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Research Article

Arbuscular Mycorrhizal Fungi Driven Phosphorus Nutrients in Paddy Soil under the Greenhouse Condition

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Abstract

Background and Objective: Arbuscular mycorrhizal fungi (AMF) involve the phosphorus solubilizing process in soil and play an important role in sustainable agriculture. The objective of this research was to investigate the impact of arbuscular mycorrhizal fungal populations on phosphorus (P) uptake of rice plants, phosphatase enzyme activity in the soil and rice yield under the greenhouse condition. **Materials and Methods:** Five AMF populations including HA, VT, LM-AG, PH and VB-BN were inoculated to the rice plant with a density of 75 spores per 100 g of dry soil each. During the experiment, the rice plant was irrigated with a low-pH-water (pH = 5) regime. **Results:** The treatments inoculated with AMF populations had more than 85% of the AMF colonization rate in rice roots at harvest. The phosphatase enzyme activity, available phosphorus content in soils and total phosphorus of straw and seeds as well as rice yield of the treatments inoculated with AMF populations got significantly higher contents than those of the control treatment. Among five potential AMF populations, the VT population showed effectively enhance plant growth, P uptake and increased rice yield and quality. **Conclusion:** The results demonstrated that the application of AMF population on rice cultivation under soil irrigated with low pH water conditions enhanced rice growth and drove the phosphorus pathway in both soil and rice plant uptake thus contributing to gained rice yield.

Key words: Arbuscular mycorrhizal fungi, available phosphorus, enzyme phosphatase, low pH water regime, paddy soil, phosphorus available

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In Vietnam, rice is a staple food for daily life and an essential income for the GDP (Gross Domestic Product) of Vietnam. According to the General Statistics of Vietnam released in 2021, the country's rice cultivation area is 7,238.86 thousand hectares and the only Mekong River Delta accounts for more than 50% of the country's arable land with 3,898.6 thousand hectares, which is more than 40% of arable land is acid sulfate soil (ASS). The major disadvantages of rice cultivation on ASS are nutrient deficiencies due to the soil's low pH. When the soil pH is below 5.5 and the solubility of phosphorus (P) obviously decreases¹. Mineral nutrient P is one of the most important macro-nutrients for plant growth and development. The rice plants are grown under the soil P deficiency, the plants are also stunted, flowering is poor, rice blooms and delayed ripening period, seeds are not full and quality is thus reduced its yield¹, the application of beneficial microorganisms, especially arbuscular mycorrhizal fungi (AMF) is considered to be the most important candidate in acid soil because these fungi are obligatory symbiosis with plants.

Arbuscular mycorrhizal fungi belonging to phylum Glomeromycota are important components of the soil microbial community². They form symbiotic relationships with most terrestrial plants, including many agricultural crops^{3,4}. The most important function of AMF fungi involves the transport of nutrients such as organic carbon in the form of sugars and fats^{5,6}, improving metabolism or uptake of mineral nutrients from the soil^{3,7} as the transfer of phosphorus and nitrogen to plants⁴, enhancing resistance to biotic stresses academic^{8,9} and abiotic^{10,11}. Therefore, the study was conducted to investigate the influence of arbuscular mycorrhizal fungal populations on rice growth, yield and the dynamic of phosphorus content in soil and rice plants under greenhouse condition.

MATERIALS AND METHODS

Study area: This study was conducted from March to June, 2022 under the greenhouse of the College of Agriculture, Can Tho University, Vietnam.

Materials

Experimental soil: Soil sample was collected at a depth of (0-20 cm) from a paddy field in Phong Dien District, Can Tho, Vietnam. The soil samples were air-dried and sieved to remove stone and root segments dried at room temperature, then passed through a 2 mm sieve. River sand was bought from a

construction company and thoroughly washed with tap water until the EC value of the water running through the sand was below $200 \mu\text{S cm}^{-1}$. The sand was air-dried. Soil and sand were mixed together at a rate of 1:1(w/w)¹². The soil mixture was autoclaved twice for 1 hr at 121°C , 1 atm and 24 hrs each. The soil mixture was filled in pots ($\varphi = 30 \text{ cm}$, $h = 25 \text{ cm}$) with 5 kg/pot. The basic soil chemical properties were as follows: $\text{pH}_{\text{H}_2\text{O}}$, 6.4, EC, $145 \mu\text{S cm}^{-1}$, Carbon: 1.38%, Total N: 0.015%, $\text{NH}_4^+\text{-N}$: 2.54 mg kg^{-1} , Total phosphorus: 0.019%, available phosphorus: 4.15 mg kg^{-1} . The AMF inoculants: Arbuscular mycorrhizal populations including HA, VT, LM, PH and VB were utilized from the Laboratory of Agricultural Microbiology, Institute of Food and Biotechnology, Can Tho University. The density of the inoculum was 75 spores per 100 g dry weight of soil mixture.

Rice seed: The rice variety OM5451 was washed in water to remove suspended particles and impurities, soaked the rice seeds in warm water (about 55°C) overnight and incubated for 24 hrs for the seeds to germinate.

Fertilizers: Fertilizer regime applied in this study consisted of urea (46% N), superphosphate (16% P_2O_5) and potassium oxide (60% K_2O).

Methods

Experimental design: The greenhouse experiment was set up as a completely randomized design, with six treatments and 5 replicates each including no arbuscular mycorrhiza (the control treatment) and five treatments of each of the AMF population of HA, VT, LM-AG, PH and VB-BN. The AMF spores were mixed on the topsoil at a depth of 2-5 cm the rice seedling was sown on the surface of the pots. During the experiment, the irrigation water was adjusted to $\text{pH} = 5$ and left for 24 hrs before irrigating for rice¹³. The low pH water was continuously applied when the rice plant was 7 days old until the end of the experiment. The fertilizer regime was applied following the recommendation for rice variety OM5451 with $100\text{N}-60 \text{ P}_2\text{O}_5-30 \text{ K}_2\text{O ha}^{-1}$.

Agronomic and yield components: For rice growth parameters, the plant height, the chlorophyll index of the leaf (SPAD) and the number of shoots per bush were collected at 15, 30, 45 and 60 days after sowing rice seedling (DAS)¹⁴. Rice seed was harvested when the rice plant was 95 days old. At harvest, the number of panicles per pot, grain filled percentage, panicle length, grain yield (14% moisture content) and straw biomass were carefully measured and weighted following Khuong *et al.*¹⁴.

Presence of arbuscular mycorrhizal fungi: The samples were collected using an auger ($\phi = 2$ cm) and three samples per pot at a distance of 5 cm from the rice stubble. These three soil samples were mixed together and were representative soil samples per pot. After that, the samples were separated into roots and soil. The roots were washed thoroughly, stained and calculated according to the method of Dalpé and Séguin¹⁵ at days 15, 30, 45, 60 and 90 DAS. The rhizosphere soil was air-dried and sieved to remove stones and root segments dried at room temperature, then passed through a 2 mm sieve for determining the number of AMF spores at the harvest following the method of Gerdemann and Nicolson¹⁶ as well as analyzing some soil chemical properties.

Soil analyses: Analyses of soil samples followed the standard methods described by Khuong *et al.*¹⁴. Briefly, $\text{pH}_{\text{H}_2\text{O}}$, electrical conductivity (EC) of soil samples were extracted using the deionized water with a 1:5 ratio of soil:water 1:5 (w/w). Total C (%C), total nitrogen (N_{tot}), total phosphorus (P_{tot}) and available phosphorus (P_{avail}) were determined following the procedures of Murphy and Riley¹⁷, Thuy *et al.*¹⁸. When rice plant got 45 days old, soil enzyme activity was determined following the method described by Tabatabai and Bremner¹⁹. For rice biomass and grain, the total phosphorus (P_{tot}) in straw and grain was analyzed following the colorimetric method using the wavelength at 880 nm¹⁷.

Statistical analysis: All data were analyzed using One-way Analysis of Variance (ANOVA) and comparison among means for significant differences using Duncan's *post hoc* Test at $p < 0.05$. These analyses were generated using the SPSS (version 23.0).

RESULTS

Percentage of AMF root colonization: The experimental results showed that the percentage of rice root colonization of

AMF populations increased through the growth stages of rice plants and reached more than 85% at harvest. During the rice crop, the rice root of the treatment inoculated with the VT population got a higher percentage of colonization than that of other treatments. The percentage of AMF colonization in rice roots increased sharply from 45 DAS to rice harvest (Table 1).

Density of AMF spores in soil: The number of spores in treatments inoculated with the AMF populations was about 8640 to 11,203 spores/100 g of dry soil at harvest and was significantly higher than that of the control treatment ($p < 0.01$) (Table 1). Among the five treatments inoculated with AMF, the VT treatment had the highest number of spores compared with that of other treatments. In addition, the proliferation rate of AMF ranged from 114 to 148 time-fold, specifically increased from 75 spores/100 g of dry soil at the sowing time to about 8640-11203/100 g of dry soil at the harvest.

Effects of AMF population on rice growth and development: Plant height, the chlorophyll (SPAD) index and the number of tillers of the rice plant treatments inoculated with the AMF were significantly higher ($p < 0.01$) than those of the control treatment across the experiment (Fig. 1a-b).

The SPAD index of the treatments showed the same pattern that these values increased from 15 up to 45 DAS and decreased at 60 DAS. When compared to the control non-AMF treatment, the SPAD index of the AMF treatments improved significantly and got a higher number of the indexes ($p < 0.01$) (Fig. 1a). In particular, the VT treatment had the maximum efficiency with the SPAD index with a difference statistically significant when compared to the control treatment. With the support of the AMF population, the yield components and grain yield in these treatments were also significantly different among the treatments (Table 2). The treatment inoculated by the VT population was the most efficient AMF enhancing rice growth and development significantly.

Table 1: Summary results of the presence of AMF population in rice root and density of AMF spore in soil

Treatment	Root colonization (%)					Number of spores per 100 g of dry soil
	15 DAS	30 DAS	45 DAS	60 DAS	90 DAS	
Control	0.60 ^d	2.40 ^e	2.40 ^e	3.20 ^e	4.0 ^d	82.2 ^a
HA	12.2 ^b	23.0 ^c	47.4 ^d	82.6 ^d	88.0 ^c	8788 ^d
VT	15.6 ^a	32.4 ^a	58.8 ^a	93.8 ^a	98.8 ^a	11203 ^a
LM-AG	10.6 ^{bc}	28.8 ^b	51.4 ^c	89.2 ^b	92.8 ^b	9991 ^b
PH	10.0 ^c	21.6 ^{cd}	50.8 ^c	85.2 ^c	88.0 ^c	8640 ^d
VB-VN	14.8 ^a	20.6 ^d	54.4 ^b	83.6 ^{cd}	90.8 ^{bc}	9480 ^c
Significant difference	**	**	**	**	**	**
CV (%)	1.30	1.29	1.28	1.28	1.28	12.3

In the same column, numbers followed by different letters are significantly different from each other by Duncan's Multiple Range Test, **Difference at $p < 0.01$ and DAS: Day after sowing

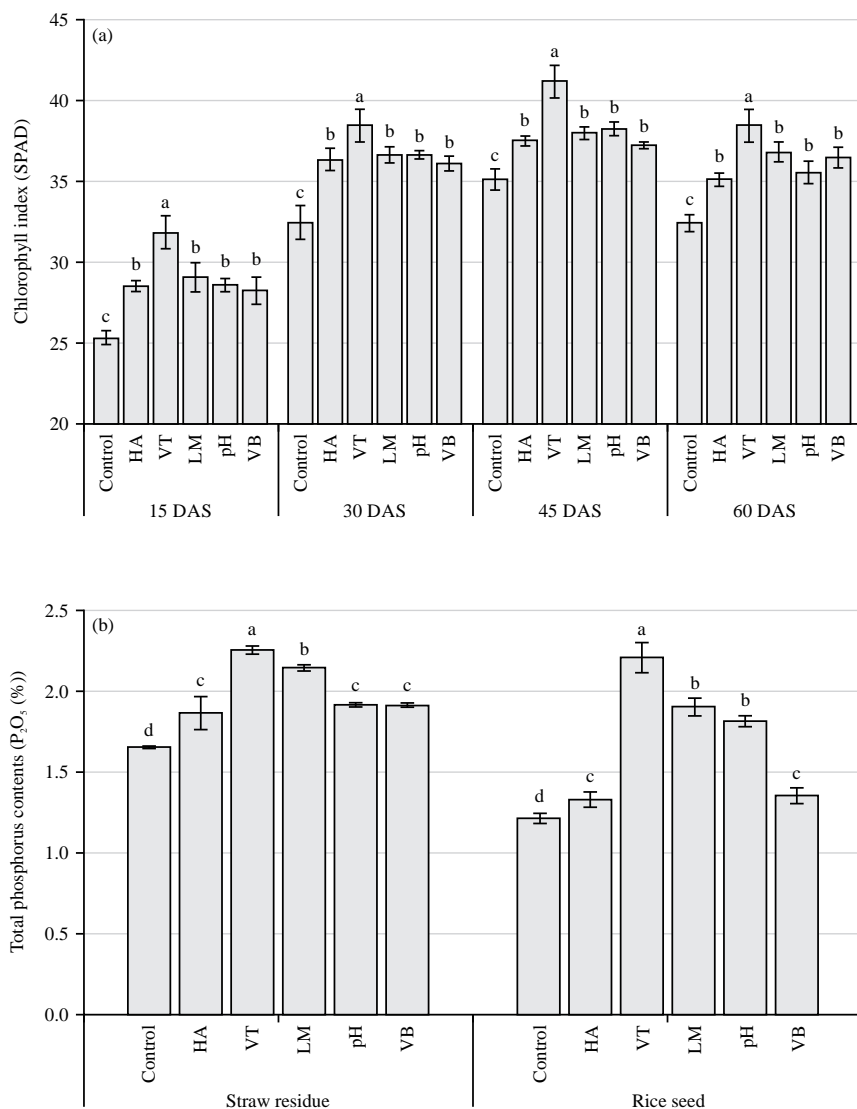


Fig. 1(a-b): Changes of chlorophyll index and total phosphorus content in rice plant, (a) Changes of chlorophyll index over the time period under the influence of AMF populations and (b) Total phosphorus contents in straw residue and rice seed at harvest

Different letters above the column are statistically different by Duncan's Multiple Range Test, With the standard deviation of the mean value ± 5 and DAS: Day after sowing

Table 2: Effects of AMF populations on rice yield components at harvest

Treatment	Number of panicles/plant	Panicle length (cm)	Filled spikelet (%)	Grain weight at 14% of humidity (g/pot)	Dry straw biomass (g/pot)
Control	4.00 ^b	20.0 ^b	89.0 ^c	27.4 ^c	18.8 ^c
HA	4.49 ^a	22.4 ^a	92.5 ^b	34.2 ^{bc}	21.3 ^{ab}
VT	4.64 ^a	23.2 ^a	93.2 ^a	36.0 ^a	19.8 ^c
LM-AG	4.52 ^a	22.6 ^a	93.3 ^a	34.5 ^b	20.0 ^{bc}
PH	4.62 ^a	23.1 ^a	92.0 ^b	33.7 ^c	22.0 ^a
VB-BN	4.46 ^a	22.3 ^a	92.9 ^a	35.4 ^a	19.9 ^{bc}
Significant difference	**	**	**	**	**
CV (%)	5.42	5.42	1.66	8.66	6.20

In the same column, numbers followed by different letters are statistically different by Duncan's Multiple Range Test and **Significant difference at $p < 0.01$

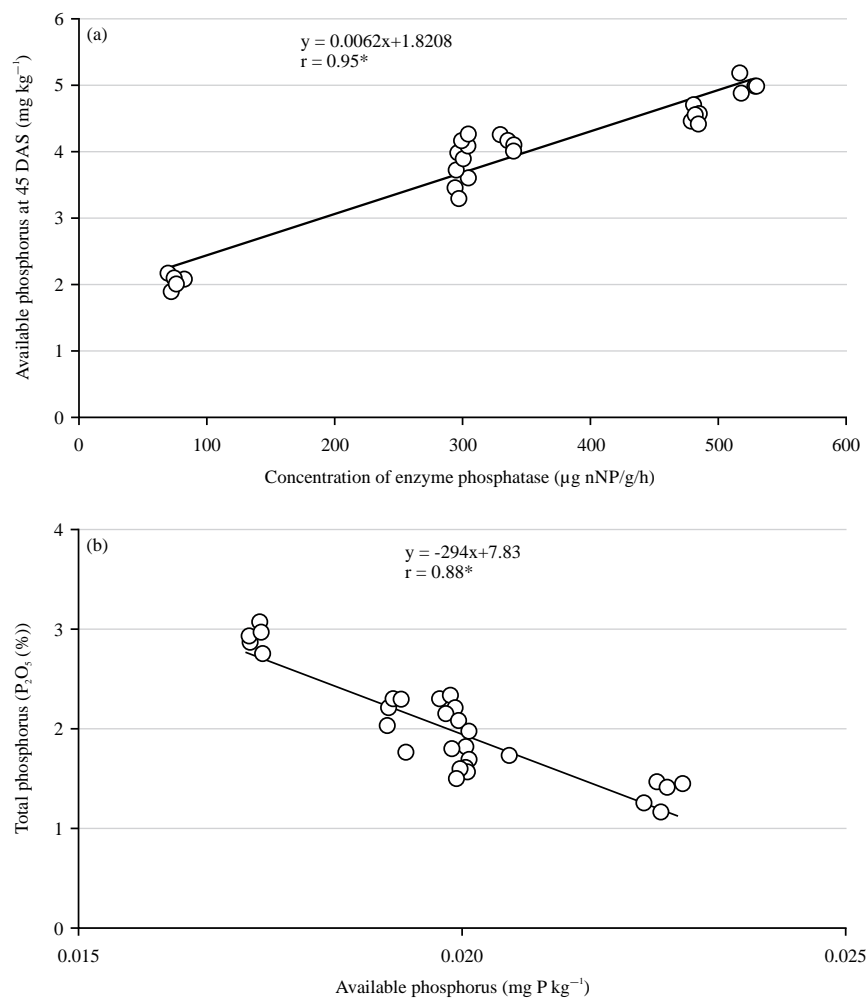


Fig. 2(a-b): Correlations between phosphorus components in soil with (a) Between available phosphorus and concentration of enzyme phosphatase at 45 DAS and (b) Between total phosphorus and available phosphorus in the soil at harvest

Effect of AMF populations on enzyme phosphatase and available phosphorus in rhizosphere soil:

At 45 DAS, the contents of enzyme phosphatases and available phosphorus in soil were demonstrated in Fig. 2a. The enzyme phosphatase was present in all treatments since the plants could release a specific amount of this enzyme as well. The content of phosphatase enzyme activity varied from 75.0 to 524 g nNP/g/hr and was significantly different among the treatments and the soil of the VT treatment exhibited the highest enzymatic activity with 524 g nNP/g/hr. The rhizosphere soil of the AMF treatments had significantly increased phosphatase enzyme activity than that of the control treatment. Similar to the trend of the enzyme activity, the available phosphorus (P_{avail}) contents of the AMF treatments showed significantly higher ($p < 0.01$) than that of the control treatment. There was a positive correlation

between the enzyme phosphatase and P_{avail} in this study ($r = 0.95^*$, $p < 0.05$) (Fig. 2a).

At the end of the experiment, the total phosphorus in the soil of the treatments inoculated with the AMF was significantly lower than that of the control treatment ($p < 0.01$). There was a negative correlation between the total phosphorus and available phosphorus in the soil of the treatments at harvest ($r = -0.88^*$, $p < 0.05$). The soil of the treatment inoculated with the VT population contained the highest available phosphorus and lowest content of P_{tot} (Fig. 2b).

Effect of AMF on phosphorus content in biomass:

At harvest, the rice seed and straw biomass of the treatments inoculated with AMF population had significantly higher contents of the total phosphorus ($p < 0.01$) than those of the control treatment

(Fig. 1b). These results could be benefits of the AMF population contributing to the plant during the symbiotic process of AMF and rice plant. With the higher content of enzyme phosphatase and soluble phosphorus in the soil, the higher content of phosphorus in the rice grain and straw residues was.

DISCUSSION

The presence and the occurrence of AMF in an agricultural ecosystem are evaluated by either the percentage of AMF root colonization or the density of sporulation in soil as well as the application of biotechnology methodologies. Most studies related to AMF focus on upland crops including upland rice plants because these plants readily form mycorrhizal symbiosis under the aerobic condition. However, under submerged conditions, the symbiosis of AMF with root is less studied due to the anoxic environment²⁰. In this study, the presence of the AMF population in the treatments were investigated during the rice crop to identify the pattern of AMF infection during the crop. The colonization rate increased significantly after 15 DAS until the rice harvest. Our results were similar to those of the results of Watanarojanaporn *et al.*²¹ and Wang *et al.*²², who showed that the roots of mature rice (blooming and ripe) stage were frequently infected more than during early development. The colonization of AMF in rice roots is due to the secretion of signaling molecules (strigolactones) by the host plant, which is a chemical signal that attracts AMF and the presence of strigolactones stimulates the growth of AMF mycelium^{23,24}. Therefore, the colonization rate of AMF in rice roots increased significantly when rice plants were in the middle of the growth stage. In this study, the results showed that the higher the colonization rate, the greater the number of spores produced, particularly in the VT treatment with 98.8% and 11 203 spores per 100 g of dry soil, respectively. The findings of this study showed that treatments with high colonization rates produced a greater density of AMF spores in paddy soils when different doses of biochar were applied.

With the presence of AMF population in rhizosphere soil and in rice root, rice plants of these treatments grew vigorously compared with that of the control treatment. The results are consistent with the previous research of Bucher *et al.*²⁵, who discovered that the presence of host plant AMF increases photosynthesis, enhancing mineral uptake and assisting the host plant in overcoming environmental stress^{26,27}. Similar to the chlorophyll indexes, results of the plant height and number of tillers of the treatments inoculated with AMF populations were significantly greater

than those of the control treatment over the crop cultivation. El-Khateeb *et al.*²⁸ discovered that the application of AMF together with a suitable inorganic fertilizer regime enhanced the height of *Chamedora elegans*. Allen *et al.*²⁹ concluded that the increase in height could be due to increased absorption of inorganic nutrients via the mycelium network and crop photosynthetic rate.

In acidic soil conditions, soil pH and endosymbiotic mycorrhizal fungi were considered to be positively affected by the number of flowering shoots, dry biomass and root length of maize³⁰. Several studies have shown that AMF fungi can increase crop output by boosting nutrient intakes, such as nitrogen³¹ and phosphorus^{32,33}. The result of research by Wangiyana *et al.*^{34,35} discovered that applying AMF-containing biofertilizers significantly increased the growth components and yield as well as anthocyanin content in the seeds of red rice varieties, particularly in terms of the percentage of firm seeds and grain yield per bush. Das *et al.*³⁶ discovered that AMF supplementation in the presence of a 25% reduction in phosphate fertilizer enhanced rice's physiological, biochemical and yield features compared to no AMF treatment under any growing circumstances. Although the study was conducted under the anaerobic condition, these findings were similar to the results from the aerobic condition. It seemed that the AMF could uptake nutrients in the soil, deliver them to the root and supply them to the plants under soil low pH.

Phosphatase is an extracellular enzyme released by microorganisms in soil and plants³⁶. These enzymes catalyze esters, phosphomonoesterases and phosphoric acid to produce Phosphates (PO_4^{3-}), which play an important role in organophosphate mineralization processes leading to the release of useful phosphorus for plants.

Several studies showed that when applying phosphorus fertilizers into soil at low pH, phosphorus is quickly absorbed and fixed by Al and Fe oxides or organic compounds, however, the main role of AMF can release enzyme phosphatases to dissolve the fixed P-forms. The effects of AMF on P and plant development seem to be very variable and they are frequently depending on the plant's innate potential along with soil properties^{4,37}. The findings were consistent with the findings of Joner and Johansen³⁸, who discovered that when phosphatase enzyme was present in the sand substrate was strained *Glomus intraradices* and *G. claroideum*, the activity of the enzyme was highest at pH levels 5.2-5.6 and decreased sharply with increasing temperature. An *et al.*³⁹ discovered a relationship between acid phosphatase activity and phosphorus absorption in soil. The enhanced phosphorus uptake is attributable to the extended AMF mycelium, which allows access to an improved soil particle surface and the

potential of AMF secretions to solubilize P. The discovery of Alarcón *et al.*⁴⁰ observed that, acid phosphatase activity was greater in *Carica papaya* when infected with the fungus *Glomus claroideum*. These results illustrated that the AMF populations were able to involve in the phosphorus transformation in the experiment.

As the role of AMF in the soil ecosystem, mycelia of mycorrhizal fungi spread into the bulk soil and they secrete complex enzymes such as phosphatases and phytases to solubilize P from the insoluble phosphorus source^{41,42}. Watts-Williams and Gilbert⁴³ found that AMF fungus was beneficial for absorbing macronutrients, particularly phosphorus, as well as improving biomass and yield in plants when compared to soils that have not been treated with AM fungi. Phosphate delivery is among the most important benefits for the host in AM symbiosis⁴⁴ and the results suggested that the arbuscules could be the site of transferring phosphate from the fungus to the plant, therefore, this nutrient boosts the sugar content of sugarcane, the quantity of flour in potatoes and the amount of protein in cereals and contribute to the quality of rice seed in this study. The current study showed that the AM populations played important roles on phosphorus availability and enhanced P uptake for rice plant under the low pH water regime. Among them, the VT population has a potential application for the rice cultivation under the soil low pH.

CONCLUSION

The results showed that AMF populations actively infected rice roots under irrigation conditions of low-pH water regime, reaching over 85% at the harvest stage and the multiplication of the number of spores reached 114-148 time-fold at the harvest and was higher than that from the beginning of the sowing rice seedling. The AMF populations enhanced rice plant growth and development, gained yield and increased rice seed quality related to phosphorus nutrients. In addition, the VT population showed its roles involved the phosphorus pathway related to enzyme phosphatase, phosphorus contents in both soil and rice grain and stubble biomass in soil under irrigation water with pH 5.

SIGNIFICANCE STATEMENT

The objective of this research was to investigate the impact of arbuscular mycorrhizal fungal populations on phosphorus (P) uptake of rice plants, phosphatase enzyme activity in the soil and rice yield under the greenhouse condition. These results clearly showed that arbuscular

mycorrhizal fungal population played an important role in solubilizing phosphorus which is supplied for rice plant uptake thus contributing to increasing rice yield. The best candidate of The VT population has a potential application for rice cultivation under the soil low pH.

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