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# Inoculation with Arbuscular Mycorrhizal Fungi or Crop Rotation with Mycorrhizal Plants Improves the Growth of Maize in Limed Acid Sulfate Soil

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**Abstract:** Arbuscular mycorrhizal fungi (AMF) improve the uptake of immobile mineral nutrients such as phosphate, thereby improving plant growth. In acid sulfate soil (ASS), AMF spore density is generally low which impacts root colonization and phosphate uptake. Thus, inoculation may help increase AMF colonization of crops grown in ASS. AMF spore density decreases after cultivation of a non-host crop or bare fallow. In addition, preceding crops affect the growth and yield of subsequent crops. The production of AMF inocula requires AMF-compatible plants. The objective of the present study is to elucidate the effect of preceding crops on the persistence of inoculated AMF and growth of succeeding maize under an ASS condition with lime application. Spore density of AMF after cultivation of preceding crops (soybean or job's tears) was maintained in comparison to fallow leading to higher AMF colonization of maize and improved plant growth. Thus, maintenance of AMF spore density, either through selection of preceding crops or application of AMF inoculum, may be a viable strategy to improve maize growth in limed ASS of Thailand.

**Key words:** Acid sulfate soil, Arbuscular mycorrhizal fungi, Maize, Preceding crops.

Acid sulfate soils (ASS) are problem soils widely distributed in tropical Asia and Africa. ASS is formed by oxidation of iron sulfate and pyrite derived from ancient seas or lagoons (Suthipradit et al., 1995; Farina et al., 2000). In most cases, ASS have a pH as low as 3.0, and are a major cause of low crop productivity (Attanandana et al., 1999). Furthermore, since ASS has a high capacity to fix phosphate, symptoms of phosphate deficiency are commonly observed in many crops (Jugsujinda et al., 1978; Moore et al., 1990; Sanyal et al., 1993).

Arbuscular mycorrhizal fungi (AMF) improve the uptake of immobile mineral nutrients such as phosphate, thereby improving plant growth (Smith and Read, 1997; Usuki and Yamamoto, 2003; Mohammad et al., 2004; Lekberg and Koide, 2005). In ASS, AMF spore density is generally low which impacts root colonization and P uptake (Isobe et al., 2005). Thus, inoculation may be useful to increase AMF colonization of crops grown in ASS.

The production of AMF inocula requires AMF-compatible plants (Norris et al., 1992). AMF spore density has been shown to decrease after cultivation of a non-host crop or bare fallow (Black and Tinker, 1977; Thompson,

1994; Karasawa et al., 2000; Karasawa et al., 2001). In addition, it is widely known that preceding crops affect the growth and yield of subsequent crops (Karlen et al., 1994). AMF infection, P uptake, growth and yield of crops have been shown to significantly decrease following a non-host crop (Arihara and Karasawa, 2000).

In the present study we evaluated 1) whether AMF inoculation promoted the growth and P uptake of crops, and 2) if the preceding crop impacts the spore density of inoculated AMF in ASS with lime application.

## Materials and Methods

### 1. Cultivation system

A field experiment was conducted at the Royal Acid Sulfate Soil Improvement Experiment Station located in Banna of the Nakhon Nayok Province of Thailand. Four plots (SB<sub>1</sub>/M, JT<sub>1</sub>/M, FW<sub>1</sub>/M and FW/M<sub>1</sub>) 2 m × 2 m were established in ASS prior to the cultivation of maize (Table 1). Four seeds of soybean (*Glycine max* (L.) Merr.) or job's tears (*Coix lacryma-jobi* L. *ma-yuen* stapf.) were sown in hills spaced at 30 cm intervals in SB<sub>1</sub>/M or JT<sub>1</sub>/M plot on 23 May, 2007. Plants were thinned to one plant per hill

Table 1. Cultivation summary of cropping system at each plot.

Plots	23 May*			16 Sep.	17 Sep.			10 Nov.
	Sowing preceding crop	AMF inoculation	Lime (Ca(OH) <sub>2</sub> )	Sampling	Sowing succeeding crop	N fertilizer	AMF inoculation	Sampling
SB <sub>1</sub> /M <sup>**</sup>	Soybean	Inoculated	Applied	Soybean, Soil	Maize	Applied	–	Maize, Soil
JT <sub>1</sub> /M	Job's tears	Inoculated	Applied	Job's tears, Soil	Maize	Applied	–	Maize, Soil
FW <sub>1</sub> /M	Non (Fallow)	Inoculated	Applied	Soil	Maize	Applied	–	Maize, Soil
FW/M <sub>1</sub>	Non (Fallow)	–	Applied	Soil	Maize	Applied	Inoculated	Maize, Soil

\*The format MM/DD. \*\*The preceding cropping (from 23 May to 16 September)/succeeding cropping (from 17 September to 10 November). SB, JT and FW show soybean, Job's tears and fallow, respectively and <sub>1</sub> meant AMF inoculation.

for a total of 49 plants per plot on 6 June, 2007. The crops used in this experiment were local varieties with uncertain names (The seeds of preceding crops were obtained from the Royal Acid Sulfate Soil Improvement Experiment Station in Thailand). FW<sub>1</sub>/M and FW/M<sub>1</sub> plots were fallow from 23 May to 16 September prior to the cultivation of maize. Four hundred g of AMF inoculum (Serakikon; mainly composed of *Gigaspora margarita*, and includes 50 spores per g, Sungreen Co. Ltd., Japan) was incorporated into SB<sub>1</sub>/M, JT<sub>1</sub>/M and FW<sub>1</sub>/M plots on 23 May, 2007. Lime (Ca(OH)<sub>2</sub>) was applied to the ASS field on 23 May, 2007 at the rate of 1.8 kg m<sup>-2</sup>, corresponding to 1 Lime Requirement (1.0 LR) as defined by Sithibush et al. (1996). No fertilizers were applied to any of the plots prior to maize. Above- and below-ground plant biomass was sampled on 16 September, 2007. After soil sampling, remaining plant biomass was plowed into the soil. AMF inoculum was incorporated into FW/M<sub>1</sub> plot on 17 September, 2007. All plots received 200 g of N fertilizer in the form of ammonium sulfate prior to seeding of maize. Three seeds of maize (*Zea mays* L.) were sown in hills spaced at 30 cm intervals on 17 September, 2007. Plants were thinned to one plant per a hill on 2 October, 2007 for a total of 49 plants per plot. The maize used in this experiment was a local variety of uncertain name (The maize seeds were obtained from the Royal Acid Sulfate Soil Improvement Experiment Station in Thailand).

## 2. Sampling and analysis of plant, measurement of AMF infection

The tops of the soybean and job's tears were cut close to ground and then the root samples (depth 10 cm, diameter 20 cm) were collected on 16 September, 2007. Ten plants (excluding border plants) were randomly sampled from soybean and job's tears fields. Above-ground plant biomass was determined after samples were oven dried at 80°C for 48 hr. Root samples from five of the ten sampled plants were used to measure AMF infection rate. AMF infection rate was estimated by the grid-line intersect method (Giovannetti and Mosse, 1980). The lowest count number of the crossing of grid and root in one plant was 200.

The tops of the 18 to 23 maize plants were cut close to ground and were randomly sampled on 10 November, 2007. The top dry weight and plant length of the maize plants were measured in all plots. Above ground plant biomass and P uptake by maize was determined after samples were oven dried at 80°C for 48 hr. P uptake was determined using the molybdenum yellow colorimetric method (Sekiya, 1970). Root samples from seven of the 18 to 23 maize plant samples were used to measure AMF infection rate. AMF infection rate was estimated by the grid-line intersect method (Giovannetti and Mosse, 1980). The count number of the crossing of grid and root in one plant was lowest 200.

Root samples of preceding crops and the succeeding maize were carefully washed with tap water. All roots of one plant were cut to 1cm segments, and 100 segments were plunged into a KOH solution (10%), heated at 105°C for 10 min with an autoclave, and rinsed in the distilled water. Then they were bleached in a H<sub>2</sub>O<sub>2</sub> solution (10%) for 1 min, and stained with a 0.05% of trypan blue solution, which were heated at 105°C for 5 min with an autoclave for the observation of AMF infection (Oba et al., 2006).

## 3. Sampling and analysis of soil, measurement of AMF spore density

The soils were randomly sampled from three points (depth 10 cm, diameter 20 cm) per plot for the measurement of soil chemical properties of the experimental field after preceding crops (16 September, 2007) and succeeding maize (10 November, 2007). Soil pH was determined according to Byju (2001) with the soil diluted with 2.5 times of distilled water. Available phosphate content was determined by the method of Bray and Kurtz (1945).

The soils were randomly sampled from four points (depth 10 cm, diameter 20 cm) per plot for the measurement of AMF spore density of the experimental field after preceding crops on 16 September, 2007. The number of AMF spores after preceding crops was determined by the method of Brundrett et al. (1996). Fifty grams of the soils were passed through 500 μm and then 53 μm mesh sieves.

The residues on the 53  $\mu\text{m}$  mesh were subjected to sucrose density gradient centrifugation to isolate AMF spores. The number of the spores in the soil was counted under a stereo microscope (SZX12, OLYMPUS, Tokyo, Japan). The measurement of the number of spores was performed four times in each plot.

### Results

The above ground plant biomass of preceding soybean and job's tears were 22.2 and 88.5 g per plant, while AMF infection rate was 4.3 and 10.0%, respectively (Table 2). Soil pH of all plots on 16 September ranged from 4.70 to 5.30. Soil pH of  $\text{SB}_1/\text{M}$ ,  $\text{JT}_1/\text{M}$  and  $\text{FW}_1/\text{M}$  plots was higher than that of  $\text{FW}/\text{M}_1$  plot about 0.5 to 0.6 (Table 3).

Table 2. The above-ground plant biomass and AMF infection rate of preceding crops under ASS with lime application.

Preceding crops	Above ground plant biomass (g plant <sup>-1</sup> )	AMF infection rate (%)
Soybean	22.2 $\pm$ 4.4*	4.3 $\pm$ 1.1
Job's tears	88.5 $\pm$ 15.7	10.0 $\pm$ 2.7

\*The values are means $\pm$ S.E. of 10 (plant biomass) or 5 (infection rate).

Significant differences in soil pH were not observed among  $\text{SB}_1/\text{M}$ ,  $\text{JT}_1/\text{M}$  and  $\text{FW}_1/\text{M}$  plots, but there was a significant difference at the 5% level between  $\text{SB}_1/\text{M}$ ,  $\text{JT}_1/\text{M}$  or  $\text{FW}_1/\text{M}$  plots and  $\text{FW}/\text{M}_1$  plot. Soil pH of all plots on 10 November was ranged from 4.93 to 5.67. Soil pH of  $\text{SB}_1/\text{M}$  plot was 0.3 to 0.7 higher than that of  $\text{JT}_1/\text{M}$ ,  $\text{FW}_1/\text{M}$  and  $\text{FW}/\text{M}_1$  plots. Significant differences were not observed between  $\text{SB}_1/\text{M}$  and  $\text{FW}/\text{M}_1$  plots, but there was a significant difference at the 5% level among  $\text{SB}_1/\text{M}$  plot and  $\text{JT}_1/\text{M}$  and  $\text{FW}_1/\text{M}$  plots. A significant difference was not observed among  $\text{JT}_1/\text{M}$ ,  $\text{FW}_1/\text{M}$  and  $\text{FW}/\text{M}_1$  plots either. On 16 September, available phosphate ranged from 8.01 to 10.45 mg per 100 g dry soil in all plots. There was no significant difference among the plots on 16 September. Moreover, on 10 November, available phosphate in all plots ranged from 5.27 to 11.16 mg per 100 g dry soil. Available phosphate of  $\text{SB}_1/\text{M}$  plot was higher than that of  $\text{JT}_1/\text{M}$ ,  $\text{FW}_1/\text{M}$  and  $\text{FW}/\text{M}_1$  plots (about 4 to 6 mg), but no significant differences in this parameter were observed in any plots on 10 November (Table 3).

Plant length, above-ground plant biomass and P uptake by maize in  $\text{SB}_1/\text{M}$  and  $\text{JT}_1/\text{M}$  plots was greater than that in the  $\text{FW}_1/\text{M}$  plots (Table 4). Significant differences at the 5% level in plant length were observed between  $\text{SB}_1/\text{M}$

Table 3. Dynamics of soil pH and available phosphate contents influenced by growing preceding and succeeding crops.

Plots	pH (H <sub>2</sub> O)		Available P <sub>2</sub> O <sub>5</sub> (mg 100g <sup>-1</sup> )	
	16 Sep.*	10 Nov.	16 Sep.	10 Nov.
	P.C Sampling	S.M Sampling	P.C Sampling	S.M Sampling
$\text{SB}_1/\text{M}^{**}$	5.30 a***	5.67 a	9.33 a	11.16 a
$\text{JT}_1/\text{M}$	5.17 a	4.93 b	9.82 a	5.27 a
$\text{FW}_1/\text{M}$	5.23 a	5.07 b	10.45 a	6.87 a
$\text{FW}/\text{M}_1$	4.70 b	5.33 ab	8.01 a	7.75 a

\*The format MM/DD. \*\*The preceding cropping (from 23 May to 16 September)/succeeding cropping (from 17 September to 10 November). SB, JT and FW show soybean, Job's tears and fallow, respectively and <sub>1</sub> meant AMF inoculation. \*\*\*Means in a column in each parameter followed by the same letters are not significantly different at 0.05 level according to Tukey's multiple range test. P.C: Preceding crops. S.M: Succeeding maize.

Table 4. Effects of AMF inoculation or preceding crops cultivation on the growth of succeeding maize plants.

Plots	Plant length (cm)	Above ground plant biomass (g plant <sup>-1</sup> )	P concentration of top (mg P g <sup>-1</sup> )	P uptake of top (mg P plant <sup>-1</sup> )
$\text{SB}_1/\text{M}^{**}$	182.8 a*	79.7 a	1.4 a	111.6 a
$\text{JT}_1/\text{M}$	171.2 ab	74.8 ab	1.7 a	127.2 a
$\text{FW}_1/\text{M}$	152.1 b	51.8 b	1.1 a	57.0 b
$\text{FW}/\text{M}_1$	166.9 b	74.8 ab	1.4 a	104.7 a

\*Means in a column in each growth parameter followed by the same letters are not significantly different at 0.05 level according to Tukey's multiple test. \*\*The preceding cropping (from 23 May to 16 September)/succeeding cropping (from 17 September to 10 November). SB, JT and FW show soybean, Job's tears and fallow, respectively and <sub>1</sub> meant AMF inoculation.

M and FW<sub>1</sub>/M plots, but there were no significant differences among JT<sub>1</sub>/M, FW<sub>1</sub>/M and FW/M<sub>1</sub> plots (Table 4). Similarly, significant differences at the 5% level in the above-ground plant biomass were observed between SB<sub>1</sub>/M and FW<sub>1</sub>/M plots, but there was no significant difference among JT<sub>1</sub>/M, FW<sub>1</sub>/M and FW/M<sub>1</sub> plots. Moreover, significant differences at the 5% level in P uptake were observed between SB<sub>1</sub>/M, JT<sub>1</sub>/M or FW/M<sub>1</sub> plots and FW<sub>1</sub>/M plot, but there were no significant differences among SB<sub>1</sub>/M, JT<sub>1</sub>/M and FW/M<sub>1</sub> plots (Table 4). In summary, maize growth parameters in SB<sub>1</sub>/M and JT<sub>1</sub>/M plots were similar to those in the FW/M<sub>1</sub> plot.

On 16 September, AMF spore density in SB<sub>1</sub>/M, JT<sub>1</sub>/M and FW<sub>1</sub>/M plots was 3.15, 2.98 and 1.29 spores per g fresh soil, respectively (Fig. 1). AMF spore density in SB<sub>1</sub>/M, JT<sub>1</sub>/M was about 1.8 spores higher than that in FW<sub>1</sub>/M plot. There was no significant difference between SB<sub>1</sub>/M and JT<sub>1</sub>/M plots. However, significant differences at the 5% level were observed among SB<sub>1</sub>/M, JT<sub>1</sub>/M and FW<sub>1</sub>/M plots.

The AMF infection rate of maize in SB<sub>1</sub>/M, JT<sub>1</sub>/M, FW<sub>1</sub>/M and FW/M<sub>1</sub> plots was 15.9, 18.1, 8.0 and 11.3%, respectively (Fig. 2). The infection rate in SB<sub>1</sub>/M and JT<sub>1</sub>/M plots were higher than that in FW<sub>1</sub>/M plot about 10% and there was no significant difference in infection rate between the SB<sub>1</sub>/M and JT<sub>1</sub>/M plots. The infection rate in the JT<sub>1</sub>/M plot was higher than that in the FW/M<sub>1</sub> plot about 7%. Significant differences at the 5% level were observed among SB<sub>1</sub>/M, JT<sub>1</sub>/M and FW<sub>1</sub>/M plots. Moreover, significant differences at the 5% level were also observed between JT<sub>1</sub>/M and FW/M<sub>1</sub> plots (Fig. 2).

### Discussion

AMF spore density increases following mycorrhizal plants in comparison to non-mycorrhizal plants (Usuki and Yamamoto, 2003) or bare fallow (Black and Tinker, 1977; Thompson, 1994; Karasawa et al., 2000; Karasawa et al., 2001). In the present study, the spore density of AMF following soybean or job's tears was greater than that after fallow and led to higher AMF infection rates in maize (Figs. 1, 2). Thus, maintaining AMF inoculum through the appropriate selection of preceding crops is important for the growth of crops in ASS.

The growth of maize following soybean and Job's tears increased the above-ground plant biomass, P uptake and plant length compared with maize grown after fallow (Table 4). However, AMF inoculation prior to maize negated the impact of fallow on maize growth parameters indicating a positive growth response to AMF inoculation. Thus, crop rotation with mycorrhizal crops or AMF inoculation is an effective strategy to improve crop performance in ASS with lime application. It is widely known that preceding crops affect the growth of the

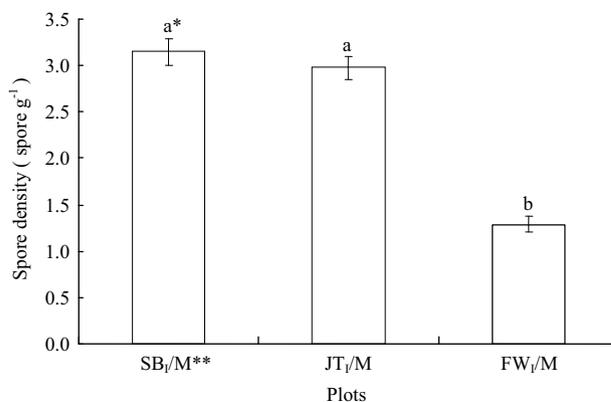


Fig. 1. AMF spore density after cultivation of preceding crops on 16 September. Vertical bars indicate  $\pm$ S.E. of the mean ( $n=4$ ). \*Means followed by the same letters are not significantly different at 0.05 level according to Tukey's multiple range test. \*\*The preceding cropping (from 23 May to 16 September)/succeeding cropping (from 17 September to 10 November). SB, JT and FW show soybean, Job's tears and fallow, respectively and <sub>1</sub> meant AMF inoculation.

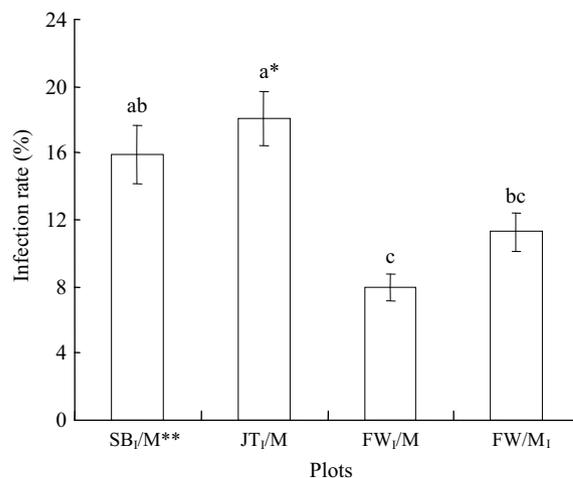


Fig. 2. Infection rate of AMF on succeeding maize plants in ASS with lime application. Vertical bars indicate  $\pm$ S.E. of the mean ( $n=7$ ). \*Means followed by the same letters are not significantly different at 0.05 level according to Tukey's multiple range test. \*\*The preceding cropping (from 23 May to 16 September)/succeeding cropping (from 17 September to 10 November). SB, JT and FW show soybean, Job's tears and fallow, respectively and <sub>1</sub> means AMF inoculation.

succeeding crops (Karlen et al., 1994). In addition to altering AMF inoculation potential, preceding crops also affect the growth of succeeding crops through changes in water use-efficiency (Karlen and Sharpley, 1994), nutrient use efficiency (Pare et al., 1992; Burle et al., 1997), quality and quantity of plant residues (Havlin et al., 1990), pest populations (Francis et al., 1986; Rush and Winter, 1990), physical and biological properties (Arihara et al., 1991; Karlen et al., 1994) and allelochemicals (Hegde and Miller,

1990; Liebman and Dyck, 1993). In the present study, soil pH and available phosphate contents were examined under ASS with lime application conditions (Table 3), however, these parameters did not account for the observed maize growth responses. Although AMF spore density, or inoculum potential, was the critical factor, other soil biological and chemical attributes cannot be ruled out.

In the present paper, the maintenance of AMF spore density, either through selection of preceding crops or application of AMF inoculum, appears to be a viable strategy to improve maize growth in limed ASS of Thailand.

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- \* In Japanese  
\*\* In Japanese with English abstract.
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