

Responses of Winged Bean (*Psophocarpus tetragonolobus*) to Mycorrhiza Inoculation in Pot and Field Trials

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ABSTRAK

Dua kajian di rumahijau dan satu kajian di ladang telah dijalankan untuk mendapat maklumat kemungkinan mengeksploit faedah kehadiran kulat mikoriza vesikul arbuskul dalam pertanian di Malaysia. Dalam kajian pertama, kacang kelisa (*Psophocarpus tetragonolobus*) telah ditanam dalam tanah Serdang disucihama. Perkembangan endofit ini dinilai setiap dua minggu. Kajian kedua menggunakan tanah Serdang dan Munchong yang tidak disucihama, dengan 3 paras baja fosforus (P) dan diberi inokulasi dengan *Acaulospora laevis*, *Glomus macrocarpum*, *Glomus mosseae*, campuran spesies *Glomus* dan *Scutellospora calospora*. Kesemua inokula kecuali *S. calospora* meningkatkan pertumbuhan kacang kelisa dengan bererti, disamping meningkatkan kepekatan tisu N, P dan K. *Glomus mosseae* merupakan spesies yang superior. Dalam kajian di ladang, *Acaulospora laevis*, *Scutellospora calospora* dan *Glomus mosseae* telah diinokulatkan kepada kacang kelisa ditanam pada tanah yang tidak disucihama, dengan atau tanpa baja fosforus. Kehadiran *Glomus mosseae* mampu meningkatkan pembentukan bunga secara bererti ($P < 0.05$) (4.5 bunga/pokok), khususnya pada kadar baja P yang sederhana (60 kg P ha^{-1}).

ABSTRACT

Two greenhouse experiments and one field trial were conducted to provide information on the possibility of exploiting the beneficial effects of vesicular-arbuscular mycorrhizal fungi in Malaysian agriculture. In the first study, winged bean (*Psophocarpus tetragonolobus*) was grown in steam-sterilized Serdang soils and the development of the endophytes evaluated fortnightly. The second experiment was conducted in unsterilized Serdang and Munchong soils respectively with three levels of P and/or inoculated with *Acaulospora laevis*, *Glomus macrocarpum*, *Glomus mosseae*, a mixture of *Glomus* species and *Scutellospora calospora*. All inocula, except for *S. calospora*, significantly enhanced growth throughout the course of the experiments and increased N, P and K concentrations in the plant tissues. *G. mosseae* was superior to the rest. In the field trial, *Acaulospora laevis*, *Scutellospora calospora* and *Glomus mosseae* were inoculated into winged bean grown in unsterilized field soil, with or without phosphate fertilizer. Inoculation of winged bean with *G. mosseae* significantly ($P < 0.05$) increased inflorescence formation (4.5/plant), particularly at an intermediate level (60 kg ha^{-1}) of P fertilizer.

INTRODUCTION

The beneficial effects of vesicular-arbuscu-

lar mycorrhizal (VAM) fungi on plant growth are well documented (Abbott and

Robson 1982; Harley and Smith 1983; Mosse 1986; Hall 1988). There is also a growing body of evidence that shows it may be possible to exploit the beneficial effects of VAM in agriculture (Daniels *et al.* 1981; Ganry *et al.* 1982; Abbott and Robson 1984). In developing countries, the relative cost of chemical fertilizer is high and consequently restricts their use. Therefore, there is considerable interest in the use of alternative fertilizers and the exploitation of symbionts such as rhizobia and VAM (Bagyaraj *et al.* 1979; Harris *et al.* 1985; Hall 1988; Azizah 1991; Kumaran and Azizah 1995). Reproducibility of results obtained from pot studies under controlled conditions is of prime importance if mycorrhiza inoculation is to be successfully introduced under uncontrolled conditions. The experiments described here were designed to screen a number of VAM fungi for effectiveness in enhancing growth of winged bean (*Psophocarpus tetragonolobus*) in Malaysian soils.

MATERIAL AND METHODS

Soils

Two greenhouse trials were established in unsterilized Serdang and Munchong soils

TABLE 1
Physicochemical properties of Serdang and Munchong soils

| Physicochemical property | Serdang | Munchong |
|-----------------------------------|---------|----------|
| Horizon | Ap | Ap |
| +Depth (cm) | 0–15 | 0–15 |
| +Coarse sand (%) | 14.0 | 7.7 |
| +Fine sand (%) | 59.0 | 27.8 |
| +Silt (%) | 3.0 | 13.3 |
| +Clay (%) | 25.6 | 51.2 |
| Organic C (%) | 1.08 | 1.68 |
| Total N (%) | 0.11 | 0.13 |
| Extractable P ($\mu\text{g/g}$) | 6.31 | 2.97 |
| pH (H_2O) | 5.10 | 4.78 |

+ Paramananthan (Pers. comm.)

(Table 1) and in Serdang soil, which had been steam-sterilized for 1 h at 100°C. The pH of the Serdang and Munchong soils was raised from the respective values of 4.6 and 4.4 to pH 6.0 by incorporating ground magnesium limestone (GML) at the rates of 2.3 and 2.7 g kg⁻¹ soil respectively.

In Experiment 1, 1 kg sterilized Serdang soil was used to fill each 12-cm diameter pot and the following basal fertilizers were added: urea, at the equivalent rate of 14 kg N ha⁻¹, triplesuperphosphate (TSP), at 12 kg P ha⁻¹ and muriate of potash (MOP), at 30 kg K ha⁻¹.

In Experiment 2, unsterilized Serdang and Munchong soils were used; 5 kg of each soil type in each 25-cm diameter pot lined with plastic. The following basal fertilizers were added: urea, at the equivalent rate of 14 kg N ha⁻¹, MOP at 60 kg K ha⁻¹, and three levels of TSP at 0, 30 and 60 kg P ha⁻¹ respectively.

In Experiment 3, the field trial was conducted 4 months later in Experimental Plot No. 2 of Universiti Pertanian Malaysia, Serdang where the mean annual rainfall is 2000 mm and the mean annual air temperature is 24°C. The soil is an alluvium (old mining land) with pH(H_2O) 4.8 and 20 μg bicarbonate extractable P. The site selected was overgrown with mixed weed species and had not received any form of fertilizer for the previous two years. After it had been ploughed three times in order to reduce soil heterogeneity, 2.3 t/ha GML was added to raise the pH to 6.0.

Endophytes

Five endophyte treatments were used in Experiment 1. They were: *Acaulospora laevis*, *Glomus macrocarpum*, *Glomus mosseae*, a mixture of *Glomus* species, and *Scutellospora calospora*. All species except *Glomus macrocarpum* were obtained from Dr. I.R. Hall of New Zealand. *G. macrocarpum* was supplied by Dr. K.R. Krishna of ICRI-

SAT, India. These species were propagated in the greenhouse of Universiti Pertanian Malaysia for a period of 9 months using *Setaria anceps* var. *splendida* as the host plant (Azizah and Omar 1987). All the VAM species above except *G. macrocarpum* were used in Experiment II, which was set up 1 month later.

Experiment 1: 50 g soil plus spores, mycelium and colonized root segments of *Setaria* were added to each inoculated pot. The inoculum was spread in a thin layer 5 cm below the level where the seeds were to be planted. Control plants were similarly treated using soil inocula grown with uninoculated *Setaria*. The plants were watered with distilled water twice daily for the first 14 days of growth and later as needed for the duration of the experiment.

Experiment 2: 100 g soil inoculum was used per pot; otherwise the procedure was identical to Experiment 1.

Experiment 3: 50 g soil inoculum containing either *A. laevis*, *G. mosseae*, *S. calospora* or sterilized soil was thinly spread 8 cm below each planting hole. In the *Rhizobium*-inoculated plots, a suspension of a *Rhizobium* culture was applied with a watering-can after the seeds were sown. Two pre-soaked seeds were sown per planting hole with 40 cm between plants and 45 cm between rows. Thinning was carried out one week after sowing. As the seedlings grew they were supported on string hung from a network of 1.5-m high wire trellises supported with wooden beams.

Experimental Design

The pots in each experiment were placed on wooden benches and randomized in blocks in the greenhouse.

Experiment 1: A 6 × 6 factorial combination consisting of the following treatments: five VAM fungal species plus one control, and six harvests. Only one plant

was planted per pot, with each treatment replicated three times. Three plants from each treatment were harvested at fortnightly intervals.

Experiment 2: A randomized complete block design comprising the following treatments: four VAM fungal species (*Acaulospora laevis*, *Scutellospora calospora*, *Glomus mosseae* and mixed *Glomus* species, designated as Alae, Scal, Gmos and Gmix respectively in the text); two soil types (Serdang and Munchong), and three levels of P fertilizer – 0, 30 and 60 kg TSP ha⁻¹ (designated as P₀, P₁ and P₂ respectively). There was also one plant per pot, with three replications per treatment. The mycorrhizal treatment (+R) was treated with a *Rhizobium* strain, RRIM 56; the control (-R) was not.

Experiment 3 (conducted four months later): The experimental design was a 3 × 5 factorial laid out in 4 randomized complete blocks giving a total of 60 plots. Each plot measured 2.6 × 2.7 m. The treatments included inoculation with: *A. laevis* + *Rhizobium*, *G. mosseae* + *Rhizobium*, *S. calospora* + *Rhizobium*, no inoculation with either VAM or *Rhizobium*, and inoculation with *Rhizobium* alone.

Harvests and Plant Analysis

Experiment 1: Successive harvests of three randomly selected replicates from each treatment were made 14 days after sowing, and continued thereafter at fortnightly intervals until 12 weeks after inoculation. At each harvest, fresh weights of root and shoots and also shoot dry weights were recorded. Randomly selected root samples were cleared of adhering debris, rinsed thoroughly and then stored in 10% formalin acetic acid (FAA). These root segments were then cleared in 10% KOH and stained with trypan blue (Phillips and Hayman 1970). A total of 90% 1mm-root sections per treatment was assessed. The

percentage of root length in the stained sample colonized by mycorrhizal fungi was recorded as positive and calculated using the following formula:

% VAM colonization =

$$\frac{\text{No. of VAM positive segments}}{\text{Total no. of segments scored}} \times 100$$

Experiment 2: The plants were harvested 16 weeks after sowing. Plant parts were weighed immediately after harvest, and again after drying to constant weights at 72°C (approx. 2 days). The dried shoots were then ground. Twenty-five mg of the ground material was then wet ashed by digesting with 5 ml concentrated H₂SO₄ and oxidised with H₂O₂ (Thomas *et al.* 1967). The digest was subsequently made up to 250 ml, and N and P levels determined with a Technicon^R autoanalyser using the ascorbic acid method for P and the alkaline salicylate method for N. K concentrations were measured with a flame photometer.

Experiment 3: Fifty days after sowing, leaf numbers from 12 plants in each plot were recorded and after a further 20 days, numbers of fully opened flowers in each plot were counted. Untimely heavy rainfall produced over-luxuriant plant growth which broke the main trellis support and

the experiment had to be terminated three weeks after the flower count was completed.

All the data obtained except percentage root colonization were subjected to analysis of variance (ANOVA) using GENSTAT. Percentage of root colonization was subjected to a chi-square test for test of significance.

RESULTS

Formation of Mycorrhizae

Good infection of winged bean roots as a result of inoculation with the five mycorrhizal species was observed (Experiment 1). However, positive signs of infection by these endophytes only became obvious 4 weeks after inoculation, after which infection of the roots increased with increase in sampling time (Table 2).

There was heavy infection (63%) of the roots inoculated with *A. laevis* from week 8 onwards. At age 4 weeks, development of mycorrhiza was greater in plants inoculated with *G. macrocarpum*, with 58% of the roots colonized. Plants inoculated with *G. mosseae* had 49% and 70% (with internal hyphae and arbuscules) mycorrhizal infection at 4 and 6 weeks after inoculation, respectively.

Results obtained from the chi-square test showed that differences in root colonization percentage by the different VAM species were only significant at the first sampling period, i.e. 4 weeks after inocula-

TABLE 2
Percentage colonization of winged bean roots by five mycorrhizal fungi with time of harvest

| | Sampling weeks | | | | |
|----------------------------|----------------|------|------|------|------|
| | W4* | W6 | W8 | W10 | W12 |
| + <i>A. laevis</i> | 38.0 | 53.5 | 63.0 | 66.3 | 70.2 |
| + <i>G. mosseae</i> | 49.0 | 70.0 | 73.3 | 74.5 | 78.8 |
| <i>G. macrocarpum</i> | 58.0 | 63.1 | 64.3 | 68.7 | 74.4 |
| + Mixed <i>Glomus</i> spp. | 34.0 | 52.2 | 26.7 | 69.4 | 75.1 |
| + <i>S. calospora</i> | 32.3 | 55.0 | 57.8 | 62.2 | 64.4 |

*For sampling week, Significant at P < 0.05

+For VAM species, Significant at P < 0.05

tion. Differences in root colonization percentage were not significant at 6, 8, 10 and 12 weeks of sampling due to the gradual increase in roots being colonized by all the VAM fungi.

Plant Growth

In Experiment 1, mycorrhiza, time of harvest (week) and interaction of these two factors significantly ($P < 0.05$) influenced growth and dry matter production of winged bean. Owing to heterogeneity in the sampling time variance data, each sampling time had to be analysed separately. However, applying a \log_e transformation allowed all the data to be analysed together.

All five VA mycorrhizal fungal species significantly ($P < 0.001$) stimulated growth of winged bean compared to control plants. Shoot dry weights of mycorrhizal plants increased curvilinearly at all harvests except for plants treated with *S. calospora*.

In these plants, shoot dry weights reached a maximum value of 2.7 g at week 10 and then declined to 2.4 g at the final harvest at week 12 (*Fig. 1*).

Fungal effectiveness was in the order: *A. laevis* = *G. mosseae* = mixed *Glomus* = *G. macrocarpum* \gg *S. calospora* \gg Control.

The root/shoot ratio values of mycorrhizal plants seemed to fluctuate with each harvest (*Fig. 2*). However, at the final harvest, all the mycorrhizal plants except for those treated with *S. calospora* had root/shoot ratios lower than the control plants. Both the *S. calospora*-treated and control plants showed a sharp increase in the root/shoot ratios from week 8 onwards.

In Experiment 2, soil, mycorrhiza and rate of phosphorus fertilization and interactions between these variables had significant ($P < 0.05$) effects on shoot (*Fig. 3*) and root dry weights (*Fig. 4*) in both Serdang and Munchong soils.

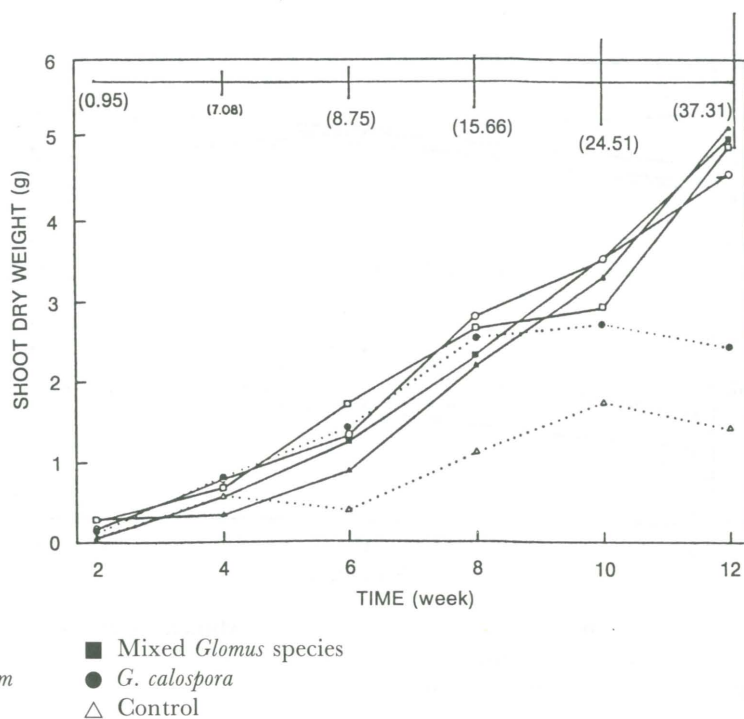
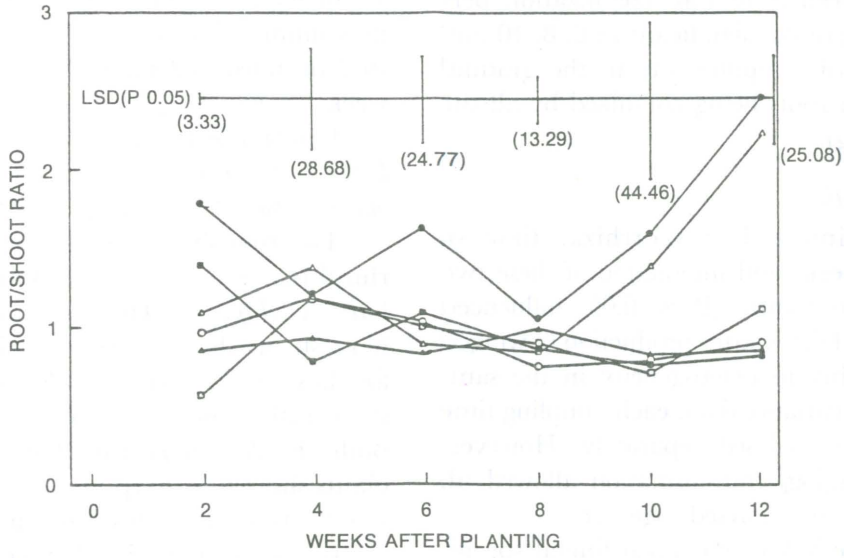
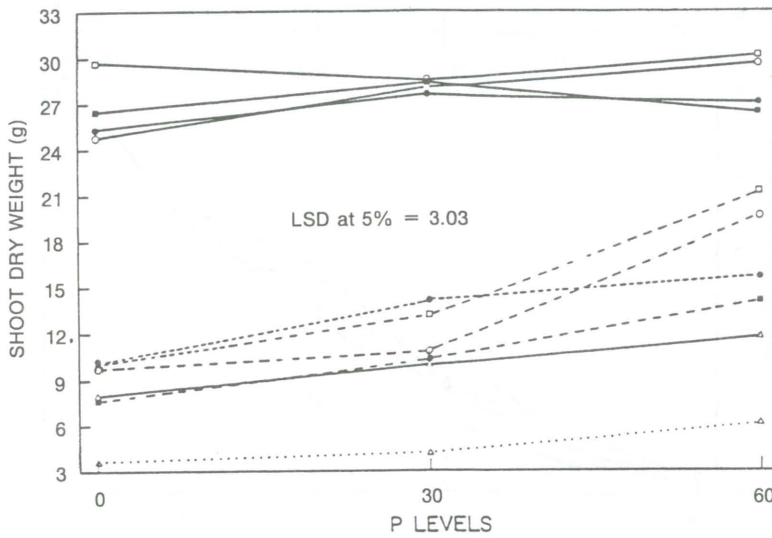


Fig. 1. Shoot dry weight of *Psophocarpus tetragonolobus* as affected by VAM inoculum and time of harvest



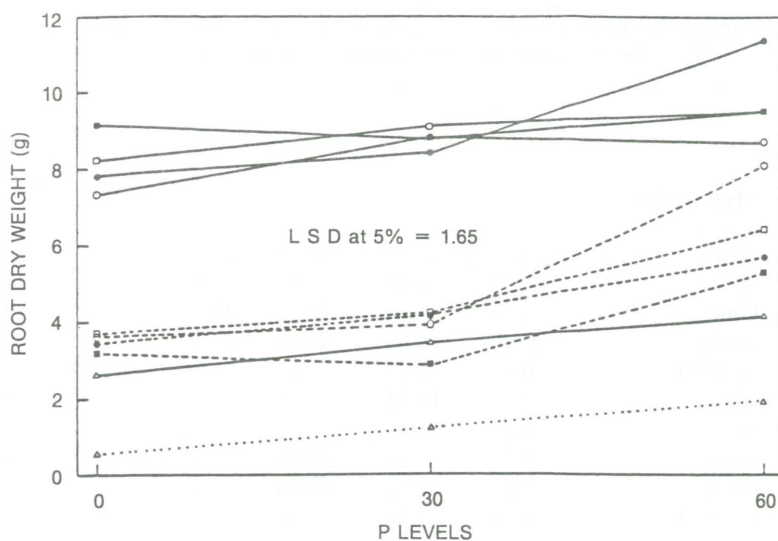
- *A. laevis*
- ▲ *G. macrocarpum*
- *G. mosseae*
- Mixed *Glomus* species
- *G. calospora*
- △ Control

Fig 2. Root/shoot ratio of *Psophocarpus tetragonolobus* as affected by VAM inoculum and time of harvest



- *A. laevis*
- *G. mosseae*
- Mixed *Glomus* species
- *G. calospora*
- △ Control
- Munchong series
- Serdang series

Fig 3. Shoot dry weight (g) of *Psophocarpus tetragonolobus* as affected by VAM inoculum and P levels in Serdang and Munchong soils



- *A. laevis* ● *G. calospora* ----- Munchong series
 □ *G. mosseae* △ Control
 ■ Mixed *Glomus* species — Serdang series

Fig 4. Root dry weights (g) of *Psophocarpus tetragonolobus* as affected by VAM inoculum and P levels in Serdang and Munchong soils

All the mycorrhizal species tested stimulated plant growth significantly ($P < 0.001$) compared to non-inoculated controls. However, differences in growth increments between the four species studied were not significant. Effectiveness of the fungal species in enhancing plant growth was in the order: *G. mosseae* > *A. laevis* = *S. calospora* > mixed *Glomus*. Addition of *Rhizobium* RRIM 56 had little effect on plant growth at P_0 and P_2 . However, the synergistic effects between RRIM 56 and the four VAM species studied became evident with the significant ($P < 0.05$) increase in plant growth at the intermediate P level of 30 kg Pha^{-1} (Table 3).

The best overall plant growth was recorded in Serdang soil; shoot dry weights increased with increasing P level except for plants inoculated with *G. mosseae* and mixed *Glomus* species. In *G. mosseae*-treated plants, shoot dry weight decreased at P_1 while in

mixed *Glomus*-inoculated plants, shoot dry weight was highest at this level of P fertilization. Overall, the highest shoot dry weight (29.4 g) was obtained from *G. mosseae*-treated plants at P_2 , followed by 27.6 g in *A. laevis*-treated plants also at the same P level. Mixed *Glomus* also appeared to work as well as the other species, with 27.1 g shoot dry weight.

In Munchong soil, increase in shoot dry weight was also seen to parallel the increase in the level of P fertilizer added. This holds true for all treatments including the control. Differences in shoot dry weight between inoculated and uninoculated plants were also significant ($P < 0.005$). As in Serdang soil, *G. mosseae* and *A. laevis*-treated plants recorded the highest shoot dry weights of 14.8 g and 13.6 g respectively. Plant growth was stimulated less in Munchong than in Serdang soil.

Results obtained from the two pot trials

TABLE 3
Shoot dry weight (g) of *Psophocarpus tetragonolobus* as affected by VAM inoculum and Rhizobium inoculation in Serdang and Munchong soils

| Phosphorus | | 0 | | 30 | | 60 | | |
|------------------|------------|-------|-------|-------|-------|-------|-------|-------|
| <i>Rhizobium</i> | | + | - | + | - | + | - | |
| Soil | Mycorrhiza | | | | | | | |
| Serdang | Alae | 24.05 | 25.55 | 30.58 | 25.52 | 31.47 | 27.67 | |
| | Scal | 23.77 | 26.90 | 32.33 | 22.86 | 26.37 | 27.67 | |
| | Gmos | 30.39 | 28.94 | 30.90 | 26.14 | 27.85 | 32.33 | SED |
| | Gmix | 26.18 | 26.89 | 31.46 | 25.23 | 24.37 | 28.33 | 2.138 |
| | Control | 8.81 | 7.08 | 10.18 | 9.77 | 11.87 | 11.65 | |
| Munchong | Alae | 4.69 | 14.84 | 17.21 | 4.64 | 16.96 | 22.34 | |
| | Scal | 7.55 | 12.76 | 21.33 | 7.08 | 13.21 | 18.12 | |
| | Gmos | 4.73 | 15.46 | 20.40 | 6.07 | 19.97 | 22.23 | LSD5% |
| | Gmix | 3.28 | 12.00 | 17.83 | 2.93 | 10.93 | 17.33 | 4.28 |
| | Control | 4.58 | 2.71 | 4.78 | 3.61 | 6.73 | 5.43 | |

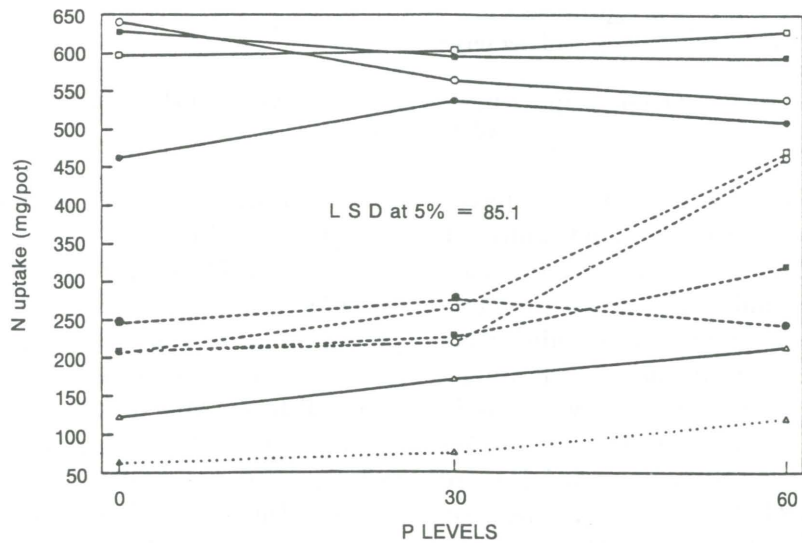


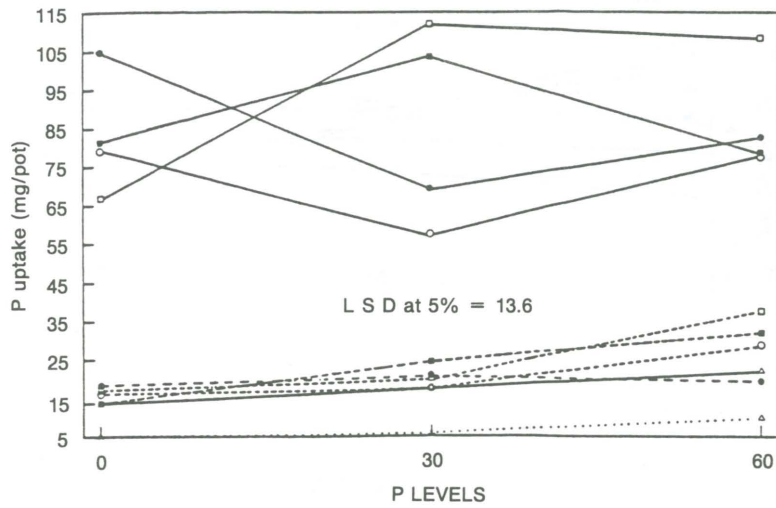
Fig 5. Uptake of N (mg/plant) by *Psophocarpus tetragonolobus* as affected by VAM inoculum and P levels in Serdang and Munchong soils

showed the same trends as those for leaf number and initiation of flowering in Experiment 3 (Table 4 and 5 respectively).

Overall, P had a significant ($P < 0.05$)

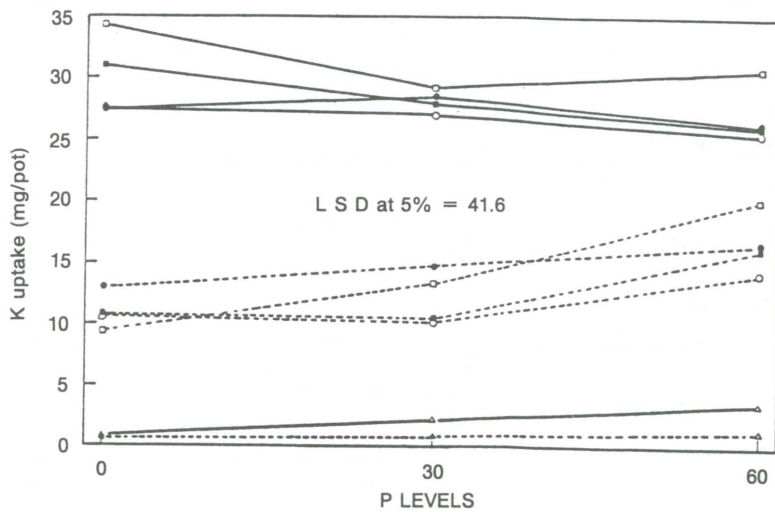
effect on leaf number (Experiment 3). Analysis of the data using linear comparison of means showed that *G. mosseae* significantly stimulated growth, particularly at 60 kg P

RESPONSES OF WINGED BEAN TO MYCORRHIZA INOCULATION



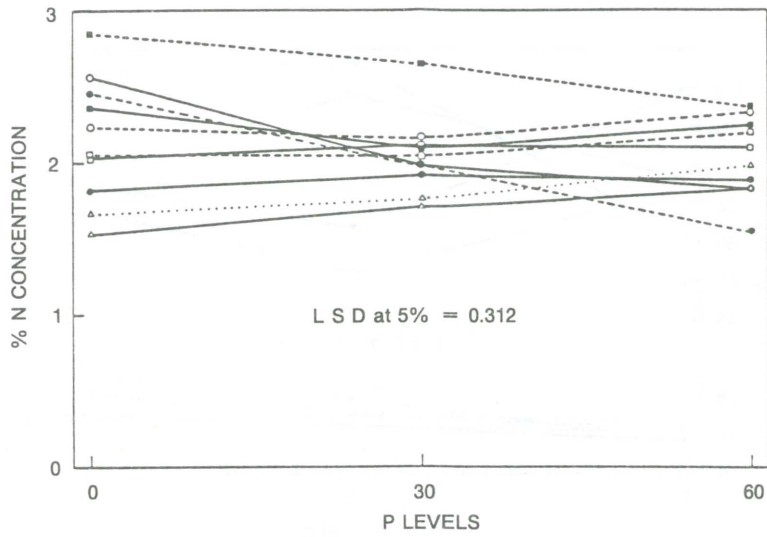
- *A. laevis*
- *G. mosseae*
- Mixed *Glomus* species
- *G. calospora*
- △ Control
- Serdang series
- Munchong series

Fig 6. Uptake of P (mg/plant) by *Psophocarpus tetragonobus* as affected by VAM inoculum and P levels in Serdang and Munchong soils



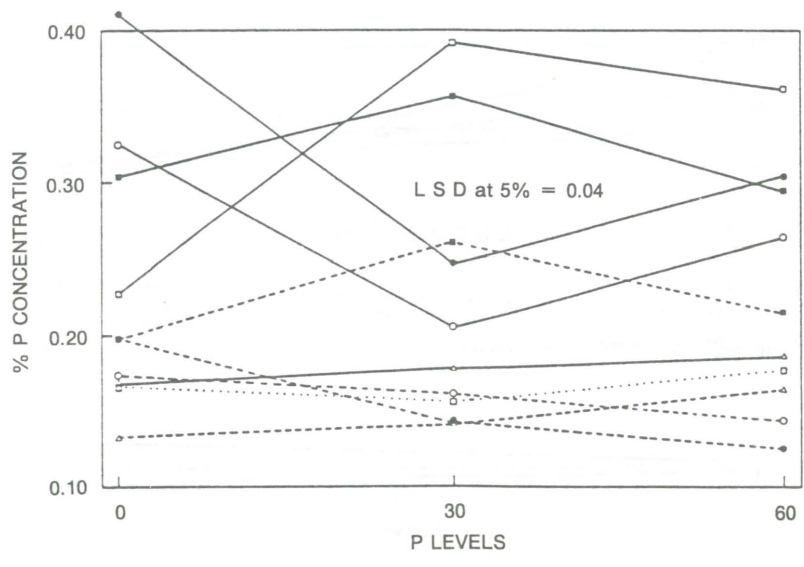
- *A. laevis*
- *G. mosseae*
- Mixed *Glomus* species
- *G. calospora*
- △ Control
- Serdang series
- Munchong series

Fig 7. Uptake of K (mg/plant) by *Psophocarpus tetragonolobus* as affected by VAM inoculum and P levels in Serdang and Munchong soils



- *A. laevis*
- *G. mosseae*
- Mixed *Glomus* species
- *G. calospora*
- △ Control
- Serdang series
- Munchong series

Fig 8. Percentage of N Concentrations in Psophocarpus tetragonolobus shoots as affected by VAM inoculum and P levels in Serdang and Munchong soils



- *A. laevis*
- *G. mosseae*
- Mixed *Glomus* species
- *G. calospora*
- △ Control
- Serdang series
- Munchong series

Fig 9. Percentage of P concentrations in Psophocarpus tetragonolobus shoots as affected by VAM inoculum and P levels in Serdang and Munchong soils

TABLE 4

Leaf number per plant of *Psophocarpus tetragonolobus* as affected by VAM inoculum and levels of P fertilizers

| Inoculum | Applied P (kg ha ⁻¹) | | |
|--------------------------------|----------------------------------|------|------|
| | 0 | 60 | 150 |
| <i>Acaulospora laevis</i> | 28.8 | 28.8 | 38.3 |
| <i>Glomus mosseae</i> | 21.3 | 41.3 | 39.0 |
| <i>Scutellospora calospora</i> | 28.5 | 25.8 | 37.5 |
| Control | 19.0 | 34.0 | 44.8 |
| <i>Rhizobium</i> | 21.8 | 27.0 | 30.5 |

LSD (5%): 13.02

ha⁻¹ whilst the effects of *A. laevis* and *S. calospora* approached significance without any P fertilizer (Table 4).

Compared with the control + *Rhizobium* treatment, the *G. mosseae* treatment significantly (P < 0.05) initiated earlier flowering in winged bean plants receiving 60 kg P ha⁻¹ (Table 5). No other effects were significant.

N, P, K Uptake and Concentrations in Shoots

N, P and K uptake by the non-inoculated plants was significantly lower than the inoculated ones. Uptake of all three nutrients increased with increased P levels (Fig. 5, Fig. 6 and Fig. 7 respectively).

TABLE 5

Initiation of flowering in *Psophocarpus tetragonolobus* as affected by VAM inoculum and levels of P fertilizers (number of flowers per plant)

| Inoculum | Applied P (kg ha ⁻¹) | | |
|--------------------------------|----------------------------------|------|------|
| | 0 | 60 | 150 |
| <i>Acaulospora laevis</i> | 3.75 | 0.75 | 1.21 |
| <i>Glomus mosseae</i> | 2.00 | 4.50 | 1.00 |
| <i>Scutellospora calospora</i> | 2.00 | 0.25 | 0.75 |
| Control | 1.00 | 2.50 | 2.00 |
| <i>Rhizobium</i> | 1.75 | 1.25 | 1.00 |

LSD (5%): 3.19

As with shoot and root dry weights, the N, P and K uptake by plant shoots was significantly (P < 0.05) influenced by soil, mycorrhiza and applied P. The uptake of all three elements was higher from Serdang than from Munchong soil.

Concentrations of N (Fig. 8), P (Fig. 9) and K (Table 6) in winged bean shoots were increased twofold through symbiosis with VAM in both plants grown at P₀ from Serdang and Munchong soils. In Serdang soil, the highest shoot P and K concentrations occurred in the *G. mosseae*-inoculated plants, while in the Munchong soils, plants inoculated with mixed *Glomus* species had the highest concentrations of P and K.

TABLE 6

K concentration (% dry weight) in winged bean as affected by VAM inoculum and P levels for Serdang and Munchong soils

| Phosphorus (kg TSP/ha) | | 0 | 30 | 60 | |
|------------------------|------------|-------|-------|-------|--------|
| Soil | Mycorrhiza | | | | |
| Serdang | Alae | 1.112 | 0.973 | 0.860 | |
| | Scal | 1.085 | 1.063 | 0.975 | |
| | Gmos | 1.162 | 1.023 | 1.023 | SED |
| | Gmix | 1.168 | 1.000 | 0.992 | 0.0855 |
| | Control | 0.105 | 0.213 | 0.298 | |
| Munchong | Alae | 1.315 | 1.263 | 0.750 | |
| | Scal | 1.375 | 1.308 | 1.063 | |
| | Gmos | 1.107 | 1.232 | 0.923 | LSD 5% |
| | Gmix | 1.692 | 1.338 | 1.198 | 0.17 |
| | Control | 0.192 | 0.197 | 0.218 | |

Shoot N concentrations were highest in plants inoculated with mixed species for both Serdang and Munchong soils.

DISCUSSION

The growth of winged bean was greatly enhanced as a result of mycorrhizal associations and addition of P fertilizers in P-deficient soils. As expected, there was, however, variability in effectivity of the different VAM species tested. Abbott and Robson (1981) and Schubert and Hayman (1986) had earlier made similar observations. Several factors have been attributed as being responsible for the differences in the degree of infectivity between species. One factor affecting effectiveness of a particular VAM species is its ability to infect roots of the host plant at a time most appropriate for increased uptake of a deficient nutrient (Abbott and Robson 1984).

In Experiment 1, the decline in shoot production after the fifth harvest in plants treated with *S. calospora* was probably the result of inter-root competition for phosphate, which becomes more serious with time as the roots become clumped at the sides and bottoms of the pot (Sander *et al.* 1977). This effect was successfully overcome by using a larger pot with 5 kg soil (Experiment 2). In this experiment, all five VAM species tested significantly increased dry matter production, especially at 30 kg P ha⁻¹. Dry matter production of winged bean in Serdang soil was increased by 169-197% following treatment with *S. calospora* and *G. mosseae*, respectively. In the higher P-fixing Munchong soil, increase in dry matter was in the range of 131-220% following infection with the mixture of *Glomus* species, and *G. mosseae*, respectively. This clearly shows that mycorrhizal plants in the Serdang soil were larger, but the actual increase as a result of inoculation with VAM was greater in Munchong soil.

The effect of mycorrhiza was less in Serdang Soil because, being a better soil, it allows better growth of the control plants, thus reducing comparatively the effects of inoculation with the mycorrhiza.

The effect of *Rhizobium* in both soils was evident at an intermediate level (P₁) of P fertilization. However, the effect of inoculation was less at P₀ and P₂. This is probably due to inefficient nitrogen fixation at P₀ because of insufficient P in the soil. However, the high rates of P applied (P₂) had adverse effects on *Rhizobium*, resulting in the lower yield of these plants at this level (60 kh TSP ha⁻¹) of P fertilization.

Greater effectiveness of *G. mosseae* and *A. laevis* over the other inocula in the Serdang soil could be indicative of (the respective) soil-endophyte and host-endophyte specificity as suggested earlier by Hayman (1982). Superiority of *G. mosseae* over the other inocula could also be attributed to the ability of this fungus to maintain low spore production for a long period of plant growth (Wilson 1984) as well as to its ability to produce rapidly growing and extensive external hyphae (Aldwell and Hall 1986). The less competitive *A. laevis* loses out to *G. mosseae* because of its slower production of external hyphae. Similar observations for these two species have been reported by Aldwell and Hall (1986). *A. laevis* has also been suspected to have a limited life cycle in the host plant (L.K. Abbott and A.D. Robson, *pers. comm.* to I.R. Hall).

In Serdang soil, the beneficial effects of inoculation with *G. mosseae* were further evidenced by the high shoot P and K content of these plants. In Munchong soil, the mixture of *Glomus* species was the most suitable inoculum in stimulating N, P and K shoot concentrations, and hence the growth of winged bean. The higher shoot K concentrations of plants in Munchong than in Serdang soil gave a clear indication of the "dilution effect" (as defined by

Jarrell and Beverly 1981) of plants in the latter soil. Positive growth responses as a result of inoculation with mixed *Glomus* species in Munchong soil indicates probable synergistic effects between these species. The use of mixed inocula (containing more than one endophyte species) has earlier been shown to give more consistent results than those containing a single species (Daft and Hogarth 1983).

VAM and P Recovery

Successful inoculation with these VAM fungi was also shown by the higher recovery of phosphate from highly phosphate-fixing Serdang and Munchong soils, with 437 and 256% P recovery respectively. The growth of winged bean was enhanced by a factor of four as a result of inoculation (Experiment 2). Greater phosphate absorption of VAM arises because of a superior efficiency of P uptake by these fungi from the labile forms of soil phosphate (Mosse *et al.* 1973; Azizah 1991).

Pot Experiments Versus the Field Trial

Although the field trial could only be regarded as a partial success, the results obtained were consistent with results obtained from the pot experiments. This is significant as conditions in the field are very different to those in pots. The volume of available soil is different, and there may be other growth-limiting factors besides insufficient P (Schubert and Hayman 1986). Consistency of results obtained from pot and field trials could also indicate that factors affecting the growth parameters in both these experiments are similar.

Results obtained from the chi-square test indicate two important points: First, there were significant differences in colonization of roots by the four VAM species four weeks after inoculation, indicating variability between VAM species in the rate of root colonization. Second, from six weeks on-

wards, there was no significant difference in root colonization between these four species, showing the ability of the slow colonizers to compete with the fast colonizers.

The ability to colonize roots rapidly was repeatedly exhibited by *G. mosseae* under both pot and field conditions. In the latter trial, significant growth stimulation of winged bean as a result of association with the mycorrhizal fungi was recorded at 60 kg P ha⁻¹. Significant growth responses from introduced VAM species strongly indicated the ineffectiveness and slow growth of the indigenous VAM fungal species, since the efficiency of these species is a major determinant governing growth responses of plants to VAM treatment in non-sterile soils.

In view of the successful field response to mycorrhizal inoculations it appears feasible in future trials to inoculate legumes in unsterilized soils. The use of mycorrhiza also seems warranted in future trials because: 1. Most Malaysian soils have low inoculum levels of the indigenous VAM species, which cannot compete with superior, introduced mycorrhiza species (Azizah 1986, 1991), 2. In winged bean, optimum performance was demonstrated at the intermediate level of P fertilization, indicating the potential of these mycorrhizal fungi in lowering mineral fertilizer input and hence a form of saving for the farmers. However, more evidence is required of successful field trials in different soil types before the biotechnology of mycorrhizal inoculation can be applied by farmers. Work is now in progress to achieve this goal.

CONCLUSION

These experiments clearly show the importance of time-course studies in elucidating the interactions between mycorrhizal fungi and the host plant. The significant and appreciable growth increases over the entire experiment obtained by inoculating with mycorrhizal fungi, especially *G. mosseae*

and a mixture of *Glomus* species, are sufficiently encouraging to warrant further utilization of these mycorrhizal fungi in other field studies in the humid tropics.

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REFERENCES

- ABBOTT, L.K. and A.D. ROBSON. 1981. Infectivity and effectiveness of vesicular-arbuscular mycorrhizal fungi: Effect of inoculum type. *Australian Journal of Agricultural Research* **32**: 631-639.
- ABBOTT, L.K. and A.D. ROBSON. 1982. The role of vesicular-arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. *Australian Journal of Agricultural Research* **33**: 389-408.
- ABBOTT, L.K. and A.D. ROBSON. 1984. The effect of mycorrhizae on plant growth. In *VA Mycorrhizas* ed. C.L.I. Powel and D.J. Bagyaraj, p. 113-130. Boca Raton: CRC Press.
- ALDWELL, F.E.B. and I.R. HALL. 1986. Monitoring spread of *Glomus mosseae* through soil infected with *Acaulospora laevis* using serological and morphological techniques. *Transactions of the British Mycological Society* **87**: 131-134.
- AZIZAH, H.C. 1986. The vesicular-arbuscular (VA) endophyte and its implications to Malaysian agriculture. Ph.D. thesis, Universiti Kebangsaan Malaysia.
- AZIZAH, H.C. 1991. Effect of fertilizer and endomycorrhizal inoculum on growth and nutrient uptake of cocoa (*Theobroma cacao L.*) seedlings. *Biology and Fertility of Soils* **11**: 250-254.
- AZIZAH, H.C. and M. OMAR. 1987. Propagation and maintenance of VAM cultures. *Pertanika* **10**: 271-275.
- BAGYARAJ, D.J., A. MAMKIMATJ and R.B. PATIL. 1979. Interaction between a-vesicular-arbuscular mycorrhiza and *Rhizobium* and their effects on soybean in the field. *New Phytologist* **82**: 141-146.
- DAFT, M.J. and B.G. HOGARTH. 1983. Competitive interactions amongst four species of *Glomus* on maize and onion. *Transactions of the British Mycological Society* **80**: 339-345.
- DANIELS, B.A., P.M. MCCOOL and J.A. MENGE. 1981. Comparative inoculum potential of spores of six vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **89**: 385-388.
- GANRY, F., H.G. DIEM and Y.R. DOMMERGUES. 1982. Effect of inoculation with *Glomus mosseae* on nitrogen fixation by field grown soybeans. *Plant and Soil* **68**: 321-329.
- HALL, I.R. 1988. The potential for exploiting vesicular-arbuscular mycorrhizas in agriculture. In *Biotechnology in Agriculture*, ed. Avshalow Mizrahi, p. 141-174. New York: Alan R. Liss, Inc.
- HARLEY, J.L. and S.E. SMITH. 1983. *Mycorrhizal Symbiosis*. London: Academic Press.
- HARRIS, D., R.S. PACOVSKY and E.A. PAUL. 1985. Carbon economy of soybean-*Rhizobium-Glomus* associations. *New Phytologist* **101**: 427-434.
- HAYMAN, D.S. 1982. Influence of soils and fertility on activity and survival of vesicular-arbuscular mycorrhizal fungi. *Phytopathology* **72**: 1119-1125.
- JARRELL, W.M. and R.B. BEVERLY. 1981. The dilution effect in plant nutrition studies. *Advances in Agronomy* **34**: 197-224.
- KUMARAN, S. and H.C. AZIZAH. 1995. Influence of biological soil conditioner on mycorrhizal versus non-mycorrhizal guava seedlings. *Tropical Agriculture (Trinidad)* **72**: 39-43.
- MOSSE, B. 1986. Mycorrhiza in a sustainable agriculture. *Biological Agricultural Horticulture* **3**: 191-209.
- MOSSE, B., D. HAYMAN and D.J. ARNOLD. 1973. Plant growth responses to vesicular-arbuscular mycorrhiza. V. Phosphate uptake by three plant species from P-deficient soils labelled with ³²P. *New Phytologist* **72**: 809-815.
- PHILLIPS, J.M. and D.S. HAYMAN. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 58-161.
- SCHUBERT, A. and D.S. HAYMAN. 1986. Plant growth responses to vesicular-arbuscular mycorrhiza. XVI. Effectiveness of different

- endophytes at different levels of soil phosphate. *New Phytologist* **103**: 79-90.
- THOMAS, R.L., R.N. SHEARD and J.R. MEYER. 1967. Comparison of conventional and automated procedure for N, P and K analysis of plants. *Agronomy Journal* **59**: 240-243.
- WILSON, J.M. 1984. Competition for infection between vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **97**: 427-435.

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