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The Effectiveness of Two Arbuscular Mycorrhiza Species on Growth of Cocoa (*Theobroma cacao* L.) Seedlings

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ABSTRAK

Satu kajian berpasu telah dijalankan untuk mengkaji keberkesanan dua spesies kulat mikoriza arbuskul ke atas pertumbuhan anak benih koko. Anak benih koko dari hibrid UIT1 × Na32 yang diinokulasi dengan Glomus mosseae *dan* Scutellospora calospora *sama ada sebagai inokulum tunggal, atau campuran dan tanpa inokulum (kawalan), ditanam pada 2 kg tanah disucihama daripada siri Tai Tak. Kajian menggunakan rekabentuk rawak lengkap (CRD) iaitu jenis inokulum sebagai rawatan, dengan empat replikasi. Di antara empat rawatan yang digunakan, pokok yang diinokulasi dengan inokulum campuran* Glomus mosseae *dengan* Scutellospora calospora *memberi tumbesaran yang lebih baik dan bererti dan peratus jangkitan akar lebih tinggi berbanding rawatan-rawatan yang lain. Tinggi pokok dan jumlah luas daun tertinggi (43.7 cm dan 1819.2 cm^2 masing-masing) diperolehi dari anak benih yang diberi inokulum campuran berbanding anak benih dirawat inokulum tunggal* G. mosseae *(37.9 cm dan 1007.2 cm^2), inokulum tunggal* S. calospora *(32.3 cm dan 1316.2 cm^2) dan kawalan (28.5 cm dan 736.7 cm^2). Peratus jangkitan akar tertinggi (72%) juga diperolehi dari anak benih yang dirawat dengan inokulum campuran diikuti oleh inokulum tunggal* S. calospora *(54%) dan inokulum tunggal* G. mosseae *(43%).*

ABSTRACT

A pot experiment was conducted to study the effectiveness of two arbuscular mycorrhiza (AM) species in enhancing growth of cocoa seedlings. Cocoa seedlings of hybrid UITI × Na32 inoculated with Glomus mosseae *and* Scutellospora calospora *either as a single inoculum or as a mixed inoculum and an uninoculated control were grown in 2 kg sterilized Tai Tak series soil. The experiment was a single factor experiment arranged in a completely randomized design, with type of inoculum as a factor with four replications. Of the four treatment used, plants inoculated with mixed inoculum* Glomus mosseae *and* Scutellospora calospora *gave more pronounced and significant vegetative growth and higher percentage of root colonization than the other treatments. Maximum plant height and total leaf area of 43.7 cm and 1819.2 cm^2 respectively were obtained from seedlings inoculated with mixed inoculum compared to seedlings given either* G. mosseae *(37.9 cm dan 1007.2 cm^2), or* S. calospora *(32.3 cm and 1316 cm^2) and control (28.5 cm and 736.7 cm^2). The highest percentage of root colonization (72%) was aslo recorded from seedlings inoculated with mixed inoculum followed by those given* S. calospora *(54%) and single inoculum* G. mosseae *(43%).*

INTRODUCTION

The importance of arbuscular mycorrhiza (AM) fungi in the improvement of plant growth under greenhouse and field conditions is now well documented (Mathew and Johri 1989; Sieverding 1991; Stanley *et al.* 1993; Azizah *et al.* 1996). It is also generally accepted that AM fungi are non-specific in their host selection, since in nature, individual species have been found to infect plant species belonging to different genera and families (Sieverding 1991). The efficiency of a particular AM fungus varies markedly between species and strains of the host plant. The response of the host plant to AM species also varies between clones. This claim was substantiated by Anand (1993), who found that of the two cocoa clones tested, mycorrhizal PBC 139 gave more pronounced and significant vegetative growth than mycorrhizal PBC 140. Hence, a suitable host-fungal combination is of prime importance in order to obtain maximum AM effectiveness in enhancing crop productivity. This study, therefore, aimed to evaluate the effectiveness of two AM species, *Glomus mosseae* and *Scutellospora calospora*, as either single or mixed inoculum on growth of cocoa seedlings of hybrid UITI × Na32 under controlled greenhouse conditions.

MATERIALS AND METHODS

A completely randomized design (CRD) pot experiment with four replications comprising the following treatments was set up: single inoculum with *Glomus mosseae* (Gm), single inoculum with *Scutellospora calospora* (Sc), mixed inoculum comprising *G. mosseae* and *S. calospora* (GmSc) and uninoculated control.

Mycorrhizal soil inoculum of *G. mosseae* [WUM 9 (6)] and *S. calospora* [WUM 12 (3)] was originally obtained from Prof. A.D. Robson, from University of Western Australia, Perth. The inoculum was mass propagated under *Setaria anceps* var. *splendida* as the host plant in the UPM greenhouse for six months (Azizah and Omar 1986).

Soils

The soil used was a clayey Tai Tak soil series (Typic Paleudult) consisting of 50.13% clay, 6.89% silt and 42.98% sand. Soil from the top 0 - 15 cm was collected from a cocoa farm in Labu, Negeri Sembilan. The soil was passed through a 2-mm diam. mesh sieve. The chemical properties of this soil after steam-sterilization for 1 hour at 100°C are as follows: 0.19% total N (determined by Kjeldahl method), 37.92 μg g^{-1} extractable P (determined by the molybdenum blue method-NH_4F and HCl), 0.18 cmol (+)/kg K, 0.87 cmol (+)/kg Ca and 0.17 cmol(+)/kg Mg (determined by the neutral ammonium acetate leaching technique) (Husni *et al.* 1990). Sixteen pots of 16-cm diameter were each filled with 2 kg of this soil. The soil was raised to 6.0 through addition of 2.8 g ground magnesium limestone (GML).

Preparation and Planting of Cocoa Seedlings

Fifty uniform-sized cocoa seeds from hybrid UIT1 × Na32 were pregerminated in sterilized sandy soil for 10 days. Sixteen uniform seedlings were then transplanted, one per pot, with 30 g AM inoculum spread as a layer 2 cm below the roots, as well as around the roots to ensure better infection. Uninoculated or control plants received 30 g of sterilized sandy soil so as to maintain similar conditions. Five grams of compound fertilizer (NPK 8:8:8) were applied as basal fertilizer at the time of transplanting. No other nutrients were added to the soil throughout the entire duration of the experiment. The plants were watered daily to field capacity.

Data Collection

The plants were harvested 12 weeks after transplanting. Plant height and stem diameter were recorded. The leaf, stem and roots were then cleaned and separated. Total leaf area per plant was recorded using a leaf area meter (Licor model 3100). The roots were carefully washed free of adhering soil particles. Three grams of roots were randomly sampled from each plant and subsequently assessed for percentage of root colonization (Giovannetti and Mosse 1980; Brundrett *et al.* 1984). The remaining root samples were then dried to constant weight at 75°C for 3 days and their dry weights determined. The plant tissues were subsequently processed for determination of N (Kjeldahl method), P, K, Ca and Mg (dry ashing method in concentrated HCl and 20% HNO_3) (Husni *et al.* 1990).

Statistical Analysis

All the data obtained were subjected to the analysis of variance (ANOVA). Treatment means were further separated by LSD for test of significance at $P < 0.05$.

RESULTS

Plant Growth

All mycorrhiza-inoculated seedlings were taller than the controls (*Fig. 1*). However, there was no significant difference between treatments Sc and Gm or between treatments Sc and control. Maximum height of 43.7 cm was obtained from seedlings inoculated with the mixed inoculum GmSc.

Inoculation with either Sc or with mixed inoculum GmSc resulted in marked

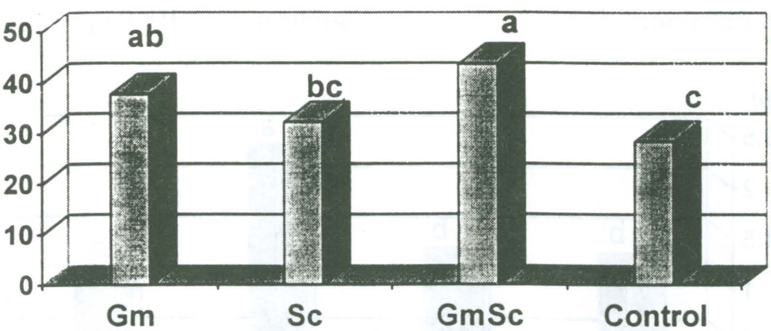

Fig. 1. Effect of Glomus mosseae *and* Scutellospora calospora *as a single or mixed inoculum on height of cocoa seedlings at week 12*

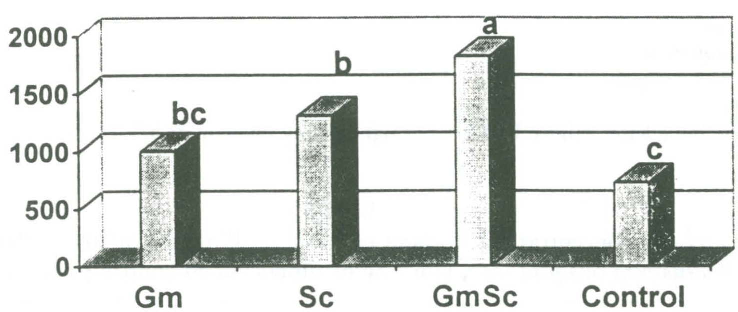

Fig. 2. Effect of Glomus mosseae *and* Scutellospora calospora *as a single or mixed inoculum on total leaf area per plant at week 12.*

Gm = *Glomus mosseae*
Sc = *Scutellospora calospora*
GmSc = *G. mosseae* + *S. calospora*

Means followed by the same letter (above bar) are not significantly different at 5% level and significant ($P<0.05$) increase in total leaf area per plant (1316.2 and 1819.2 cm^2, respectively) compared with 736.7 cm^2 for the uninoculated control (*Fig. 2*). Cocoa seedlings responded most positively to the mixed inoculum, as shown by the maximum and significantly greater total leaf area when compared to single inoculum inoculations.

A similar trend was noticed for root dry weight (*Fig. 3*). A significant ($P<0.05$) increase in root dry weight per plant was observed only from plants given mixed inoculum. The rest of the treatments were not significantly different.

Nutrient Uptake

In line with the other parameters recorded, seedlings inoculated with mixed inoculum GmSc resulted in maximum and significantly higher tissue P, K, Ca and Mg concentrations (Table 1). However, tissue N, K and Mg in Gm or Sc treated plants were at par with those of the control.

Mycorrhizal Infection

The cocoa seedlings responded positively to all mycorrhizal inoculation but exhibited various degrees of mycorrhizal colonization (*Fig. 4*). The highest colonization of 72% (significant at $P<0.05$) was observed in

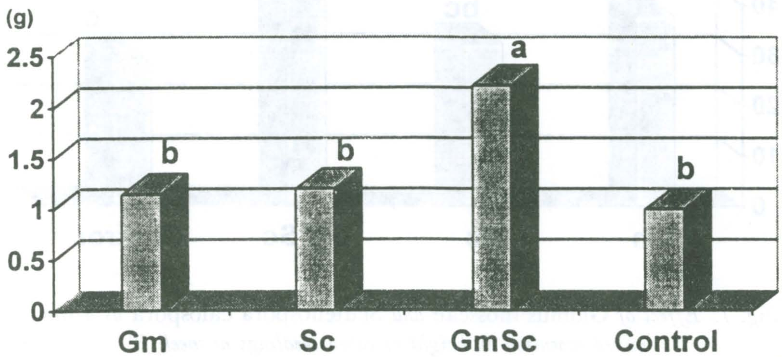

Fig. 3. Effect of Glomus mosseae *and* Scutellospora calospora *as a single or mixed inoculum on root dry weight of plant*

Gm = *Glomus mosseae*
Sc = *Scutellospora calospora*
GmSc = *G. mosseae* + *S. calospora*

Means followed by the same letter (above bar) are not significantly different at 5% level

TABLE 1
N, P, K, Ca and Mg concentrations in shoot of cocoa seedlings inoculated with *Glomus mosseae* (Gm), *Scutellospora calospora* (Sc), Gm + Sc or uninoculated control plant at week 12

AM Inoculum	N	P	K	Ca	Mg
			(%)		
G. mosseae (Gm)	1.99^a	0.176^b	1.85^b	0.25^b	0.28^b
S. calospora (Sc)	1.90^a	0.165b^{bc}	1.98^b	0.27^b	0.29^b
Gm + Sc	2.08^a	0.205^a	2.44^a	0.31^a	0.34^a
Control	2.11^a	0.155^c	2.10^b	0.17^c	0.27^b

In a column, the means followed by the same letter are not significantly different at 5% level.

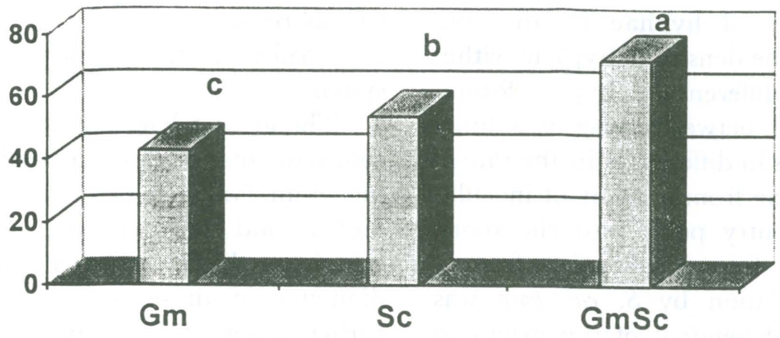

Fig. 4. Percentage root colonization of plants inoculated with Glomus mosseae *(Gm) and* Scutellospora calospora *(Sc) as a single or mixed inoculum*

Gm = *Glomus mosseae*
Sc = *Scutellospora calospora*
GmSc = *G. mosseae* + *S. calospora*

Means followed by the same letter (above bar) are not significantly different at 5% level.

seedlings inoculated with GmSc. Seedlings inoculated with Gm or Sc had 43 and 54% respectively of their roots infected. None of the roots from control plants were colonized by AM.

DISCUSSION

There was variability in the ability of *G. mosseae*, *S. calospora* and a mixture of *G. mosseae* and *S. calospora* to stimulate plant growth and nutrient uptake of hybrid cocoa seedlings. A similar trend in variability in effectiveness of different AM species in stimulating growth of winged bean was earlier reported by Azizah (1986). The effectiveness of a particular AM species appears to be directly related to the rate and time of formation of mycorrhiza as well as to the amount of infection developed by the fungi (Abbott and Robson 1981). Pearson and Schweiger (1994) found that the isolate *Glomus* sp. maintained a similar level of colonization on subterranean clover root at all harvests, but percentage root length colonized by the isolate *S. calospora* increased until the seventh week after sowing, after which it decreased until the end of the experiment (11 weeks). In contrast, in this study, percentage root infection by *S. calospora* in cocoa was higher than infection by *G. mosseae* even at 12 weeks after transplanting. This is probably due to the longer duration of the present experiment, different soil type, host plant and isolate of *S. calospora* used in this study.

AM species may also differ in their ability to form hyphae in soils, both in distribution and in quantity (Bevege and Bowen 1975). Abbott and Robson (1984) hypothesized that low initial levels of infective hyphae of *Glomus* sp. (WUM 10(1)) in the soil may lead to small amounts of hyphae in the soil in relation to the quantity present in the roots. In contrast, presence of high density infective hyphae in the soil could result in greater extension of the exponential phase of colonization in the roots, which subsequently stimulate massive development of hyphae in the soil. An association of this nature, i.e. between formation of hyphae in the soil and within the roots may not occur for *S. calospora*, which consistently produces large quantities of hyphae in the soil, irrespective of the density of hyphae within the roots. The difference in hyphal formation in the soil between these two fungi probably results in differences in the rate of spread of hyphae from a point of inoculation (i.e. the entry point into the root). Hyphal spread from roots away from a point of inoculation by *S. calospora* was found to be independent of the extent to which the individual roots are colonized. However, for *Glomus* sp., the growth of hyphae in the soil and subsequent spread are strongly dependent on the rapid and extensive colonization within the plant roots (Abbott and Robson 1984).

Differences in effectiveness between VAM species have also been related to their pattern and mechanism of sporulation (Abbott and Robson 1981). Fungi which sporulate gradually may not deplete nutrient resources available for the hyphae within plant roots, whereas species which sporulate excessively or suddenly may deplete the hyphae from substances necessary for continued or renewed growth. Azizah (1986) found that the superiority of *G. mosseae* over the other inocula lies in the low spore production of *G. mosseae* for a long period of plant growth as well as its ability to produce rapid and extensive external hyphae.

In the present study, the mixed inoculation (*G. mosseae* and *S. calospora*) gave the highest plant growth increment and nutrient concentrations in cocoa shoot compared to the control. Positive growth responses as a result of inoculation with the mixed inoculum GmSc clearly indicate the probable synergistic effects between these species, even though they exhibit different growth pattern. Using a split-root technique, Pearson *et al.* (1993) reported that root weight and total root length of one half of the root system of subterranean clover inoculated with *Glomus* sp. increased when *S. calospora* formed mycorrhizae in the second half of the root system.

The greater length and more intensive root hairs on plants inoculated with mixed inoculum GmSc subsequently resulted in higher total root dry weight. Higher and significant higher root length and root diameter of the inoculated plants were earlier reported by Hardey and Leyton (1981). The greater root length and number of root branches probably indicate that the mycorrhizal plant has a higher potential for uptake and absorption of relatively mobile nutrients through exploration of a greater soil volume. This subsequently results in higher nutrient concentrations in the shoots of these plants.

Mycorrhiza treatment, either as a single or mixed inoculum, also gave significantly higher P concentration in shoots compared to the control. The highest concentration of 0.205% was obtained from plants inoculated with GmSc followed by Gm (0.176%) and Sc (0.165%). These findings are in agreement with results obtained earlier by Pearson *et al.* (1994) and Azizah *et al.* (1996). *S. calospora* has been shown to be a significant drain of host photosynthates compared to *Glomus* sp., probably in part due to its inability to supply the host with sufficient P, especially in soils with low to moderate P levels (Pearson *et al.* 1994).

High root colonization of plants inoculated with GmSc and, subsequently, greater absorption of nutrients could also result in greater production of leaf area, i.e. increases the area for photosynthesis, and hence produce more carbohydrate to support better plant growth (Sieverding 1991; Kumaran and Azizah 1995), as well as to support growth of the VAM fungi in the plants. Jakobsen and Rosendahl (1990) reported that mycorrhizal plants have to pay a price of 10-20% of the net photosynthates, which is required for formation, maintenance and function of the mycorrhizal symbionts. In spite of this, the host plant still benefits from the symbiotic association with these mycorrhiza fungi.

CONCLUSION

Under greenhouse conditions, mixed inoculum of *G. Mosseae* and *S. Calospora* was far superior in enhancing growth and nutrient uptake in cocoa seedlings (hybrid UITI × Na32) compared to inoculation with either single inoculum of *G. mosseae* or *S. calospora*.

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