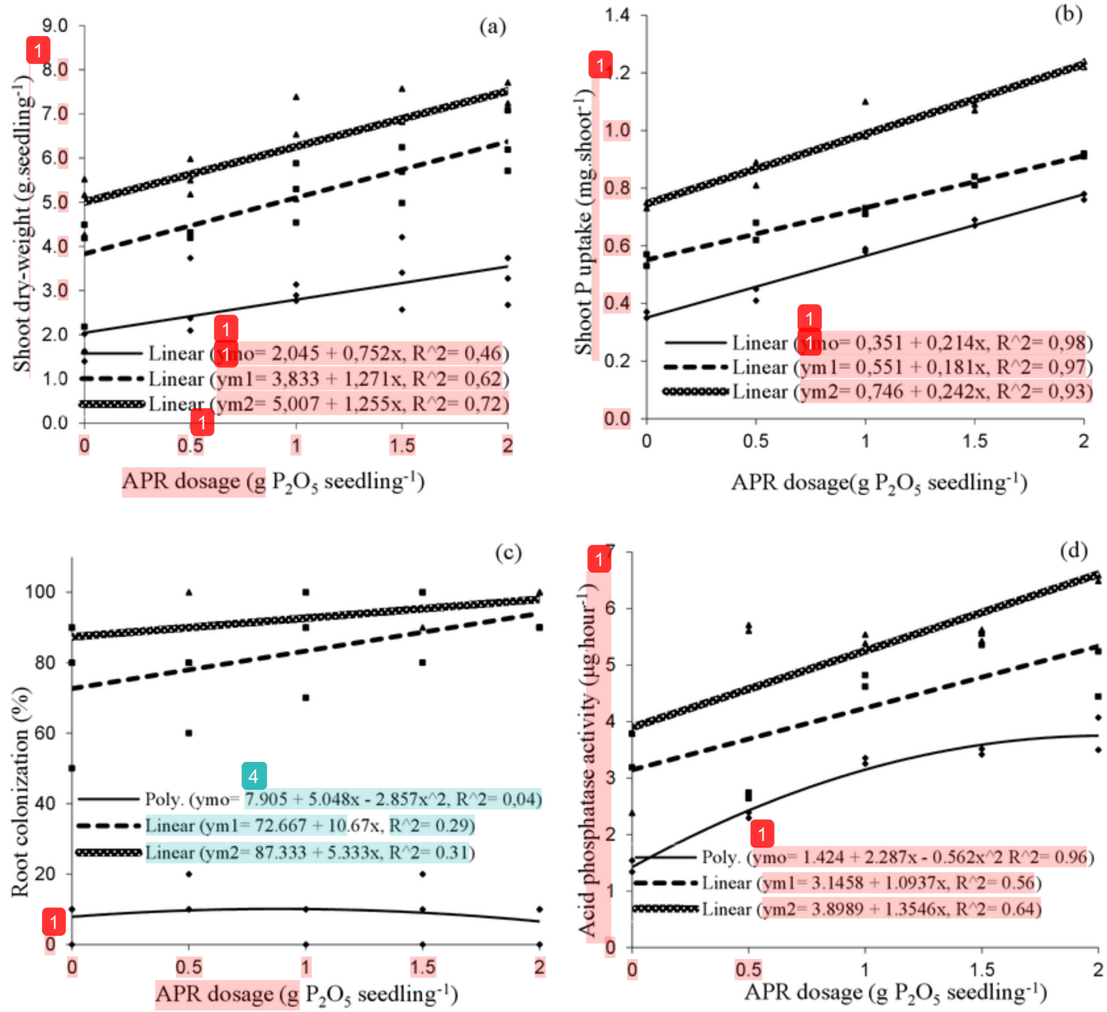


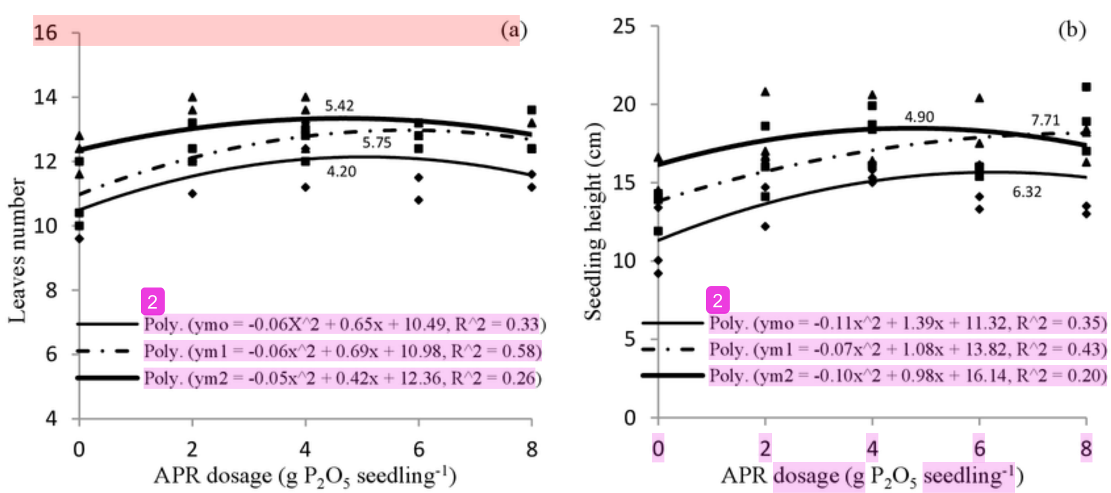
Figure 2. The relationship of Shoot dry-weight (a), shoot P uptake (b), root colonization (c), and acid phosphatase activity (d) of cocoa seedlings to APR dosage levels. *Note*: mo= non AMF, m_1= Manokwari indigenous AMF, m_2= Mycofer AMF.

The interaction between AMF inoculation and APR dosage level affected leaf number, shoot dry-weight, P uptake, and root AMF colonization of cocoa seedling, whereas it affected on root AMF colonization only in the coffee seedling (Table 3; Figure 3,4). However, when APR dosage level was increased to 8 g P_2O_5.seedling^{-1} on coffee seedling, the level of root AMF colonization, acid phosphatase activity, leaves number, stem diameter, seedling height, shoot dry weight decreased, while P uptake had still increased. However, AMF Mycofer always produced good results in all dosage levels both on cocoa and coffee seedlings. This shows that effective AMF inoculation is needed to produce high quality seedlings.

Table 3. Effect of APR dosage and AMF Inoculum on leaves number, seedling height, stem diameter, shoot dry weight, root dry weight, and root-shoot ratio of coffee seedling

Treatment	Leaves number	Seedling height (cm)	Stem Diameter (mm)	Shoot dry weight (g)	Root dry weight (g)	Root-shoot ratio
APR dosage (g.seedling^{-1}):						
Control	11.16 b	13.38 b	2.41 b	3.74 b	1.52 b	344.79 a
2	12.47 a	16.28 a	3.19 a	5.36 a	1.96 ab	317.94 a
4	12.73 a	17.36 a	3.07 a	6.07 a	2.86 a	242.69 a
6	12.52 a	16.03 a	3.09 a	5.28 a	2.28 ab	278.80 a
8	12.48 a	17.50 a	3.13 a	5.98 a	2.56 ab	276.45 a
SP-18 (2 g. seedling^{-1})	12.22 a	15.80 a	3.08 a	5.84 a	2.80 a	229.83 a
LSD Test (95%)	*0.86*	*1.93*	*0.51*	*1.29*	*1.12*	*120.33*
AMF inoculum:						
m_0	11.52 C	14.00 C	2.58 B	3.44 C	1.41 C	304.70 A
m_1	12.32 B	16.34 B	3.05 A	5.39 B	2.24 B	293.85 A
m_2	12.96 A	17.82 A	3.36 A	7.30 A	3.34 C	246.69 A
Effectiveness (%):						
m_0 vs m_1	6.94	16.71	18.22	56.69	58.88	3.56
m_0 vs m_2	12.50	27.29	30.23	112.21	136.88	19.04
m_1 vs m_2	5.19	10.57	10.16	35.44	49.11	16.05

Note : The number in each column followed by the same letter are not significantly different in LSD Test for 95%. m_0= without AMF, m_1= Indigenous AMF, m_2= AMF Mycofer, SP= Super Phosphate, vs= versus.



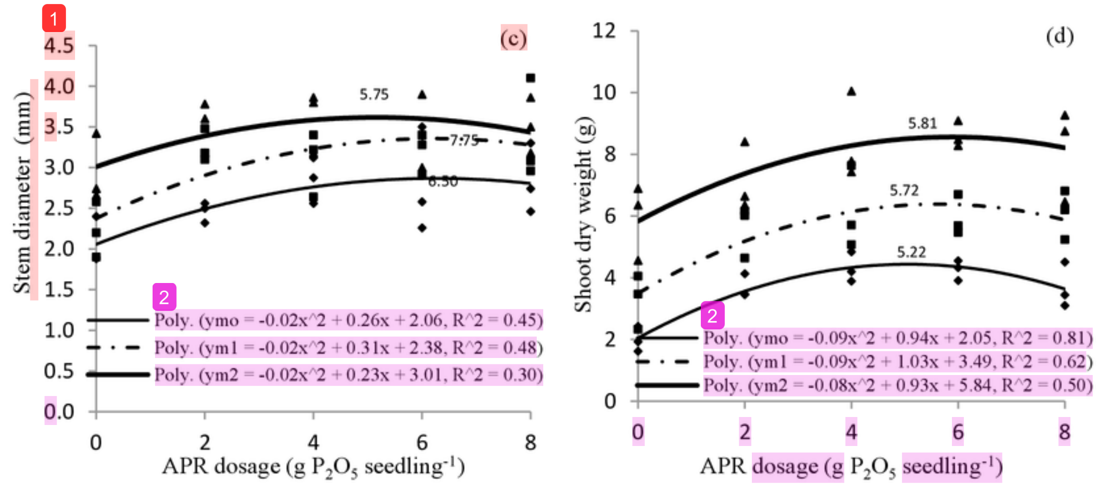
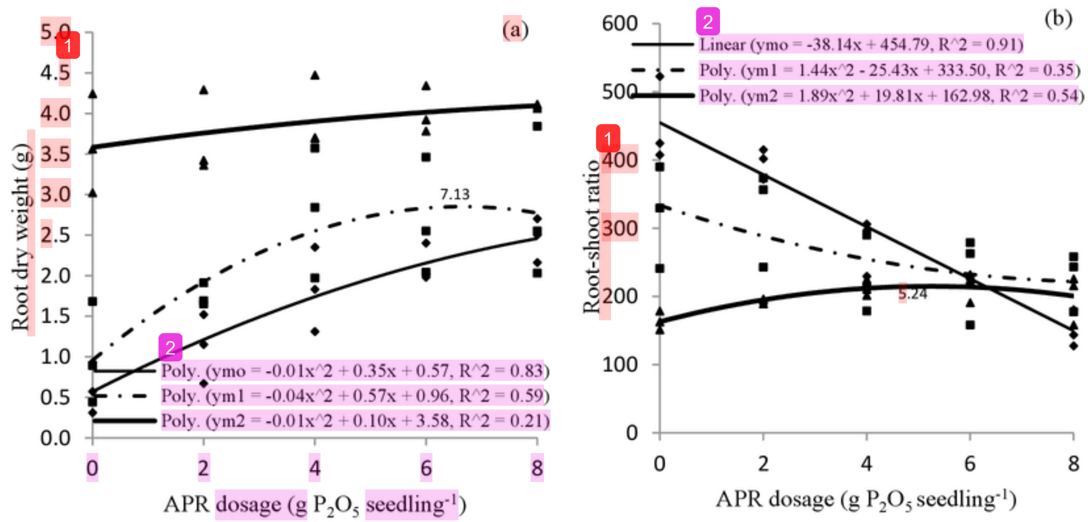


Figure 3. The interaction curve response of leave number (a), seedling height (b), stem diameter (c) shoot dry weight (d) as resulted from APR dosage levels with different AMF inoculum on coffee seedling. *Note*: mo= non AMF, m_1= Indigenous AMF, m_2= Mycofer AMF.



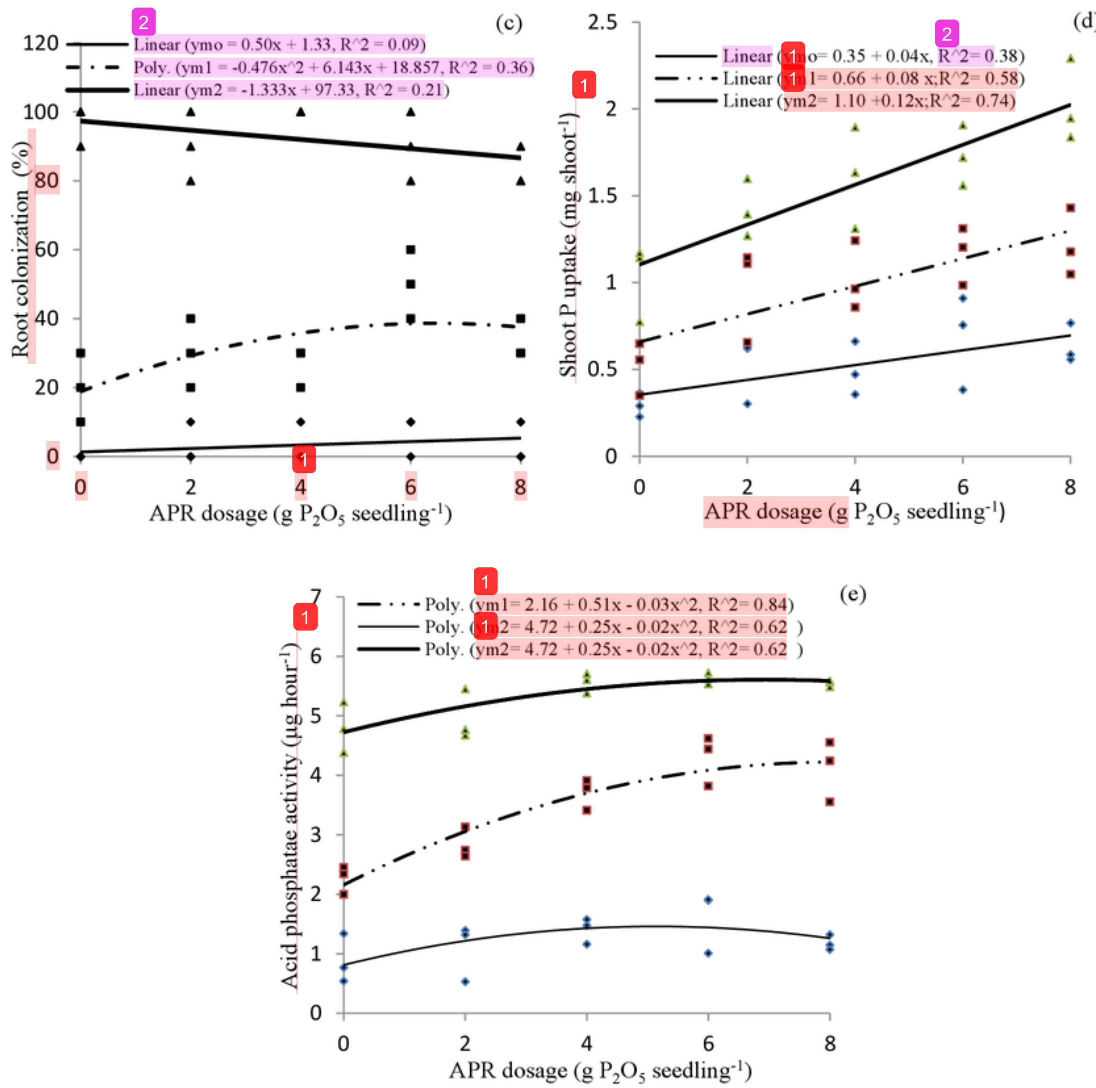


Figure 4. The interaction curve response of root dry-weight (a) root-shoot ratio (b) root colonization (c) shoot P uptake (d) acid phosphatase activity (e) as resulted from APR dosage levels with different AMF inoculum on coffee seedling. *Note*: mo= non AMF, m_1= Indigenous AMF, m_2= Mycofer AMF.

The utilization of low release P such as APR and bones powder even though in high dosage application, was effective to maintain AMF and to increase plant growth (Cardoso, 1996; Nikolaou et al., 2002). P content was reported to negatively correlated ((Amijee et al., 1989; Koide & Li, 1990), no correlation (Tawaraya et al., 1996), or positively correlated (Carrenho et al., 2001) with AMF, depends on AMF species, plant species, P source and its solubility (Cardoso, 1996; Tawaraya et al., 1996; Nikolaou et al., 2002; Bhadalung et al., 2005). Maximum AMF symbiotic was achieved if soil P content was not more than 50 $mg.kg^{-1}$ (50 ppm) ((Ishii, 2004).

Cocoa and coffee seedling which are inoculated with AMF grew well because the AMF infected the root, intraradical hyphae (IH) and extraradical hyphae (EH) developed and spread, hence it produced positive effect on plants. The spreading of EH in soil was soon formed after root colonization. EH produce multiple function such as the transport of water and essential mineral, spore production, soil aggregation, and host plant protection from pathogen. The role of EH in essential mineral transport especially organic P, is important because EH is able to reach out the depletion zone, that is unreachable and unavailable to plant roots (Zhu et al., 2001).

The contribution of AMF on water and organic P uptake was affected by AMF species, plant, and environment which indicated that the functional suitability between AMF and plant/crop is not always be related to its colonization (Pearson & Jakobsen, 1993; Burleigh et al., 2002). AMF inoculation was done one time only, which was in planting time, but the result will be in a long time effect. This method could reduce the investment on fertilizer and pesticide supply, and therefore farmer able to produce safe agriculture products with high quality and lower in cost.

The use of AMF inoculation is proven in improving the resistance of plant to biotic and abiotic stress that result to reduce fertilizer supply and pesticide investment (Herrera et al., 1993; Douds & Johnson, 2007; Vosatka & Albrechtova, 2009; Koltai & Kapulnik, 2010). The application of AMF inoculant could produce more productive plants and from stable ecosystem (van der Heijden et al., 1998; Siddiqui et al., 2008; Cameron, 2010), especially in restoring the degradation land (Herrera et al., 1993). AMF mycofer inoculant shows better effect to the growth of cocoa and coffee seedling. This is presumably because of AMF Mycofer consists of four species, and hence the benefit of each species can support one another.

Mycofer AMF is more effective because it consists of four AMF species i.e *Gigaspora margarita, Acaulospora crobiculata, Glomus manihotis,* and *Glomus etunicatum.* Positive effect of *G. etunicatum* could be nutrient functionality such as increasing in macro-nutrient uptake (Govindarajulu et al., 2005; Li et al., 2006), micro-nutrient (Purakayastha et al., 2001; Nogueira & Cardoso, 2002; Andrade et al., 2010), and non-nutrient functionality such as secondary metabolic production (Yao et al., 2003; Fester & Hause, 2007).

AMF has been reported engaged in rhizodeposition process to enrich the soil with carbon substrate with the result of the increasing of the soil biological activity (Rillig et al., 2001), and soil aggregation (Rillig et al., 2002; Rillig & Mummey, 2006). Symbiosis of *G. etunicatum* has the impact to plant root morphology as well (Bressan & Vasconcellos, 2002), hence it speeds up the early growing of vascular plant seedling planted at post mining area (Flores-Aylas et al., 2003; Santos et al., 2008).

Generally, plants that are symbiotic with AMF have lower shoot-root ratio than plants without AMF (Piccini et al., 1988; Khalil et al., 1994). Shoot-root ratio is the description of carbon translocation from top to the root of plants. The shoot-root ratio value get smaller due to the changing on rhizosphere dynamic that is reflected by carbon translocation to roots. And this shows one of the plant mechanisms to find out the limited nutrition. Carbon translocation to roots was showed by an increasing of root biomass, therefore it increases the root surface colonized by AMF.

The success of AMF inoculation was commonly observed based on plant indicators such as the increasing of plant dry-weight and yield dry-weight, nutrition uptake-Phosphor in particular (Rossiana & Supriatun, 2003; Simarmata & Herdiani, 2003). In fact, cocoa and coffee seedling inoculated with AMF always produce better growth in all of APR dosage levels.

AMF is widely known producing various benefits so the plant is capable to cope with biotic and abiotic stress (Smith & Read, 2008; Gianinazzi et al., 2010; Smith et al., 2010). The real advantage of AMF inoculation on seedling production is in an increasing growth of seedlings when transplanted to the field. The growth of seedlings without AMF are often dwarf and leaf suffer from necrosis (Landis & Amaranthus, 2009).

The diameter of AMF hypha is smaller than root hair, therefore it was able to reach out soil water and nutrient which were difficult to get to by plant roots. *Glomus etunicatum* has greater IH diameter (2.2 – 18.0 µm), greater number of hypha and spreads all over the root, however EH developed slowly and non-extensive. *A. tuberculata's* has smaller IH diameter (1.2 – 9.1 µm), fever hypha number but in developed faster and spread extensively on root surface (Dodd et al., 2000; Smith & Read, 2008). Greater hypha diameter will enables greater carbon volume transported to the rhizosphere for EH formation and extraradical spore.

Better nutrient availability determine better growth of host plant and better establishment of AMF intra-structure (IH, vesicle, and arbuscul) and extra-structure (EH and spore) (Olsson et al., 2010). The increasing of nutrient uptake by host plants ensured to vigorous plant in order to continually supply carbon to rhizosphere (Kaschuk et al., 2010) which is needed for the formation of EH and AMF sporulation.

4. Conclusion

Mycofer inoculum is more effective to increase the potency of APR as fertilizer and the growth of coffee and cocoa seedling. Cocoa seedling applied with APR up to 4 g P_2O_5.seedling^{-1} and coffee seedling applied with APR up to 8 g P_2O_5.seedling^{-1} linearly increased the growth of both cocoa and coffee seedling. Compared to control (without AMF), inoculation of AMF Mycofer increased the cocoa seedling's height up to 50.58% and shoot dry-weight 127.55%, whereas height of coffee seedling increased by 27.29% and shoot dry-weight by 121.21%. For all APR dosage, the growth of cocoa and coffee seedling inoculated with AMF Mycofer inoculum was better than inoculated by indigenous AMF and without AMF (control). This showed that AMF inoculation in both cocoa and coffee seedling increased the APR's effectiveness.

Acknowledgments

Our great gratitude goes to the Agroclimate Laboratory, Agriculture Faculty of Papua University Manokwari-Indonesia, Laboratory of Biotechnology on Forest and Environment, Research Center of Natural Resource and Biotechnology IPB, Bogor-Indonesia, the Indonesian Coffee and Cocoa Research Institute (ICCRI) Jember, Directurate General of Higher Education, and everyone who supported this research.

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