Biol Fertil Soils (1997) 24:175–178

ORIGINAL PAPER

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Differential effect of various endomycorrhizal fungi on nodulating ability of green gram by *Bradyrhizobium* sp. (*Vigna*) strain S24

Received: 26 June 1995

Abstract *Bradyrhizobium* sp. (*Vigna*) strain S24 interacted differentially with eight vesicular-arbuscular mycorrhizal (VAM) fungi and caused significant variations in nodulation and growth parameters of green gram. Coinoculation with *Scutellospora calospora* resulted in the highest nitrogenase activity and dry biomass. The nodulation competitiveness of strain S24 was significantly higher (60–63%) in the presence of *Glomus mosseae, G. fasciculatum* and *Scutellospora calospora* when compared to treatment with single inoculation of S24 (51%). Percentage VAM colonization was higher in treatments having higher nocule occupancy of introduced strain (S24).

Key words Acetylene reduction assay · ARA · *Bradyrhizobium* · Green gram · Inoculation · Nitrogenase activity · Nodule occupancy · VAM colonization

Introduction

The tripartite symbiosis between leguminous plants, *Rhizobium* spp. (and *Bradyrhizobium* spp.) and vesicular-arbuscular mycorrhizal (VAM) fungi has been the subject of intensive research in recent years (Thiagarajan et al. 1992; Daniels-Hylton and Ahmad 1994). Synergistic effects of dual colonization of roots with VAM fungi and rhizobia on growth, nutrient uptake and nitrogen fixation in soybean (Bethlenfalvay et al. 1990), chickpea (Champawat 1990) and cowpea (Islam et al. 1990) have been reported. Specificity between the two endosymbionts in the root cortical cells, a region of their activity, have also been reported (Ames et al. 1991; Azcon and Rubio 1990). These studies indicated the importance of compatibility between the strains of VAM fungus and *Rhizobium* spp. However, there is only one report on the influence of a VAM fungus (*Glomus pallidum*) on the competitive ability of introduced and native *Bradyrhizobium* strains to nodulate cowpea (Thiagarajan and Ahmad 1993). *Bradyrhizobium* spp. (*Vigna*) strain S24 exhibits superior nodulation competitiveness in greenhouse experiments (Sreekumar and Sen 1989). The present study was carried out with the following objectives: (1) to determine the effect of VAM fungi-*Bradyrhizobium* sp. (*Vigna*) S24 on nodulation, nitrogen fixation and growth of green gram and (2) to determine the effect of different VAM fungi on the nodulation competitiveness of strain S24 and the latter's effect on the colonization of VAM fungi in green gram.

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Materials and methods

Bacterial culture

Bradyrhizobium sp. (*Vigna*) strain S24 isolated from green gram [*Vigna radiata* (L.) Wilczek] was obtained from the Department of Microbiology, Haryana Agricultural University, Hisar, India. It was maintained on yeast extract mannitol agar (YEMA) at 28°±1°C. The intrinsic antibiotic resistance pattern was examined according to Josey et al. 1979. Stock solutions (10 mg ml^{-1}) of antibiotics, sterilized by millipore filtration (0.45 μm), were added to pre-cooled (50°C) tryptone yeast extract (TY) medium to give final concentrations of 5, 10, 20, 50, 75 and 100 μg ml^{-1} for ampicillin, chloramphenicol, nalidixic acid and tetracycline. S24 was resistant to antibiotics (μg ml^{-1}): chloramphenicol (100), ampicillin (100), nalidixic acid (20), kanamycin (100) and tetracycline (5).

VAM culture

Eight culture of VAM fungi, *Glomus mosseae, G. fasciculatum, G. versiforme, G. macrocarpum, Gigaspora gilmorei, G. margarita, Scutellospora calospora* and *Endogone duseii*, were obtained from the Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India. The cultures were obtained as sand and soil (1:1) mixtures containing spores, hyphae and infected mycorrhizal root segments of *Cenchrus* sp. The inocula were maintained at 4°C until use.

Pot culture

The soil used was collected from the farms of the Indian Agricultural Research Institute, New Delhi, India. The sandy loam soil contained 4.48 mg kg^{-1} Olsen P, 6 mg kg^{-1} NO_3^--N and had a pH (H_2O) of 7.2. The indigenous population of *Rhizobium* spp. specific to green gram was estimated by the plant infection test using a most probable number method (Vincent 1970) and was found to be 21×10^2 cells g^{-1} soil. Seeds of green gram [*Vigna radiata* (L.) Wilczek] cultivar Pusa 105 were sown in earthen pots filled with 14 kg unsterilized farm soil. Inoculation with VAM fungus was done by the layering method (Jackson et al. 1972). A broth culture (10 ml) of *Bradyrhizobium* strain S24 was pipetted over the seeds. Water was added to make up about 75% of water-holding capacity (WHC) (WHC of soil – 28%). Upon germination, the plants were thinned to six per pot. A total of ten treatments, including dual inoculation of S24 with one each of the eight VAM cultures, S24 alone and an uninoculated control, in a completely randomized design was used with six replicates.

The plants were harvested (three replications) 40 days after seedling emergence. The roots were washed with tap water and dried between the folds of filter paper. N_2-fixation activity of root nodules was assayed by the C_2H_2 reduction technique immediately after harvesting (Hardy et al. 1968). The nodulated roots were incubated at 28°C for 60 min in 500-ml plastic bottles containing 10% acetylene. C_2H_4 production was analysed by gas chromatography using the Shimadzu Model equipped with a flame ionization detector (110°C) and a Porapak N column (80°C). N served as a carrier gas (30 cm^3 min^{-1}). The acetylene reduction assay (ARA) was expressed as micromoles C_2H_4 h^{-1} g^{-1} fresh weight of nodules. The nodules were detached and fresh weight recorded. The dry matter yield of roots and shoots were recorded separately after drying them at 80°C for 48 h. The nodules were surface sterilized by immersion in 0.1% $HgCl_2$ for 5 min and 70% alcohol for 30 s rinsed 6 times in sterile water. A total of 60 nodules per treatment selected randomly were aseptically crushed and streaked in duplicate on the tryptone yeast extract (TY) plates with and without antibiotics (Josey et al. 1979). The plates were incubated at 28°C for 5–7 days and observed for growth. The competitive ability of a strain was determined by calculating the percentage nodule occupancy was follows.

$$\%\ \text{Nodule occupancy} = \frac{\text{number of nodules occupied by inoculant strain}}{\text{total number of nodules tested}} \times 100\ .$$

The plants from three replicates per treatment were harvested after 10 weeks (green pod formation stage). The roots were cut into 1- to 2-cm segments and stained with trypan blue-lactophenol mixture (Phillips and Hayman 1970), and frequency of VAM infection as percentage colonization was evaluated microscopically according to Jalali and Domsch (1975).

Statistical analysis

The data were statistically analysed using the one-way analysis of variance (ANOVA) and differences were assessed for significance.

Results

Bradyrhizobium spp. (*Vigna*) strain S24 interacted differentially with VAM fungi and caused significant variations in nodulation, nitrogen fixation and growth parameters of green gram cultivar Pusa 105 (Table 1). Co-inoculation of all cultures of VAM fungi except *Endogone duseii* produced significantly higher nodule fresh weights and root and shoot dry weights than single inoculation with S24 treatment and uninoculated control. Maximum nodule weight was obtained for treatment inoculated with *Glomus mosseae.* S24 in combination with *Scutellospora calospora* significantly increased the root and shoot dry biomass and nitrogenase activity over S24 treatment. The ARA activity increased to varying amounts from 8% to 72% in association with all VAM fungi except *E. duseii* although the differences were not significant (Table 1).

The nodule occupancy of strain S24 as determined by intrinsic antibiotic resistance profile varied with the VAM species (Table 2). In the presence of *Glomus mosseae, G. fasciculatum* and *Scutellospora calospora* the nodule occupancy of strain was significantly (P=0.05) higher (60–63%) than single inoculation of S24 (51%). However, co-inoculation of *G. versiforme, G. macrocarpum, Gigaspora margarita* and *Endogene duseii* exhibited a decreasing trend on the nodule occupancy of strain S24 (26–43%).

Table 1 Influence of VAM fungi and *Bradyrhizobium* sp. (*Vigna*) strain S24 on growth, nodulation and nitrogen fixation of green gram (cv. Pusa 105)

Treatment	Nodule fresh weight (mg)	Root dry weight (mg)	Shoot dry weight (g)	ARA (μmol C_2H_4 h^{-1} g^{-1} fresh wt. of nodules)
Uninoculated	78	186	0.81	3.6
S24	195	260	1.64	8.7
S24 + *Glomus mosseae*	408	413	3.30	11.8
S24 + *G. fasciculatum*	371	426	2.52	9.4
S24 + *G. versiforme*	343	303	2.25	13.6
S24 + *G. macrocarpum*	345	520	2.63	11.6
S24 + *Gigaspora gilmorei*	206	436	2.84	12.1
S24 + *G. margarita*	287	586	2.26	10.7
S24 + *Scutellospora calospora*	394	673	3.57	15.0
S24 + *Endogone duseii*	80	166	0.79	6.0
LSD (P=0.05%)	125	245	1.03	NS

NS non-significant

Table 2 Nodule occupancy of *Bradyrhizobium* sp. (*Vigna*) strain S24 and root colonization by VAM fungi under various S24-VAM fungi combinations

	Nodule occupancy (%)	VAM colonization (%)
Uninoculated	–	3
S24	51	6
S24 + *Glomus mosseae*	63	32
S24 + *G. fasciculatum*	60	30
S24 + *G. versiforme*	34	25
S24 + *G. macrocarpum*	35	24
S24 + *Gigaspora gilmorei*	50	23
S24 + *G. margarita*	43	20
S24 + *Scutellospora calospora*	61	40
S24 + *Endogone duseii*	26	18
LSD (P=0.05%)	8	9

Root colonization by VAM varied from 18 to 40% in green gram plants inoculated with different VAM fungi in the presence of strain S24 (Table 2). VAM infection was negligible in roots inoculated with S24 alone and in the uninoculated control.

Discussion

In this study we examined the effect of different vesicular-arbuscular mycorrhizae (VAM) on the competitive ability of *Bradyrhizobium* sp. (*Vigna*) strain S24 and the latter's effect on percentage root colonization by different VAM fungi on green gram. The results demonstrate that dual inoculation of green gram increased the growth, nodulation and nitrogenease activity to varying levels for all pairs except S24+*Endogone duseii* over single inoculation with S24 or the uninoculated control. The variations in the growth and nodulation parameters indicate the existence of specificity between the two endosymbionts. Pairing of S24 with *Scutellospora calospora, Glomus mosseae* and *G. fasciculatum* produced the best results and appeared to be compatible pairings. However, pairing of S24 with *Endogone duseii* was incompatible and gave a negative response.

Specific compatibilities between VAM fungi and *Rhizobium* strain have been reported in cowpea (Thiagarajan et al. 1992; Ames et al. 1991), *Medicago* (Azcon and Rubio 1990) and kidney bean (Daniels-Hylton and Ahmad 1994). Pacovsky et al. (1986) have also claimed that, by changing the *Bradyrhizobium* strain, mycorrhizal plants showed an improvement in the yield of soybean. In the present study, a negative response in the presence of *Endogone duseii* indicates antagonism between VAM fungi and S24. Antagonistic effects between mycorrhizal colonization and nodulation have been reported for *Glycine-Glomus-Rhizobium* spp. (Bethlenfalvay et al. 1985) and *Phaseolus-Sclerocystis-Rhizobium* (Daniels-Hylton and Ahmad 1994), symbiosis.

An increase or decrease in dry biomass, nodulation and nitrogen-fixation reflects indirect relationships between VAM fungi and rhizobia. More direct relationships were obtained by studying the effect of VAM fungus on competitiveness of *Bradyrhizobium* spp. strain S24 for root nodulation and inversely of *Bradyrhizobium* on VAM infection and colonization. The data on nodule occupancy and percentage VAM colonization confirmed the selection of compatible pairs based on growth performance. Maximum nodule occupancy and percentage root colonization was obtained in treatments where S24 was combined with any of the three VAM fungi, namely *Glomus mosseae, G. fasciculatum* and *Scutellospora calospora.* Thiagarajan and Ahmad (1993) also reported an increase in the competitive ability of introduced *Bradyrhizobium* spp. strain in the presence of VAM fungus (*Glomus pallidum* Hall). However, they studied the competitive ability of *Bradyrhizobium* strain in the presence of only one VAM fungi selected on the basis of their earlier studies (Thiagarajan et al. 1992). VAM fungi and rhizobia influence each other's activities in both the rhizosphere and cortical cells. VAM-colonized roots exhibit a different physiology and have altered root exudation (Schwab et al. 1983) and thereby alter growth conditions for general flora including *Rhizobium.* The root exudates contain host signals for nodule initiation by *Rhizobium.* Strains that can quickly and efficiently respond to host signals, and initiate and sustain an infection attempt, dominate nodulation and appear highly competitive.

The present study demonstrated that the treatments having a higher nodule occupancy of introduced strain also exhibit greater VAM colonization and in the process produce positive effects on growth and nodulation of plant. Thus to obtain maximum benefit from dual inoculation, the compatibility between both the partners for nodulation competitiveness and percentage VAM colonization should be tested.

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