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The Effectiveness of *Entrophospora colombiana* and *Scutellospora* sp. Associated with Soybean on Phosphate Solubilization

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ABSTRACT

The maximum yield of soybean (5.72 g plant^{-1}) was obtained from plots treated with *E. colombiana* + TSP (triple superphosphate) at 30 kg P_2O_5 rai^{-}. Application of TSP increased soybean yield but the enhancement of VAMF (Vesicular Arbuscular Mycorrhizal Fungi) on soybean growth was reduced. The enhancement of VAMF in RPP (rock phosphate from Petchabun) and RPK (rock phosphate from Kanchanaburi) was significantly greater than in TSP treatment. Increasing the level of phosphate fertilizer reduced VAMF enhancement. The promotion of plant yield by *E.colombiana* together with RPP at 10 kg P_2O_5 rai^{-1} was the greatest (2.63 g plant^{-1}) whereas the minimum promotion (0.06 g plant^{-1}) was found in *Scutellospora* sp. + TSP at 30 kg P_2O_5 rai^{-1}. P uptake was highly correlated with plant yield and the r^2 was 0.8984. The remaining available P and citrate soluble P were also highly correlated to each other at the r^2 of 0.9989. *E. colombiana* could solubilize P from rock phosphate better than that of *Scutellospora* sp. The application of *E. colombiana* + RPP at 30 kg P_2O_5 rai^{-1} may be an appropriate option to reduce cultivation cost. VAMF could solubilize P from cheaper rock phosphate and release enough available P to promote soybean yield as much as 4.95 g plant^{-1} which was similar to the maximum yield of *E. colombiana* + TSP at 30 kg P_2O_5 rai^{-1} treatment.

Key words: phosphate solubilization, soybean, vesicular-arbuscular mycorrhizal fungi

INTRODUCTION

The increase of plant growth by VAMF may be largely due to the enhancing absorption of nutrient from soil solution. The rate of phosphate uptake in mycorrhizal plant is faster than that in non-mycorrhizal root. Bolan (1991) reported that the inflow rate of phosphate in mycorrhizal roots was 17×10^{-14} moles cm^{-1}s^{-1} whereas that into non-mycorrhizal roots was 3.6×10^{-14} moles cm^{-1}s^{-1}. Therefore, the rate of phosphate inflow into the hyphae is about six times greater than the rate found into the root hair. Exploration through the soil volume of VAMF hyphae will decrease the distance for diffusion of phosphate ions, and increase the surface area of absorption at the same time

Hattingh *et al*. (1973) found that VAMF hyphae could intercept phosphate placed 27 cm from onion root. The radius of depletion zone for phosphate around mycorrhizal root was twice that of non-mycorrhizal root. Previous estimation revealed that the increase of phosphate uptake was 60 fold when diffusion limits the uptake, whereas the increase was only 10 fold when diffusion does

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not limits the uptake. Bolan (1991) suggested that mycorrhizal infection increase phosphate uptake by decreasing the distance that phosphate ions must diffuse to the plant roots.

Direct modification of the rhizosphere may occur by excretion of certain organic compounds to surrounding soil. The increase of P uptake from goethite-phosphate complexes by ryegrass inoculated with VAMF may be due to the increasing production of citrate and other organic compounds. Jayachandran *et al.* (1989) observed that in the presence of synthetic chelates (EDDHA), VAMF caused greater P uptake than in the absence of these chelates, meanwhile non-mycorrhizal plants were unable to use P released by chelation. They suggested that siderophore production by VAMF could significantly increase P availability in low pH soils. This is a feasible mechanism that VAMF plants could acquire P sources which unavailable to non-mycorrhizal plants. Mycorrhizal roots have a larger area of close contact with surface where phosphate ions are chemically dissociated. When these ions are taken up more effectively by external hyphae, more ions will chemically dissociate to restore the equilibrium.

Generally, indigenous VAMF species exist ubiquitously in soils. These are not specific to plant species, but their root colonization and plant growth contribution seem to be significant. Therefore, it is necessary to inoculate the appropriate species of VAMF to the rhizosphere of certain plant. Vejsadova *et al*. (1993) concluded from their experiments that the *Glomus claroideum* was capable of competing with the native VAMF populations in the greenhouse and in the field experiment. The VAMF positively influenced nitrogen fixation, nodulation and N, P, K and Mg concentration in leaves. In pots, VAMF plants produced a 24 % greater biomass than non-inoculated plant colonized by native mycorrhizal species.

Khalil *et al*. (1994) examined the soybean cultivars and found that soybean vary in mycorrhizal dependency. Three improved and two unimproved soybean cultivars were evaluated for growth response, nutrient uptake (N, P, K, Ca, Mg, Zn) and root phosphatase activity. The colonization of *Gigaspora margarita* and *Glomus intraradices* on soybean root ranged from 62-87 %. The relative growth of two unimproved soybean cultivars was significantly greater with mycorrhizal colonization than without mycorrhizal colonization. However, relative growth in improved soybean cultivars was less enhanced with colonization.

Plenchette and Morel (1996) found that barley did not respond to *Glomus intraradices* inoculation. They concluded that P nutrition was not the limiting factor on growth of this low mycotrophic plant, but, mycorrhizal inoculation stimulated growth of soybeans. The external P requirements were 0.11 $\mu g\ ml^{-1}$ for mycorrhizal and 0.148 $\mu g\ ml^{-1}$ for non-mycorrhizal soybeans in order to obtain 80 % of maximum yield.

The effectiveness of VAMF on phosphate solubilization, plant growth and P uptake is evaluated. The remaining P in growth medium after harvest including water soluble P, citrate soluble P and total P are also determined.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse of the Soil Science Department, Kamphaengsaen Campus, Kesetsart University, Nakhon Pathom province, during 1998-1999. The methods and materials were described as follows:

Experimental design

Four replications of 3×3×3 factorial in completely randomized design were employed using soybean SJ5 in 6- litre clay pot. The factors used in this experiment were illustrated in Table 1.

Table 1 Various factors used in the experiment.

VAMF	Kinds of phosphate	Levels of phosphate
Non-VAMF inoculation	Rock phosphate from Petchabun province (RPP, 18 % total P_2O_5, 2.63 % available P_2O_5)	10 kg P_2O_5 rai^{-1} (98 mg P pot^{-1})
E. colombiana	Rock phosphate from Kanchanaburi province (RPK, 22 %total P_2O_5, 3.16 %available P_2O_5)	20 kg P_2O_5 rai^{-1} (196 mg P pot^{-1})
Scutellospora sp.	Triple superphosphate (TSP, 46 % available P_2O_5)	30 kg P_2O_5 rai^{-1} (294 mg P pot^{-1})

Preparation of VAMF inoculum

The inoculum of VAMF, *E. colombiana* and *Scutellospora* sp, was produced in sterilized Yang Talad soil using sorghum as host plant. The inoculum production in this experiment was prepared as described by Mala (1998). The host plant, sorghum, had been grown for 14 weeks. After harvest the inoculum, the amount of spore in each species was checked. The inoculum with the number of spore above 40 spore g^{-1} was selected for further experiment.

Sand and pot preparation

River sand from Kanchanaburi was collected and prepared for growth medium of soybean. The sand was washed and sifted through 2.00 mm diameter sieve in tap water and then sand bed was raised on plastic sheet to the size of 1.00x2.00x0.15 m^3. Fumigation of the sand was done by adding 50 g of basamid-G to the pile of sand, then, incorporated thoroughly by hoe. Water was applied and adjusted to appropriate moisture, then the sand bed was covered with plastic sheet. After one week, plastic sheet was removed and the sand was aerated using cleaned-hoe. The aeration of sand bed was operated for one week to ensure that hazardous gas was released completely.

The individual six-litre clay pot was cleaned using tap water and the inner surface was sterilized with 70 % alcohol. Each pot contained seven kilogram of sterilized sand. Certain amount and kind of phosphate depending upon individual treatment was added and mixed thoroughly.

Seed preparation

SJ5 soybean seeds were cleaned using tap water, then they were soaked in 10 % clorox solution (sodium hypochlorite 5.25 % w/w) for 10 minutes. The remaining clorox was washed out from seeds using sterilized water.

VAMF inoculation and planting

After pouring 7 kilogram of sterilized sand into each pot, inoculation of VAMF was done. VAMF was inoculated to growth medium by mixing 1,000 ml of sterilized sand with 300 ml inoculum. This mixture of inoculum, or inoculum layer, was spreaded and pressed down into the pot. Then, 600 ml of sterilized sand was topped over the inoculum layer, leveled and pressed down. Sterilized inoculum was used in non-inoculated treatment (control).

Five surface-sterilized soybean seeds were placed 2 cm under the soil surface. Three hundred millilitre of nutrient solution was applied daily as described by Asher (1975), but without phosphate. After 10 days, seedlings were thinned to one plant per pot and the pots were kept in the greenhouse until harvest. During the course of experiment, soybean was sprayed with cypermethrin every 2 weeks to control white flies and leaf roller caterpillars.

At harvest, shoot dry weight, root dry weight and grain yield was determined. Plant P content and P uptake were determined using the method of Attanandana *et al*. (1989). After harvest, the remaining fractionated-P included water soluble P, citrate soluble P and total P in growth medium were determined using the method of Page *et al*. (1984). The remaining available P in growth medium was also determined using Bray II method as described by Attanandana *et al*. (1989). As plant takes up P from a part of solubilized-P, thus the remaining solubilized-P (SOP) still present in growth medium. Therefore, SOP can be calculated using equation below.

SOP = P uptake + Remaining citrate soluble P

The variance of individual parameter was calculated and the comparisons among relevant means were also determined using Duncan's new multiple range test at 95 % level of confidence.

RESULTS AND DISCUSSION

Shoot dry weight, root dry weight and yield of soybean SJ5

Components of soybean growth in this experiment were presented in terms of shoot dry weight, root dry weight and plant yield as shown in Table 2. The maximum shoot dry weight of plant was found in the treatment of *E. colombiana* + TSP at 20 kg P_2O_5 rai^{-1} at 11.09 g $plant^{-1}$ while those of *E. colombiana* + TSP at 30 kg P_2O_5 rai^{-1} and *E. colombiana* + RPP at 30 kg P_2O_5 rai^{-1} were 10.65 and 10.57 g $plant^{-1}$, respectively. However, amounts of shoot dry weight among these treatments were not significant.

Shoot dry weight among different VAMF treatments were highly significant. It was increased when inoculated with *Scutellospora* sp. and *E. colombiana* (Table 5). Similarly, kinds of phosphate affected shoot dry weight at 99 % level of confidence. The maximum shoot dry weight of soybean was found in TSP treatment. However, the application of RPP and RPK had no influence on shoot dry weight of soybean (Table 6).

High P level (30 kg P_2O_5 rai^{-1}) influenced shoot dry weight. Shoot dry weight of plant applied with 30 kg P_2O_5 rai^{-1} was the greatest and it decreased with decreasing levels of phosphate (Table 7).

The maximum root dry weight was found in plants treated with *E. colombiana* + TSP at 30 kg P_2O_5 rai^{-1}. The plant root in this treatment was intensively developed with the root dry weight of 2.81 g $plant^{-1}$ (Table 2). Root dry weight of *Scutellospora* sp.+ TSP at 30 kg P_2O_5 rai^{-1} and *E. colombiana* + TSP at 20 kg P_2O_5 rai^{-1} treated plants were 2.75 and 2.66 g $plant^{-1}$, respectively. However, root dry weights among these treatments were not significantly different.

Root dry weight of non-VAMF plant was rather low. It was slightly increased to 2.09 g pl^{-1} when inoculated with *Scutellospora* sp. which was not significantly different from non-VAMF treatment. However, the root dry weight of *E. colombiana* treatment was the greatest (Table 5). Kinds of phosphate significantly affected root growth, therefore root dry weight among the application of RPP, RPK and TSP fertilizer were highly significant. Root dry weight in RPP treatment was less than that of RPK and TSP (Table 6). Different levels of phosphate fertilizer had highly significant difference on root dry weight. Root dry weight of plant applying with 30 kg P_2O_5 rai^{-1} was the greatest, while the lesser weights were found in plants treated with 20 and 10 kg P_2O_5 rai^{-1} (Table 7).

The minimum yield was found in non-VAMF inoculation+RPP at 10 kg P_2O_5 rai^{-1} (1.63

g plant^{-1}). The yield of group a (Table 2), non-VAMF inoculation + TSP at 20 kg P_2O_5 rai^{-1}, *E. colombiana* + TSP at 10 kg P_2O_5 rai^{-1}, *E. colombiana* + RPP at 30 kg P_2O_5 rai^{-1}, *E. colombiana* + TSP at 20 kg P_2O_5 rai^{-1}, *Scutellospora* sp. + TSP at 20 kg P_2O_5 rai^{-1}, non-VAMF inoculation+TSP at 30kg P_2O_5 rai^{-1}, *Scutellospora* sp. + TSP at 30 kg P_2O_5 rai^{-1}, and *E. colombiana* + TSP at 30 kg P_2O_5 rai^{-1} ,were 4.67, 4.72, 4.95, 5.02, 5.05, 5.52, 5.58 and 5.72 g plant^{-1}, respectively.

Table 2 Effects of VAMF, kinds and levels of phosphate on growth of soybean SJ5.

VAMF species	Kinds of phosphate	Levels of phosphate (kg rai^{-1})	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Yield (g plant^{-1})
Non-VAMF inoculation	RPP(18 %P_2O_5)	10	5.06 $^{h-i}$	1.41^{h}	1.63^{j}
		20	5.78 $^{g-i}$	1.71^{f-h}	2.23 $^{i-j}$
		30	6.54 $^{e-i}$	1.72^{e-h}	2.82 $^{g-i}$
	RPK(22%P_2O_5)	10	4.94 ^{i}	1.54^{g-h}	2.32 $^{i-j}$
		20	6.08^{f-i}	2.05^{d-f}	2.76 $^{h-i}$
		30	6.86^{e-i}	2.09^{c-f}	3.77 $^{e-g}$
	TSP(46%P_2O_5)	10	7.59^{c-g}	2.44^{a-c}	3.58 $^{f-h}$
		20	8.34^{b-f}	2.46^{a-c}	4.67 $^{a-f}$
		30	9.67^{a-c}	2.62^{a}	5.52 $^{a-b}$
Scutellospora sp.	RPP(18 %P_2O_5)	10	8.29^{b-g}	1.61^{g-h}	4.12 $^{c-f}$
		20	7.22^{c-i}	1.82^{d-g}	4.48 $^{b-f}$
		30	7.41^{c-h}	1.83^{d-g}	4.52 $^{b-f}$
	RPK(22%P_2O_5)	10	7.03 $^{d-i}$	1.76^{e-h}	3.85 $^{d-f}$
		20	8.21 $^{b-g}$	2.06^{d-f}	4.18 $^{c-f}$
		30	8.18 $^{b-g}$	2.04^{d-f}	4.32 $^{c-f}$
	TSP(46%P_2O_5)	10	7.65 $^{c-g}$	2.48^{a-b}	4.20 $^{c-f}$
		20	8.43 $^{b-f}$	2.5^{a-b}	5.05 $^{a-c}$
		30	9.71 $^{a-c}$	2.75^{a}	5.58 $^{c-b}$
E. colombiana	RPP(18 %P_2O_5)	10	7.57 $^{c-g}$	1.63^{g-h}	4.26 $^{c-f}$
		20	7.44 $^{c-h}$	1.79^{d-h}	4.58^{b-f}
		30	10.57 $^{a-b}$	2.19^{b-d}	4.95 $^{a-d}$
	RPK(22%P_2O_5)	10	9.46 $^{a-d}$	2.06^{d-f}	4.36 $^{c-f}$
		20	8.95 $^{a-e}$	2.06^{d-f}	4.47 $^{b-f}$
		30	8.27 $^{b-g}$	2.12^{b-e}	4.63 $^{b-f}$
	TSP(46%P_2O_5)	10	10.23 $^{a-b}$	2.65^{a}	4.72 $^{a-e}$
		20	11.09^{a}	2.66^{a}	5.02^{a-c}
		30	10.65 $^{a-b}$	2.81^{a}	5.72^{a}
F-test			**	**	**

Means in each row followed by the same letter are not significantly different by DMRT at 95% confidence.
** = highly significant, * = significant, ns = non-significant.

The yield of non-VAMF plant was the least in this experiment. Inoculation of both VAMF increased soybean yield significantly. However, the roles of both species in enhancing grain yield were not different (Table 5). RPP and RPK had no significant effect on increasing soybean yield. In contrast, TSP significantly increased soybean yield as compared with those of RPP and RPK (Table 6).

Table 3 The amount of fractionated-P in growth medium after harvest soybean SJ5.

VAMF species	Kinds of phosphate	Levels of phosphate (kg rai^{-1})	Water P (mg P pot^{-1})	Citrate soluble P (mg P pot^{-1})	Total P (mg P Pot^{-1})
Non-VAMF inoculation	RPP(18 %P_2O_5)	10	4.15	5.23^{f}	79.26^{i}
		20	4.17	6.48^{f}	166.41^{f-g}
		30	4.14	7.83^{f}	280.37^{a}
	RPK(22%P_2O_5)	10	4.14	5.01^{f}	75.25^{i}
		20	5.15	7.02^{f}	172.79^{f}
		30	5.15	8.09^{f}	268.78^{a-b}
	TSP(46%P_2O_5)	10	4.17	51.94^{e}	66.40^{i-j}
		20	5.14	117.27^{d}	150.19^{h}
		30	3.13	183.11^{b}	242.05^{d-e}
Scutellospora sp.	RPP(18 %P_2O_5)	10	5.15	6.08^{f}	70.23^{i-j}
		20	4.14	6.26^{f}	163.42^{f-h}
		30	4.14	7.65^{f}	260.01^{b-c}
	RPK(22%P_2O_5)	10	4.15	4.93^{f}	71.36^{i-j}
		20	4.14	6.04^{f}	168.15^{f-g}
		30	5.17	7.99^{f}	259.56^{b-c}
	TSP(46%P_2O_5)	10	5.66	56.09^{e}	66.27^{i-j}
		20	4.12	129.02^{c}	155.44^{g-h}
		30	4.14	190.66^{b}	231.61^{e}
E. colombiana	RPP(18 %P_2O_5)	10	5.18	6.08^{f}	69.66^{i-j}
		20	5.16	7.27^{f}	159.00^{f-h}
		30	4.21	7.58^{f}	250.50^{c-d}
	RPK(22%P_2O_5)	10	4.17	4.94^{f}	66.96^{i-j}
		20	4.16	5.99^{f}	160.17^{f-h}
		30	5.18	8.00^{f}	257.20^{b-c}
	TSP(46%P_2O_5)	10	4.16	50.81^{e}	57.67^{j}
		20	4.14	131.07^{c}	154.87^{g-h}
		30	4.15	198.29^{a}	233.69^{e}
F-test			ns	**	**

Means in each row followed by the same letter are not significantly different by DMRT at 95% confidence.
** = highly significant, * = significant, ns = non-significant.

Soybean yield among different P levels were highly significant (Table 7). The minimum yield was found in plant receiving 10 kg P_2O_5 rai^{-1} (3.67 g $plant^{-1}$). Increased phosphate level up to 20 and 30 kg P_2O_5 rai^{-1}, the yield was increased to 4.13 and 4.65 g $plant^{-1}$, respectively.

P uptake of soybean

P uptake of soybean among various treatments as shown in Table 4 was highly

Table 4 Plant P uptake, available-P and SOP (solubilized P) in growth medium after harvest soybean SJ5.

VAMF species	Kinds of phosphate	Levels of phosphate (kg rai^{-1})	P uptake P (mg $plant^{-1}$)	Available P (mg P pot^{-1})	SOP (mg P pot^{-1})
Non-VAMF inoculation	RPP(18 %P_2O_5)	10	17.98^{j}	4.99^{g}	23.21^{j}
		20	24.61^{j}	5.89^{g}	31.08^{i-j}
		30	27.38^{I-j}	7.98^{g}	35.21^{i}
	RPK(22%P_2O_5)	10	26.31^{i-j}	3.35^{g}	31.32^{i-j}
		20	29.22^{h-g}	5.00^{g}	36.23^{h-i}
		30	38.83^{g-i}	7.00^{g}	46.91^{g-h}
	TSP(46%P_2O_5)	10	50.17^{c-g}	48.67^{f}	102.09^{e}
		20	54.69^{c-f}	116.63^{d}	171.96^{d}
		30	64.13^{a-c}	185.64^{b}	247.24^{b}
Scutellospora sp.	RPP(18 %P_2O_5)	10	43.39^{e-h}	4.15^{g}	49.48^{g}
		20	43.63^{e-h}	6.00^{g}	49.89^{g}
		30	47.96^{d-g}	7.45^{g}	55.61^{f-g}
	RPK(22%P_2O_5)	10	42.06^{f-h}	4.86^{g}	46.99^{g-h}
		20	43.39^{e-h}	5.98^{g}	49.42^{g}
		30	48.85^{c-g}	8.10^{g}	56.84^{f-g}
	TSP(46%P_2O_5)	10	51.05^{c-g}	59.11^{e}	107.15^{e}
		20	55.26^{c-f}	124.36^{d}	184.30^{c-d}
		30	75.12^{a}	185.89^{b}	265.76^{a}
E. colombiana	RPP(18 %P_2O_5)	10	43.97^{e-g}	5.46^{g}	50.04^{g}
		20	51.68^{c-g}	7.12^{g}	58.95^{f-g}
		30	57.90^{b-e}	7.66^{g}	65.48^{f}
	RPK(22%P_2O_5)	10	49.05^{c-g}	5.01^{g}	53.98^{f-g}
		20	49.80^{c-g}	6.21^{g}	55.78^{f-g}
		30	48.88^{c-g}	7.65^{g}	56.88^{f-g}
	TSP(46%P_2O_5)	10	59.39^{b-d}	54.43^{e-f}	110.20^{e}
		20	60.94^{a-d}	135.12^{c}	192.01^{c}
		30	70.81^{a-b}	194.49^{a}	269.09^{a}
F-test			**	**	**

Means in each row followed by the same letter are not significantly different by DMRT at 95% confidence.
** = highly significant, * = significant, ns = non-significant.

Table 5 Effect of VAMF inoculation on phosphate solubilization and soybean growth.

	Non-VAMF inoculation	*Scutellospora* sp.	*E.colombiana*	F-test
Shoot dry weight (g plant^{-1})	6.76^b	8.01^b	9.36^a	**
Root dry weight (g plant^{-1})	2.02^b	2.09^b	2.22^a	**
Yield (g plant^{-1})	3.26^b	4.45^a	4.75^a	**
P uptake (mg plant^{-1})	37.81^c	50.07^b	54.71^a	**
SOP (mg pot^{-1})	81.36^c	96.14^a	101.38^a	**
Water soluble P (mg pot^{-1})	4.37	4.54	4.50	ns
Citrate soluble P (mg pot^{-1})	43.54^b	46.08^a	46.67^a	*
Available P (mg pot^{-1})	42.80^b	45.10a^b	47.02^a	**
Total P (mg pot^{-1})	166.81^a	160.68^b	156.64^b	**

Means in each row followed by the same letter are not significantly different by DMRT at 95% confidence.
** = highly significant, * = significant, ns = non-significant.

Table 6 Effect of P kinds on phosphate solubilization and soybean growth.

	RPP(18%P_2O_5)	RPK(22%P_2O_5)	TSP(46%P_2O_5)	F-test
Shoot dry weight (g plant^{-1})	7.32^b	7.55^b	9.26^a	**
Root dry weight (g plant^{-1})	1.76^c	1.97^b	2.60^a	**
Yield (g plant^{-1})	3.73^b	3.85^b	4.87^a	**
P uptake (mg plant^{-1})	39.83^b	41.81^b	60.95^a	**
SOP (mg pot^{-1})	46.55^b	48.26^b	184.07^a	**
Water soluble P (mg pot^{-1})	4.49	4.60	4.31	ns
Citrate soluble P (mg pot^{-1})	6.72^b	6.43^b	123.14^a	**
Available P (mg pot^{-1})	6.30^b	5.91^b	122.7^a	**
Total P (mg pot^{-1})	166.54^a	166.67^a	150.92^b	**

Means in each row followed by the same letter are not significantly different by DMRT at 95% confidence.
** = highly significant, * = significant, ns = non-significant.

Table 7 Effect of P levels on phosphate solubilization and soybean growth.

	10 kgP_2O_5 rai^{-1}	20 kgP_2O_5 rai^{-1}	30 kgP_2O_5 rai^{-1}	F-test
Shoot dry weight (g plant^{-1})	7.54^b	7.95^b	8.65^a	**
Root dry weight (g plant^{-1})	1.97^c	2.12^b	2.24^a	**
Yield (g plant^{-1})	3.67^c	4.13^b	4.65^a	**
P uptake (mg plant^{-1})	42.59^c	46.69^b	53.31^a	**
SOP (mg pot^{-1})	63.81^c	92.96^b	122.11^a	**
Water soluble P (mg pot^{-1})	4.55	4.48	4.40	ns
Citrate soluble P (mg pot^{-1})	21.22^c	46.27^b	68.80^a	**
Available P (mg pot^{-1})	21.11^c	45.82^b	67.98^a	**
Total P (mg pot^{-1})	69.21^c	161.16^b	253.76^a	*

Means in each row followed by the same letter are not significantly different by DMRT at 95% confidence.
** = highly significant, * = significant, ns = non-significant.

significant difference. The maximum P uptake, 75.12 mg P $plant^{-1}$, was found in plant treated with *Scutellospora* sp. + TSP at 30 kg P_2O_5 rai^{-1}. Plants in other treatments which took up large P (group a) were found in *E. colombiana* + TSP at 30 kg P_2O_5 rai^{-1}, non-VAMF inoculation + TSP at 30 kg P_2O_5 rai^{-1}, non-VAMF inoculation + TSP at 20 kg P_2O_5 rai^{-1} and *E. colombiana* + TSP at 20 kg P_2O_5 rai^{-1}. Those P uptake were 70.81, 64.13, 61.69 and 60.94 mg P $plant^{-1}$, respectively, whereas the minimum P uptake, 17.78 mg P $plant^{-1}$, was found in plant applied with non-VAMF inoculation + RPP at 10 kg P_2O_5 rai^{-1}.

Highly significant differences were observed in VAMF inoculation treatment for P uptake (Table 5). The minimum P uptake was found in non-VAMF treatment. On the contrary, P uptake of plant was increased in VAMF inoculation. P uptake of soybean among treatments of RPP and RPK factors were not significant (Table 6). However, P uptake in RPK tended to be higher than that of RPP. Application of TSP had highly significant differences on P uptake. P uptake in plant of TSP application was increased and was greater than those of RPP and RPK. Levels of P had highly significant differences on P uptake. Increasing P level increased P uptake of plant (Table 7). Adding 10 kg P_2O_5 rai^{-1} decreased P uptake, but P uptake could be increased at 20 and 30 kg P_2O_5 rai^{-1}.

The results coincided with earlier report by Mala *et al.* (1997). They examined the effectiveness of VAMF on plant growth and P uptake in corn and soybean and found that P uptake of VAMF plant ranged from 3.86-6.79 mg P $plant^{-1}$ which greater than that of non-VAMF plant (3.69 mg P $plant^{-1}$). Shoot P concentration of mycorrhizal soybean tended to be greater than that of non-mycorrhizal soybean. The maximum shoot P concentration in mycorrhizal soybean was 0.076 % meanwhile that in control (without VAMF inoculation) contained shoot P of 0.042 %.

Quantity of the remaining phosphate in growth medium after harvest

Water soluble P and citrate soluble P in growth medium after harvest showed not only the potential of solubilzation of P from various factors but also the availability of P for the next cropping. Amounts of water soluble P among various treatments were not significant. These amounts ranged from 3.13-5.66 mg P pot^{-1} which reflecting the concentration of remaining water soluble P in growth medium was less than 1 mg kg^{-1}. However, most of citrate soluble P in RPP and RPK from this experiment was water soluble P.

Citrate soluble P in growth medium, after harvest, among various treatments were highly significant difference (Table 3). The maximum citrate soluble P, 198.29 mg P pot^{-1}, was found in *E. colombiana* + TSP at 30 kg P_2O_5 rai^{-1}. The minor group was found in two treatments, *Scutellospora* sp. + TSP at 30 kg P_2O_5 rai^{-1} and non-VAMF inoculation + TSP at 30 kg P_2O_5 rai^{-1} at 196.66 and 183.11 mg P pot^{-1}, respectively. Citrate soluble P of other treatments was low.

Significant differences in amount of citrate soluble P were observed in VAMF inoculation. Citrate soluble P in VAMF-inoculated growth medium was greater than that of non-VAMF one (Table 5). Citrate soluble P in TSP was greater than that of RPP and RPK treatments (Table 6) and citrate soluble P of RPP treated plant was in the vicinity to that of RPK. Most phosphate in RPP and RPK were in citrate insoluble form. There was slightly citrate soluble P in both kinds of rock phosphate. Generally, the amount of citrate soluble P in rock phosphate is no more than 3 % P_2O_5. In TSP application, nearly all phosphate was citrate soluble P. Plant took up this P and P fixation in growth medium (sand) occurred slightly. Therefore, the main form of P remaining in growth medium was citrate soluble P. Application of P at various levels affected the amount of citrate soluble P. At 10 kg P_2O_5 rai^{-1}, citrate soluble P was the least. Adding P at 20 and 30 kg P_2O_5 rai^{-1} increased citrate soluble P about 2 and 3 times (Table 7).

Amounts of remaining total P among various treatments were highly significant difference (Table 3). The maximum, 280.37 mg P pot^{-1}, was found in non-VAMF inoculation + RPP at 30 kg P_2O_5 rai^{-1}. The minor group of total P were found in non-inoculation + RPK at 30 kg P_2O_5 rai^{-1}, *Scutellospora* sp. + RPP at 30 kg P_2O_5 rai^{-1}, *Scutellospora* sp. + RPK at 30 kg P_2O_5 rai^{-1}, *E. colombiana* + RPK at 30 kg P_2O_5 rai^{-1} and *E. colombiana* + RPP at 30 kg P_2O_5 rai^{-1} at 268.78, 260.01, 259.56, 257.20 and 250.50 mg P pot^{-1}, respectively. Total P of other treatments was low.

Total P in VAMF treatment was less than that of non-VAMF treatment. VAMF inoculation enhanced phosphate solubilization through activity of extraradical hyphae and excretion of certain organic acid, then plant took up greater amount of soluble P and consequently left the lesser amount of total P as compared with that of non-VAMF treatment (Table 5). Highly significant differences were observed in TSP application for total P. Plant took up greater amount of P in TSP treatments. Therefore, total P remained in TSP treatment was less than that of RPP and RPK (Table 6). Total P in RPP was similar to that of RPK, but significantly differed from that of TSP. Levels of P application affected the amount of total P in growth medium. Total P among P levels were highly significant (Table 7). At 10 kg P_2O_5 rai^{-1}, the quantity of total P should be enough for second crop in the event that most of the remaining total P was either plant available form or slowly released form which can be solubilized and released to plant available form during plant growth. At P level of 10 kg P_2O_5 rai^{-1}, total P was at 69.21 mg P pot^{-1}, meanwhile plant P uptake at this P level ranged from 40-50 mg P pot^{-1}. Adding P 20-30 kg P_2O_5 rai^{-1}, the remaining total P was expected to be enough for soybean in the next crop.

The available P in growth medium after harvest was extracted and determined with Bray II procedure. These P among treatments were highly significant. The available P of individual treatment showed similar trend to that of citrate soluble P. The maximum available P (194.99 mg P pot^{-1}) was found in *E. colombiana* + TSP at 30 kg P_2O_5 rai^{-1}, while the minor group were found in *Scutellospora* sp. + TSP at 30 kg P_2O_5 rai^{-1} and non-VAMF inoculation + TSP at 30 kg P_2O_5 rai^{-1} at 185.89 and 185.64 mg P pot^{-1}, respectively. Application of rock phosphate (RPP and RPK) at certain level gave the similar amount of available P to the other levels. However, the available P tended to be increased as increased P level. The available P of this group was the least at 3.35 mg P pot^{-1} in non-VAMF inoculation + RPK at 10 kg P_2O_5 rai^{-1} and the greatest amount, 8.10 mg P pot^{-1}, was found in *Scutellospora* sp. + RPK at 30 kg P_2O_5 rai^{-1}. However, amounts of available P in various levels of TSP were highly significant difference (Table 4).

The effect of VAMF inoculation on the quantity of available P in growth medium after harvest was highly significant difference. The most effective species of VAMF was *E. colombiana* which could induce greater P solubilization. While the available P among non-VAMF inoculation and *Scutellospora* sp. treatment was not significant, but, that amount in *Scutellospora* sp. tended to be slightly greater than that of non-VAMF treatment. The available P among different P kinds was highly significant (Table 6). The maximum amount was released from TSP, while that amount of RPP and RPK was not significant difference from each other. P level had highly significant differences on the quantity of available P (Table 7). The available P at 20 and 30 kg P_2O_5 rai^{-1} was increased 2 and 3 times greater than that of the 10 kg P_2O_5 rai^{-1} .

SOP in this experiment, obtained from the sum of plant P uptake and the remaining citrate soluble P after harvest time, was illustrated in Table 4. The maximum SOP was found in *E. colombiana* + TSP at 30 kg P_2O_5 rai^{-1} and *Scutellospora* sp. + TSP at 30 kg P_2O_5 rai^{-1} at 269.09 and 265.76 mg P pot^{-1}, respectively. These SOPs among P kinds were not significantly different from each other, but significantly differed from SOP of non-VAMF inoculation + TSP at 30 kg P_2O_5 rai^{-1} (247.24 mg P pot^{-1}). SOP amount of other treatments were low, while the minimum SOP (23.21 mg P pot^{-1}) was found in non-VAMF inoculation + RPP at 10 kg P_2O_5 rai^{-1} .

VAMF inoculation increased P solubilization. The P solubilization among the activity of *E. colombiana* and *Scutellospora* sp. were not significant, but that of the former tended to be greater than the later. The solubilization of P in non-VAMF treatment was significantly different from VAMF treatment (Table 5). Kinds of P affected solubilization of P. SOP among RPP and RPK application were not significant. In contrast, SOP of TSP application significantly differed from those of rock phosphate application (Table 6). P levels affected the amount of SOP and these SOP among P levels was highly significant (Table 7). Increase P levels resulted in increasing SOP up to nearly twofold.

The results from this experiment suggested that growth and yield of soybean in VAMF-plant were greater than non-VAMF plant. The main reasons had been described by Bolan (1991). Firstly, VAMF produce extraradical hyphae intensively through soil volume. These hyphae absorb and take up a large amount of nutrient particularly phosphate beyond the zone of normal root. Consequently, part of the nutrient in VAMF hyphae translocate to plant root tissue. Secondly, the production and excretion of some organic acids by VAMF hyphae around the root zone will be occurred in VAMF-plant. These organic acids can dissolve and change insoluble P into plant available form.

Correlation between plant P uptake and plant yield was observed (Figure 1). Plant P uptake was highly related to soybean yield and the value of coefficient of determination (r^2) was 0.8984. The yield of soybean was increased as P uptake of plant increased. The linear correlation between them is illustrated in the following equation.

$$Y = 0.0701X + 0.829$$

when Y = soybean yield (g $plant^{-1}$)

X = P uptake of soybean (mg P $plant^{-1}$)

The remaining citrate soluble P of individual treatment and part of fractionated-P in growth medium were closely related to its available P. Highly relationships between both values was

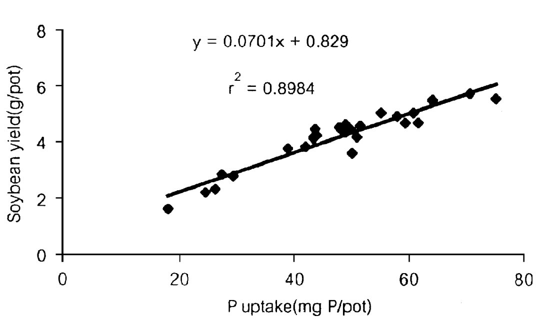

Figure 1 Correlation between soybean yield and P uptake.

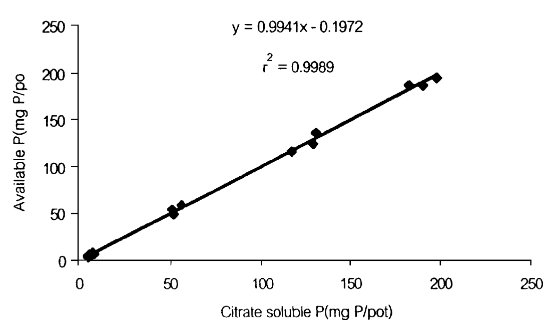

Figure 2 Correlation between available P and citrate soluble P in growth medium.

observed and the r^2 was 0.9989 (Figure 2). The correlation is illustrated in the linear equation as follows.

$$Y = 0.9941X - 0.1972$$

when Y = available P (mg P pot^{-1})

X = citrate soluble P (mg P pot^{-1})

After P application, available P will be partially taken up by plant and microorganisms. The remaining available P will be retained in soil either in form of organic or inorganic fixation, then left smaller amount of available P. In this experiment, due to sand was used as growth medium, phosphate fixation therefore was slightly. Then, the amount of citrate soluble P of each treatment was closed to that of available P. However, partial P may be immobilized by fungi, but, this process may slightly occurred.

VSOP (VAMF solubilized-P) of individual treatment could be obtained by finding the difference between SOP of VAMF treatment and their corresponding SOP of non-VAMF treatment. The VSOP of *Scutellospora* sp. with the application of RPP and RPK, illustrated in Table 3, were tended to decrease when increased P levels. Contrastingly, the result of TSP was significantly differed. VSOP in TSP was increased when increased P level. While VSOP in *E. colombiana* + RPP among P levels were not significantly differed from each other. In contrast, VSOP of *E. colombiana* + RPK and *E. colombiana* + TSP were similar to that of *Scutellospora* sp. + TSP. However, it can be suggested that *E. colombiana* had greater ability on P solubilization than did *Scutellospora* sp. Moreover, *E. colombiana* was more tolerant to higher level of rock phosphate than did *Scutellospora* sp. The activity of *E. colombiana* on P solubilization had been done continuously even the level of P either in form of RPP or TSP was increased.

In the condition which P limited growth and yield of plant, increase solubilized-P affected the increase in plant P uptake and influenced on plant yield. VEY (VAMF-enhanced yield) of soybean in Figure 4 illustrated that most treatments inoculated with *E. colombiana* had greater ability to enhance plant growth than did *Scutellospora* sp. On the other hand, VEY in *E. colombiana* and *Scutellospora* sp. inoculation applying TSP at 20 kg P_2O_5 rai^{-1}were not significantly differed from each other. However, VEY was decreased as P level increased. In case of VAMF inoculation + kinds of P , *E. colombiana* had greater trend of VEY than did *Scutellospora* sp. RPP had the greatest trend of VEY, while that of TSP was the least.

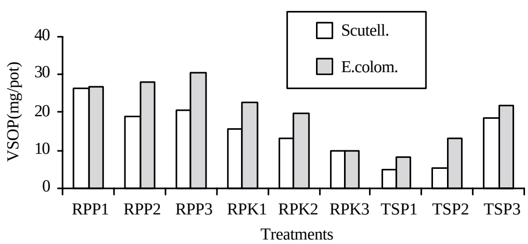

Figure 3 The effectiveness of phosphate solubilization of *Scutellospora* sp. and *E. colombiana* associated with soybean SJ5 at different kinds and levels of phosphate.

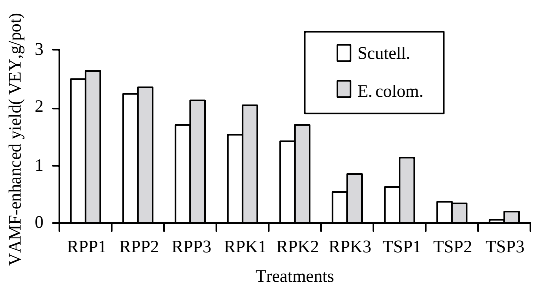

Figure 4 The yield increment of soybean SJ5 as caused by *Scutellospora* sp. and *E. colombiana* at different kinds and levels of phosphate.

E. colombiana had greater ability on increasing yield than did *Scutellospora* sp. VEY of soybean applying RPP was greater than that of RPK and TSP. However, increasing P level reduced VEY. It may be suggested that the effectiveness of VAMF on promoting plant growth will be decreased as P level increased. RPP in this experiment has the least amount of soluble P and total P whereas the soluble P in TSP is the greatest. Therefore, plant growth enhancement of VAMF in high available P treatment seemed to have lesser benefit.

Decreasing plant growth enhancement of VAMF may be caused by lesser development of extraradical hyphae. Amijee *et al*. (1989) concluded that increasing the levels of soluble phosphate to soil reduced the overall VAMF colonization on plant root. This phosphate decreased the extension of extraradical hyphae and arbuscule formation, but increased the root and plant growth. Addition of phosphate fertilizer to soil reduced both colonization and sporulation in various crops including corn, soybean, clover and small grain crops.

The effectiveness of various VAMF on plant promotion in various conditions is different. Miranda and Harris (1994) found that *Scutellospora heterogama* was highly sensitive to soil P supply. But, Kurle and Pfleger (1996) found that the populations of *Gigaspora magarita* were positively correlated with soil P level, meanwhile the populations of *Glomus aggregatum* were negatively correlated with soil P. However, Mala *et al*. (1998) found that *Entrophospora* sp. showed the greatest effectiveness on colonizing soybean root. They concluded that VAMF had different ability to colonize plant root even at high P level of phosphate. The enhancement of VAMF on plant P uptake and yield were observed and found that all mycorrhizal plants took up more phosphate and then obtained greater yield.

CONCLUSION

The comparison of plant growth promotion and phosphate solubilization between *E. colombiana* and *Scutellospora* sp. as associated with soybean SJ5 was studied. VAMF had important role on phosphate solubilization, P uptake, growth and yield of soybean. During growth of soybean, *E. colombiana* showed greater ability on phosphate solubilization from rock phosphate than did *Scutellospora* sp. The effectiveness of both VAMF was decreased at high level of phosphate. Increasing phosphate levels resulted in increasing plant yield, remaining P in soil, but decreasing the enhancement of VAMF.

The solubilization of phosphate from rock phosphate is one of the important option to reduce cost in cultivation. The result indicated that the utilization of *E. colombiana* and applying RPP at 30 kg P_2O_5 kg rai^{-1} enhanced the plant ability to take up more phosphate and obtained the yield as much as that of the plant applying with TSP at 30 kg P_2O_5 kg rai^{-1} in non-VAMF inoculation treatment. The production cost of the former is less than the later and some parts of remaining phosphate will be solubilized by VAMF in the next crop. However, the study of VAMF mechanisms should be further verified in various conditions including in the soil where phosphate availability and plant growth were limited by phosphate fixation.

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