Phosphate Response by Indigenous Microbes and Mycorrhizal Inoculation on Corn in Inceptisol Soil

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Abstract. The low availability of phosphate is due to fixation by Al and Fe at acid pH, making it unavailable to plants. These problems can be overcome through the utilization of soil microbes and Arbuscular Mycorrhizal Fungi (AMF) that can associate with corn plants. This study aims to determine the effect of indigenous microbes and arbuscular mycorrhiza on phosphate nutrients. The experiment used a nested design with three factors. The first factor is land use, consisting of inceptisol moorland soil and inceptisol forest soil. The second factor is soil sterilization consisting of sterile and non-sterile. The third factor is mycorrhiza, consisting of non mycorrhiza and mycorrhiza inoculation. The results showed that land use had a significant effect on total P, potential P, Al oxide, and Fe oxide. However, it did not significantly affect available P, P uptake, and root CEC. Soil sterilization and mycorrhiza inoculation significantly affected available P, total P, potential P, P uptake, Al oxide, Fe oxide, and root CEC. The treatment interaction significantly affected all parameters, except P uptake and root CEC. However, there was a negative relationship between Al and Fe oxide content on available P and P uptake. The best phosphorus response was non sterile forest inceptisol soil with mycorrhizal inoculation.

Keywords: Arbuscular Mycorrhizae · Inceptisol · Indigenous Microbes · Phosphate

1 Introduction

The fertility of inceptisol soil in Indonesia varies from fertile, moderately fertile and less fertile. One of the factors is also influenced by land use, which affects the availability of nutrients including phosphorus. The presence of phosphorus although abundant in the soil, both in organic and inorganic forms, is limited because most of it occurs in insoluble form. The average soil P content is about 0.05%, but only 0.01% of the total P is available to plants due to low solubility and fixation in the soil [1]. Phosphorus accounts for about 0.2–0.8% of plant dry weight. To meet the nutritional needs of plants, P is usually added

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to the soil as P chemical fertilizer, however, the synthesis of P chemical fertilizer is a very energy intensive process, and has long term impacts on the environment in terms of eutrophication, soil fertility depletion, carbon footprint. Moreover, plants can use only a small amount of this P as 75–90% of the added P is precipitated by metal cation complexes, and quickly becomes fixed in the soil. Such environmental concerns have led to the search for a sustainable means of plant P nutrition [2].

These problems can be solved by the presence of microorganisms in the soil. Soil microorganisms play an important role in the ecosystem, carrying out various activities that interact with other microorganisms. Similarly, interaction with biological factors and non biological factors (environment) also plays a very important role in the soil, especially in the process of decomposing organic matter into nutrients. The metabolism of soil microbial diversity is an active driver, or contributor to major nutrient cycles including phosphorus. Soil microbes, bacteria, archaea and fungi play diverse and often important roles in this ecosystem. The metabolism of soil microbial diversity is an active driver of, or contributor to, the cycling of all major elements (e.g. C, N, P), and these cycles influence the structure and function of soil ecosystems as well as the ability of the soil to produce nutrients [3].

Previous research has been conducted by [4] related to the interaction of AMF and soil bacteria. However, research information related to the interaction of indigenous microbes and mycorrhizae on differences in land use has not been comprehensively studied. The plant that is usually associated with the presence of mycorrhizal fungi is c because the root system supports the growth of mycorrhizae and supplies carbon in exchange for nutrients, especially phosphorus.

A holistic understanding of soil to plant P dynamics is needed with the aim of reducing the consumption of chemical P fertilizers, maximizing the exploitation of the biological potential of root/rhizosphere processes for efficient mobilization, and the uptake of soil P by plants. Overall, P dynamics in soil plant systems are a function of the integrative effects of P transformation, availability, and utilization induced by soil, rhizosphere, and plant processes. The problem with P in acidic inceptisol soils is that it is easily fixed by Al or Fe. One way is through the help of phosphate solubilizing microbes, including fungi. There are mycorrhizae as root fungi whose role is to reach water, protect from pathogens, and can increase the solubility of P so that it becomes available.

2 Research Methods

This research was conducted from April to June 2021. The research was conducted in Greenhouse, Faculty of Agriculture, Universitas Gadjah Mada. Soil and plant tissue analysis (P uptake) was conducted at the Soil Science Laboratory, Faculty of Agriculture, Gadjah Mada University. The treatment design used was a nested design. Consists of three factorials and produces 8 combinations. Repeated 3 times so that there were 24 experimental units. Treatment factor 1 (main plot) is land use, consisting of inceptisol moorland (A1), and inceptisol forest soil (A2). Treatment factor 2 (nest plot) is soil sterilization, consisting of sterile soil (B1), and non sterile soil (B2). Treatment factor 3 (nest plot) was mycorrhiza inoculation, consisting of control (C1) and arbuscular mycorrhiza inoculation (C2).

Table 1. P.value from ANOVA test on soil chemical properties (5%)

Parameter	Available P	Total P	Potential P	P Uptake	AI Oxide	Fe Oxide	Root CEC
F1	0.055ns	0.007**	0.000**	0.117ns	0.005**	0.000**	0.182ns
F2	0.000**	0.000**	0.000**	0.009**	0.000**	0.000**	0.019**
F3	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
F1(F2(F3))	0.000**	0.000**	0.000**	0.871ns	0.000**	0.000**	0.058ns

Remarks: F1 = land use; F2 = sterilization soil; F3 = inoculation mycorrhizae; CEC = Cation Exchangeable Capacity; ns = no significant difference; *** = highly significant difference.

The main materials used were inceptisol soil from moorland and Balai KPH (Forest Management Unit) in Gunung Kidul, rock phosphate, arbuscular mycorrhiza, Bisi 2 hybrid corn seeds, urea and KCL as base fertilizers, and chemicals for soil and plant tissue analysis. The equipment used were electric scales, sieves, ovens, mortars, pipettes, test tubes, erlenmeyer tubes, autoclaves, spectrophotometers, distillation, titration, and deconstruction tools.

The soil to be used as a planting medium is sterilized before planting with the aim of eliminating the presence of unwanted soil microorganisms. Soil sterilization before according to the research method [5] was carried out using an autoclave by evaporating (steaming) at 121 °C for 30 min, repeated twice in a row with an interval of 24 h. The soil was wrapped in 3 layers of plastic. The dose of mycorrhiza used was 5 gr/plant.

The parameters analyzed were available P (Olsen method), total P (deconstruction with a mixture of $HClO_4$ and concentrated HNO_3 (10:1), potential P (HCL 25%). Measurement of P content in plants using wet digestion method with a mixture of concentrated H_2SO_4 and H_2O_2 (5:3) and measured using UV-VIS spectrophotometer at 889 nm. Determination of Al and Fe using ammonium oxalate extraction method [6]. Root CEC was analyzed with ammonium chloride extract [7]. The results were analyzed using ANOVA test on SPSS, if significantly different, followed by DMRT test at 5% error level. Correlation analysis using Pearson correlation test.

3 Results

Land use has a significant effect on total P, potential P, Al oxide, and Fe oxide. However, it had no significant effect on available P, P uptake, and root CEC. Soil sterilization and mycorrhizal inoculation had a significant effect on available P, total P, potential P, P uptake, Al oxide, Fe oxide, and root CEC ($p = 0.000$). Treatment interactions had a significant effect on all parameters, except P uptake and root CEC (Table 1).

The highest available P content, total P, and potential P were non sterile inceptisol forest soil with mycorrhizal inoculation, each value ($p = 28.54$); ($p = 44.14$); ($p = 41.05$) (Table 2), not significantly different from non sterile inceptisol moorland soil with mycorrhizal inoculation. The value of P uptake by corn was highest on non sterile inceptisol forest soil with mycorrhizal inoculation ($p = 36.61$) (Table 2). The highest values of Al and Fe oxides were found in sterile inceptisol moorland soil without mycorrhizal

Table 2. Effect of land use, soil sterilization, and mycorrhizae on P content and P Uptake Plant

Treatment			Available P	Total P	Potential P	P Uptake Plant
A1	B1	C1	7.49b	14.27c	12.04d	5.48e
		C2	10.02b	20.11b	16.78bc	21.19bcd
	B2	C1	9.61b	20.15b	15.80c	12.97cde
		C2	**25.64a**	**40.51a**	**39.41a**	27.37ab
A2	B1	C1	8.22b	17.68c	16.11bc	10.50de
		C2	11.15b	22.50b	19.56b	23.54bc
	B2	C1	10.81b	20.35b	17.67bc	21.06bcd
		C2	**28.54a**	**44.14a**	**41.05a**	**36.61a**

Remarks: the means followed by the same letters in the same column indicate no significant difference according to DMRT (a = 5%); A1 = Inceptisol moorland soil; A2 = Inceptisol forest soil; B1 = Sterile soil; B2 = Non sterile soil; C1 = non mycorrhizae; C2 = mycorrhizae inoculation.

Table 3. Effect of land use, soil sterilization, and mycorrhizae on Al oxcide, Fe oxcide, and root CEC

Treatment			Aluminum (Al)	Iron (Fe)	Root CEC
A1	B1	C1	**8.98a**	**14.73a**	12.28d
		C2	**8.41a**	13.36ab	18.60c
	B2	C1	6.10bc	4.12d	13.47d
		C2	3.87d	3.51a	25.19b
A2	B1	C1	6.99b	12.06b	13.29d
		C2	5.48c	6.26c	20.72c
	B2	C1	6.43bc	3.81d	14.15d
		C2	2.38e	3.45d	**30.97a**

Remarks: the means followed by the same letters in the same column indicate no significant difference according to DMRT (a = 5%); A1 = Inceptisol of moorland; A2 = Inceptisol of forest; B1 = Sterile; B2 = Non-sterile; C1 = non mycorrhizae; C2 = mycorrhizae; CEC = Cation Exchangeable Capacity.

inoculation, each value (p = 8.98) and (p = 14.73). The highest root CEC was found in non sterile inceptisol forest soil with mycorrhizal inoculation (p = 30.97) (Table 3).

Available P was positively correlated with P uptake (r = 0.755*). However, it was negatively correlated with the content of Al oxide (r = -0.784**), Fe oxide (r = -0.539**). P uptake was negatively correlated with Al oxide (r = -0.648**), Fe oxide (r = -0.470*). However, it was positively correlated to root CEC (r = 0.873**). Al and Fe oxides were negatively correlated with root CEC, respectively (r = -0.677*) and (r = -0.383) (Table 4).

Table 4. Variable correlation

Variable	P Available	P Uptake	Al Oxcide	Fe Oxide
P Uptake	0.755**			
Al Oxcide	-0.784**	-0.648**		
Fe Oxide	-0.539**	-0.470*	0.800**	
CEC Root	0.880**	0.873**	-0.677*	-0.383

Remarks: ** = correlation is significant at the 0.01 level; * = correlation is significant at the 0.05 level.

4 Discussion

4.1 Phosphorus

4.1.1 Phosphorus Available

Land use has a significant effect on total P and potential P content. Forest land has the highest phosphorus content. In general, land that does not receive high disturbance such as intensive soil management will not support a more diverse soil microbial community [8]. In contrast to dry lands that receive more intensive management, the soil will be degraded and the diversity of soil microorganisms will decrease. The sterile soil treatment has a significant effect on P content, because the presence of microorganisms can dissolve organic P into inorganic P which is absorbed by plants.

Soil microorganisms such as mycorrhizal fungi and phosphate solubilizing bacteria use different strategies to convert unavailable phosphate forms into more available ones. Generally, mycorrhiza can absorb nutrients with the help of hyphal elongation so that the roots can reach deeper zones. In most bacteria, organic acid production was shown to be associated with the dissolution of glucose minerals into gluconic acid (GA) as the main mechanism for dissolving phosphate minerals in gram negative bacteria. Organic acids released by microorganisms act as good chelators of the divalent cation Ca^{2+} coupled with the release of phosphate from insoluble complexes, organic acids can also form soluble complexes with metal ions coupled with insoluble phosphorus, thus releasing part of the phosphorus [9].

4.1.2 Total Phosphorus

Single or interaction treatments had a significant effect on total P content. Phosphate is widely available in soil in both organic and inorganic forms, but 95–99% of phosphate is in insoluble, immobilized and precipitated forms. Therefore, it is difficult for plants to absorb it. Plants absorb phosphate only as ions (H2PO4-) and (HPO4-2). The role of soil microorganisms in the form of bacteria or fungi can provide P nutrients in the soil. Phosphorus solubilization and mineralization by phosphate-solubilizing bacteria is an important property that can be achieved by PGPR. These bacteria secrete various types of organic acids (e.g. carboxylic acids), which lower the pH in the rhizosphere and thus release bound phosphates such as $Ca_3(PO_4{}^{-2})$ in calcareous soils [1]. Mycorrhizal inoculation also has a significant effect because based on research results, there is a correlation with the high spore density produced by AMF [10]. Therefore, AMF has a high interaction with total phosphorus.

4.1.3 Potential Phosphorus

Potential P is the total amount of P in the soil that cannot all be absorbed by plant roots. In the soil biogeochemical process, the potential P content in the soil is a substrate that will be used in the mineralization process so that P nutrients can be available to plant roots. Some case studies show that continuous application of chemical fertilizers reduces soil microbial population and earthworm activity, which in turn will reduce the quality of soil fertility [11]. The main problem of P fixation in acid soils results in reduced nutrient uptake. This can be overcome by direct application of natural P as a phosphate source, which is an alternative to make phosphate fertilizer more efficient. Therefore, the phosphate rock used in this study will dissolve in the acid soil environment. Either organic acids released by plants and microbes or acidic conditions will support the dissolution of P from rock phosphate, so that the potential P on non sterile soil with mycorrhizal inoculation is higher [12].

4.2 Al and Fe

The availability of P that can be absorbed by plants in the soil is generally very low, because P in the soil is mostly in adsorbed form. The adsorption ability of P by soil is generally influenced by the content of clay, Al and Fe oxidation [13]. In acid soils, P is mostly adsorbed by allophane, klei, Al, and Fe. The effect of adding organic matter can increase the availability of P in the soil, because it is organically acidic. The decomposition of organic matter has the ability to chelate Al and Fe so that P can be available. The content of Al and Fe in the soil is negatively correlated with available P and P uptake in plants. The higher the Al and Fe content in the soil, the lower the available P in the soil and can be absorbed by plants.

4.3 Root CEC

AMF infects plant roots, so it is thought to have an influence on the cation exchange capacity of plant roots. Root CEC is the ability of plant roots to absorb and exchange cations in soil solution. Adsorbed cations are usually available to plants by exchanging them with H+ produced by plant root respiration. The highest root CEC value was found on non sterile inceptisol forest soil with mycorrhizal inoculation. In accordance with the results of research [14], it shows that forest land has a higher CEC than cultivated land. This is due to the higher base cation content that is not easily leached in forest land compared to moorland due to management.

There is a positive correlation between root CEC and P. Mycorrhizae form a symbiotic correlation of mutualism in most plant roots. This symbiosis is highly relevant for sustainable agriculture due to its ability to increase productivity, nutrient uptake [15]. Endophytic and symbiotic mycorrhizal fungi directly interact with living host plants.

4.4 P Uptake Plant

Soil sterilization and mycorrhizal inoculation significantly affected the P uptake of corn plants. AMF applied to corn plants will release hyphae that will infect plant roots. Hyphae coming out of germinated spores will seek root exudates secreted by plants as a food source for AMF to penetrate the roots. In addition, AMF in the root cells will produce an external hyphal network that develops expansively in the form of hyphae colonization that grows and develops through the hairs of plant roots, thus expanding the absorption area and increasing nutrient uptake by plants, especially phosphate (P).

There is a correlation between the presence of soil microorganisms between bacteria and fungi. The results showed that in sterile soil conditions without mycorrhizal inoculation, P absorbed by plants was lower. On the other hand [16] also stated that increasing AMF infection would increase P uptake in plants, another study confirmed the relationship between AMF and plant growth promoting rhizobacteria (PGPR). PGPR is one of the soil bacteria that can stimulate AMF to develop and colonize roots. These bacteria also work together with AMF in increasing crop yields [4].

5 Conclusions

The results showed that land use significantly affected total P, potential P, Al oxide, and Fe oxide. However, it did not significantly affect available P, P uptake plant, and root CEC. Soil sterilization and mycorrhiza inoculation significantly affected available P, total P, potential P, P uptake plant, Al oxide, Fe oxide, and root CEC. The treatment interaction significantly affected all parameters, except P uptake and root CEC. However, there was a negative relationship between Al and Fe oxide content and available P and P uptake. The best phosphorus response was non sterile inceptisol forest soil with mycorrhizal inoculation.

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