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Interaction Between the Arbuscular Mycorrhizal Fungus *Glomus mosseae* and Two *Streptomyces* Species in Soybean Growth, Nutrition, Nodulation and Nitrogenase Activity

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THIS INVESTIGATION aimed at increasing soil fertility through root amendment with natural biological factors. It was designed to explore the potential of *Streptornyces violochromogenes* and *S. glauccescens* on the interaction with *Glomus mosseae* in sterile, phosphorus (P)-deficient soil in the rhizosphere of soybean plants. These organisms interacted synergistically when added consecutively at 2-week intervals, where sporulation, root infection with *G. mosseae* and population of either *Streptomyces* spp. were significantly stimulated with dual inoculation especially when *Streptomyces* spp. were inoculated 2 weeks prior to sowing.

Dry weight of shoot and root, nitrogen (N) and P content of the shoots, as well as nodulation and nitrogenase activity of soybean roots were improved by inoculation with either *G. mosseae* or the *Streptomyces* spp. *S. violochromogenes* was more stimulatory than *S. glaucescens*. Dual inoculation was more effective on growth, nutrition, nodulation and nitrogenase activity than individual inoculation.

Accordingly soil inoculation with these microorganisms participated in better growth of soybean plants and may be applied to other crop plants for better yield.

Key words: Mycorrhiza in soybean, *Glomus mosseae,* Mycorrhiza-*Streptomyces* interaction, Symbiotic nitrogen Fixation, *streptomyces* and Soybean nutrition.

Arbuscular mycorrhiza (AM) are widespread in nature and are fundamental component of the agroecosystem. They are stable mutually beneficial plant-fungus associations, in which the fungus is partly inside and partly outside the host and form a living link between root and soil (Bethlenfalvay *et al.*, 1997). External factors, such as the presence of soil microorganisms affect this symbiotic development. Mycorrhizal fungi also exert profound effects on other rhizosphere microorganisms, either through indirect effects on host physiology and changes in root exudation or through direct effects via fungal exudates *i.e.* mycorrhizosphere effect (Poulitz and Linderman 1991)

Interaction between mycorrhizal fungi and other soil microorganisms may occur widely. Shifts in the presence or abundance of microbial species occur in the rhizosphere of mycorrhizal plants (Linderman and Poulitz, 1990). The interaction between mycorrhizal fungi and other rhizosphere inhabitants can be detrimental to the mycorrhizal fungi (Krishna *et al.*, 1982), to certain rhizosphere microorganisms (Posta *et al.*, 1994) and to pathogens (Secilia and Bagyaraj, 1987). In contrast, Calvet *el al.* (1993) claimed the favourable effect of other rhizospheric microorganisms to mycorrhizal fungi dependent on their host plants.

The metabolic activity of rhizospheric streptomycetes play an important role in the growth of the plants. Streptomycetes from *Pinus sylvestris* L. mycorrhizosphere produced organic acids and free amino acids (Rozychiand and Strezlezyk, 1986), vitamins (Strezelezyk *et al.*, 1987). S. *orientalis* stimulated germination of mycorrhizal fungus spores (Munjier and Mosse, 1987). They may produce degrading enzymes, growth promoting or growth retarding substances (Kamel *et al.*, 1988; Elshanshory, 1995). The role of rhizospheric *Streptomyces* species reflected beneficial effects to plant growth (Naguib *et al.*, 1987; Shalaby, 1996) and stimulatory effects on the N-fixing rhizospheric bacteria (Elshanshory, 1995).

The purpose of this study was to investigate the interaction between the arbuscular mycorrhizal fungus, *Glomus mosseae* and two *Streptomyces* spp. (*S. violochromogenes* and *S. glaucescens*) and their effects on soybean plants.

Materia1 and Methods

Soybean seeds (*Glycine max* L. Merr.) were surface sterilized by shaking in 7% calcium hypochlorite for 10 min, rinsed with sterile distilled water (Asimi *et al.*, 1980) and sown (4 seeds per pot) in 20-cm diameter plastic pots, each containing 4kg sterilized clay loam soil (pH 7.4), deficient in P. The soil was

autoclaved twice at 1.2 kg cm^2 pressure and 121°C for one hour (Krishna *et al.*, 1982).

Bradyrhizohium japonicum USDA 110, obtained from the Biofertilizer Unit, Soil and Water Research Institute, Agriculture Research Center, Giza, was used for the inoculation of soybean plants. The bacterium was cultured on yeast extract-mannitol broth (Trinick and Parker, 1982), at 27°C on a rotary shaker (140 rpm). Bacterial cells were harvested at the late logarithmic growth phase, centrifuged, washed with sterile distilled water, and diluted with sterile distilled water to a cell density of approximated 10^5 cells per ml. During seeding the bacterial suspension (10ml) was inoculated into each pot.

The spores of the mycorrhizal fungus *G. mosseae* were isolated by wet sieving the soil (Gerdemann and Nicolson, 1963) from alfalfa plants pot cultures, stored in sterile distilled water at 4°C until used. The amount of inoculum was adjusted to give about 10^4 spores per pot at sowing.

Two *Streptomyces* species (*S. violochromogenes* and *S. glaucenscens*) were isolated from rhizosphere of soybean plant and identified (Kamel *et al.*, 1988). One-week slants, on starch-nitrate agar (Kuster and Williams, 1964) were scraped into sterile water to give a suspension of 10^6 spores per ml, 10 ml of which were added per required treatment pot. The six treatments were: uninoculated control (C); inoculation with *G. mosseae* (Gm); *S. violochromogenes* (Sv); *S. glauscescens* (Sg); *G. mosseae* and *S. violochromogenes* (Gm + Sv); *G. mosseae* and *S. glausescens* (Gm + Sg).

Each *Strepromyces* species was inoculated at 2-week intervals before or after sowing. The pots were irrigated to field capacity (53%)during the experimental period under greenhouse conditions. After emergence, the seedlings were thinned to two uniform plants per pot. Plants were harvested 42 days after sowing. The root systems of each six pot replicates per treatment were divided into three batches. In the first, mycorrhizal root infection was measured after clearing and staining 1 cm root segments with trypan blue (Phillips and Hannan, 1970). In the second, the number and dry weight of the nodules and the dry weight of the roots were determined. In the third, nitrogenase activity of nodulating soybean root was assayed by the acetylene reduction (Hardy *et al.*, 1973).

Nutrient content (N and P) of the shoots were determined colorimetrically (Raveh and Avininelech, 1979 and Murphy and Riley, 1962) respectively. The population of each *Streptomyces* species, in the rhizosphere soil, 2, 4 and 6 weeks after sowing, was measured by dilution counts on starch-nitrate agar medium. The number of *G. mosseae* spores was also recorded after removal from the soil, applying the wet sieving and decanting method (Gerdemann and Nicolson, 1963).

The experimental design was complete randomized block with six replicates of each treatment. Statistical analysis was carried out according to Snedecor and Cochran (1980), using L.S.D. to compare the significance of the results.

Results

S. violochromogenes or *S. glaucescens* significantly stimulated sporulation and mycorrizal infection when inoculated with *G. mossea*, either pre- or at sowing soybean seeds (Table 1). The response was very apparent when inoculation was carried out 2 weeks prior sowing, and more prominently when S. *violochromogens* was applied. Post-sowing inoculation of *Streptomyces* species did not affect sporulation or mycorrhizal infection.

TABLE 1. Interaction between *G. mosseae*, *S. violochromogenes* and *S. glausescens* in the rhizosphere soil of soybean plants.

Inoculation time for *Strepto-myces*	Inoculation treatment	*G. mosseae* spore count per 1g dry soil	Infection by *G. mosseae* %	*Streptomyces* population Weeks after sowing		
				2	4	6
				c.f.u. $x10^3$ g^{-1} dry soil		
At sowing (Treatment I)	C	0	0	0	0	0
	Gm	32	45	0	0	0
	Sv	0	0	2.8	5.5	10.2
	Sg	0	0	2.9	4.9	7.0
	Gm + Sv	48	59	3.9	7.8	15.4
	Gm + Sg	39	52	3.8	7.0	13.6
2 weeks pre-sowing (Treat-ment II)	C	0	0	0	0	0
	Gm	32	45	0	0	0
	Sv	0	0	3.5	6.7	12.2
	Sg	0	0	3.1	5.2	11.1
	Gm + Sv	52	72	4.6	8.6	19.8
	Gm + Sg	45	65	4.2	7.2	17.1
2 weeks post-sowing (Treatment III)	C	0	0	0	0	0
	Gm	32	45	0	0	0
	Sv	0	0	0.9	2.6	5.3
	Sg	0	0	0.8	2.3	5
	Gm + Sv	37	56	1	4.4	9.5
	Gm + Sg	33	49	0.9	4	8.8
L. S. D.	5%	9.8	11.4	0.9	1.7	2.9

C: uninoculated soil; Gm: *G. mosseae;* Sv: *S. violochromogenes;* Sg: *S. glausescens*

Coupling *G. mosseae* with either of the two *Streptomyces* species in the rhizosphere significantly increased the population density when the latter was inoculated 2 weeks prior to sowing (Table 1). This response did not occur with inoculation 2 weeks post-sowing. Irrespective of application time, the population density of either *Streptomyces* species doubled every 2 weeks. The population of *S. violochromogenes* was higher than that of *S. glaucescens,* more especially 2 weeks after sowing.

Regardless of inoculation time, neither *Streptomyces* species affected the dry weight of either shoot or root of soybean plant, except when *S. violochromogenes* was inoculated 2 weeks before sowing; this raised the dry weight of both organs, almost to that of the *G. mosseae* treatment alone (Table 2). When coupled together, the dry weight gain significantly increased above that of the *G. mosseae* treatment, reaching maximum when inoculation was performed 2 weeks before sowing. The same trends were followed for N and P content per plant shoot.

On dry weight basis, inoculation of *Streptomyces*, either at or 2 weeks post sowing soybean seeds or in the presence of *G. mosseae* did not affect soybean shoot N content (Table 2). On the other hand, coupling either *Streptomyces* species with *G. mosseae*, inoculated 2 weeks before sowing, stimulated N accumulation in the *S. violochromogenes* inoculated pots without affecting that of the S. *glausescens*. The P shoot content was hardly affected by the presence of either *Streptomyces* species except *S. violochromogenes* inoculated 2 weeks before sowing that accelerated phosphorus accumulation whether per plant or on dry weight basis. *G. mosseae* alone or coupled with *Streptomyces* species incrreased P accumulation in soybean shoots.

Under all inoculation conditions, neither *Streptomyces* species significantly affected nodule number, dry weight per plant nor the nitrogenase activity (Table3); combination with *G. mosseae* significantly increased nodulation number and weight as well as nitrogenase activity per unit nodule dry weight. *G.mosseae* alone had no effect on nitrogenase activity per unit nodule dry

weight, but the coupling of either *Streptomyces* species stimulated nitrogenase activity per unit nodule dry weight, almost to the same extent regardless of time of application of these organisms.

TABLE 2. Interaction between *G. mosseae, S. violochromogenes* and *S. glausescens.*

Inoculation time for *Strepto-myces*	Inoculation treatment	Dry Weight		N content / 1g d. wt.Shoot	P content / 1g d. wt.Shoot
		Shoot	Root		
At sowing (Treatment I)	C	0.96	0.68	28.3	1.58
	Gm	1.34	0.87	28.6	2.10
	Sv	1.13	0.77	28.4	1.50
	Sg	1.02	0.70	29.7	1.55
	Gm + Sv	1.56	1.07	29.6	2.06
	Gm + Sg	1.43	0.98	28.5	2.04
2 weeks pre-sowing (Treatment II)	C	0.96	0.68	28.3	1.58
	Gm	1.34	0.87	28.6	2.10
	Sv	1.22	0.83	30.5	1.71
	Sg	1.07	0.71	29.8	1.64
	Gm + Sv	1.65	1.13	31.9	1.95
	Gm + Sg	1.60	0.98	30.3	1.93
2 weeks post-sowing (Treatment III)	C	0.96	0.68	28.3	1.58
	Gm	1.34	0.87	28.6	2.10
	Sv	1.12	0.74	27.3	1.51
	Sg	0.98	0.71	29.0	1.61
	Gm + Sv	1.45	1.09	31.6	2.18
	Gm + Sg	1.35	0.99	29.6	2.17
L. S. D.	5%	0.28	0.13	2.6	0.23

C: Uninoculated control; Gm: with *G. mosseae;* Sv: with *S. violochromogenes;* Sg: with *S. glausescens*

TABLE 3. Interaction between *G. mosseae*, *S. violochromogenes* and *S. glausescens* on nodulation and nitrogenase activity in soybean plants.

Inoculation time for *Strepto-myces*	Inoculation treatment	Nodulation		Nitrogenase Activity nm C_2H_4 h^{-1}	
		Number per plant	mg D. Wt/ plant	Per plant	per mg D. Wt. Nodule
At sowing (Treatment I)	C	38	73	128	1.8
	Gm	52	99	183	1.9
	Sv	43	80	138	1.7
	Sg	40	79	137	1.7
	Gm + Sv	62	107	212	2.0
	Gm + Sg	57	104	198	1.9
2 weeks pre-sowing (Treatment II)	C	38	73	128	1.8
	Gm	52	99	183	1.9
	Sv	46	92	158	1.7
	Sg	44	92	149	1.6
	Gm + Sv	68	112	217	1.9
	Gm + Sg	61	109	212	1.9
2 weeks post sowing (Treatment III)	C	38	73	128	1.8
	Gm	52	99	183	1.9
	Sv	42	79	136	1.6
	Sg	41	80	136	1.7
	Gm +Sv	60	105	206	2.0
	Gm + Sg	58	94	189	1.9
L. S. D.	5%	8.5	13	31	0.2

C: Uninoculated soil; Gm: *G. mosseae;* Sv: *S. violochromogenes;* Sg: *S. glausescens*

Discussion

In this investigation, a significant increase in infection and spore production of *G. mosseae* as a result of its association with either *S. violochromogenes* or *S. glaucescens* was highly apparent, the former being more effective than the latter. In contrast, Krishna *et al.* (1982) showed that S. *cinnamomeous* reduced the sporulation and infection of *G. fasciculatum*. Time of *Streptomyces* inoculation

played a role in sporulation and infection of mycorrhizal fungus. Two weeks post sowing, seemed to have lower effect. This might be attributed to the age of *Streptomyces* (being only 4 weeks) compared with inoculation 2 weeks pre-sowing (8 weeks old). Under the latter condition, the *Streptomyces* count was double that under the former condition. *S. glaucescens* seemed to secrete moderately harmful metabolites which hindered, to a certain extent, spore formation by *G. mosseae*. Munjier and Mosse (1987) showed that the soil actinomycete *S. orientalis* stimulated germination of mycorrhiza fungus spores. It is apparent that at 4-week age of *Streptomyces* inoculation, the *Streptomyces* density varied, being highest after Treatment III and least by Treatment II. This indicates that age of sopybean plant and/or micorrhiza, have its role in secretion of activators and/or inhibitors of *Streptomyces* growth, being higher bt progress of age of both organisms. McAllister *et al.* (1995) reported that the population of *Aspergilus niger* decreased when inoculated to the rhizosphere of plants, at the same time, or 2 weeks after *G. mosseae*, but not when it was inoculated before the latter. Ravnsko and Jakobsen (1999) indicated that the stimulatory effect of *Pseudomonas fluorescens* on AM fungus was due to release of biologically active molecules from bacteria.

The stimulatory effects of *G. mosseae* on the activity of either test *Streptomyces* species may be attributed to alterations in the root exudates. The composition of the rhizosphere soil solution, induced by mycorrhizal fungus, may activate the two applied *Streptomyces* species. In this concept, Christensen and Jakobsen (1993) and Posta *et al.* (1994) reported that mycorrhizal infection influences the composition of the rhizosphere microbial community by stimulating some groups and decreasing others. Marshner *et al.* (1997) concluded that mycorrhizal infection may decrease root exudation and alter the composition of the rhizosphere soil solution, altering the density of certain bacterial groups in the rhizosphere. Kurtboke *et al.* (1993) showed that the soil *Streptomyces* significantly increased exudate production by a basidiomycete (the sterile red fungus) which had antifungal and growth-promoting properties. Andrade *et al.* (1997) showed that, six arbuscular isolates had effects on the composition of microbial community of the rhizosphere. They suggested that different effects of the AM fungi reflect on the release of root exudates differently which, in turn, is responsible for the different responses of the microbial community to AM fungi.

The results further show that, under all conditions, *G. mosseae* stimuated growth, N or P content in the soybean shoot system, regardless of inoculation time, whereas *S. glausescens* alone seemed without effect. The same applies to *S. violochromogenes* when inoculated 2 weeks before sowing; an indication of the difference in metabolic activity of these two organisms. The former seemed to produce metabolic activators (bio-regulators) that stimulated the activity of soybean plant.

Both *S.violochromogenes* or *S.glausescens* stimulated growth and nodulation of soybean plants in the presence of calcium salts (Naguib *et al.*, 1985). El-Sayed *et al.* (1987) showed that *Streptomyces* spp. secreted plant growth regulators that stimulated the soil microbial population in the rhizosphere which caused improvement in the tomato growth. Shalaby (1996) revealed a significant increase in growth and nodulation of soybean plant, when treated with culture filtrates of either *S. violochromogenes* or *S. glausescens*.

Coupling mycorrhiza with either *Streptomyces* species highly stimulated the metabolic activity in the soybean shoots regardless of the type of treatment, indicating the synergistic effect between the two types of organisms. In the meantime, the efficacy of these organisms, when prevailing singly, for N content in soybean shoot (N per unit dry weight) was unaffected by either type of treatment, except Treatment II, indicating that the *Streptomyces* species has its significant role in the synergistic efficacy of these organisms. The efficacy of *G. mosseae* for phosphorus accumulation was highly apparent (increased P content per unit dry weight) whereas that of *S. glausescens* was absent under all treatments; that of *S. violochromogenes* was only apparent at Treatments II and III, indicating that age of the Streptomyces and/or age of the plant before inoculation (Treatment III) has a significant role. Coupling either *Streptomyces* with *G. mosseae* furthered the efficacy of either organisms to P content.

Similarly, nodule number, dry weight and nitrogenase activity were highly apparent by treatment with *G. mosseae*. Either *Streptomyces* species had no effect except for the synergistic effect on *G. mosseae* activity, reflecting itself on the efficacy of this organisms for nitrogen fixation. Coupling either *Streptomyces* species with *G. mosseae*, in the soil, under the test conditions, increased the efficacy of the latter organism to initiate nodulation, better nitrogenase activity and growth of the soybean plant.

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العلاقة التبادلية لفطرة الميكوريزا الداخلية *Glomus mosseae* ونوعين من الاستربتوميسس وتأثير ذلك على نمو وتغذية وتكوين العقد الجذرية ونشاط إنزيم النيتروجينيز لنبات فول الصويا

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قسم النبات – كلية العلوم – جامعة القاهرة – مصر.

صمم هذا البحث لتباين القدرة التبادلية لكل من *Streptomyces violochromogenes* و *Streptomyces glaucescens* مع فطرة الميكوريزا الداخلية *Glomus mosseae* وذلك فى منطقة جذور نبات فول الصويا فى تربة معقمة وشحيحة الفوسفور. وقد دلت النتائج على أن العلاقة المتبادلة للكائنات تكون معاونة عند إضافته كل أسبوعين حيث كان هناك زيادة معنوية فى تجرثم وإصابة الجذور بفطرة الميكوريزا وذلك عند التلقيح المزدوج وخاصة عند إضافة الاستربتوميسس قبل الزراعة بأسبوعين.

أوضحت الدراسة أيضاً، تحسن النمو للمجموع الجذرى والخضرى والمحتوى النيتروجينى والفوسفورى للمجموع الخضرى وكذلك تكوين العقد الجذرية ونشاط إنزيم النتروجينيز عند التلقيح بكل من *Glomus mosseae* أو إحدى نوعى الاستربتوميسس. وكان نشاط *S. violochromogenes* أعلى من *S. glaucescens* بينما كان التلقيح المزدوج أكثر تأثيراً على النمو والتغذية وتكون العقد الجذرية ونشاط إنزيم النيتروجينيز مقارنة بالتلقيح بإحدى الكائنات منفرداً.

وعلى ذلك فإن التلقيح بالكائنات السابقة قد يصبح مفيد فى النمو والتغذية وتكوين العقد الجذرية ومن الممكن استخدامها فى نباتات أخرى لزيارة المحصول.