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STUDIES OF THE INTERACTION BETWEEN VA MYCORRHIZAL FUNGUS *Glomus mosseae*, *Rhizobium phaseoli* AND *Xanthomonas phaseoli* IN BEAN PLANTS.

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ABSTRACT

The tripartite association of bean plants VA Fungi and *Rhizobium phaseoli* was studied in attempt to evaluate this association regarding plant growth. The results of this study reveal that co-inoculation of mycorrhizal fungi with *Rhizobium phaseoli* increased the shoot dry weight, nodulation, accumulated nitrogen and phophorus as well as nitrate reductase activity in bean plants. VA prevented *Xanthomonas phaseoli* to infect bean plants and improved plants growth. Nitrogen and phosphorus accumulation and nitrate reductase activity were also improved . The presence of mycorrhizal fungi suppressed the population density of the pathogenic bacterium in the rhizosphere of bean plants. The mycorrhiza increased the plant resistance to *X. Phaseoli* more prominently in the presence of *R. phaseoli*.

INTRODUCTION

Beans (*Phaseolus Vulgaris*) is one of the most widely cultivated legumes in Egypt, for food and as a rich source of plant protein. Most legumes are symbiotically associated with arbuscular mycorrhizal (AM) fungi and rhizobia. The effect of this dual symbiosis on plant growth is well documented (Attia, 1994). As the arbuscular fungi are partly inside and partly outside the host, external factors such as the presence of soil microorganisms affect the development of the symbiosis. VAM fungi are more effective for biological control when they interact with the plant pathogens and reduce disease incidence (Clavet *et al.*, 1995 and Liu, 1995). However, in cultivated soils, the effect of dual symbiosis may fluctuate and the beneficial effects may be lost through cultural practices. In particular the use of pesticides is known to affect VAM fungi and rhizobia and previous studies have shown that pesticides may have either adverse or in noculous effects on rhizobial growth, nodulation and N_2 fixation, both *in vitro* and under field conditions (Udalyan *et al.*, 1995). The possibility of mycorrhizae for increasing the effectiveness of *Rhizobium* in soybean plants was suggested by several investigators (Soliman *et al.*, 1996 and Fathy *et al.*, 2000) who observed that VAM fungi strongly stimulated the growth and nodulation of plant. The inoculation of *Glomus mosseae* with *Bradyrhizobium japonicum* increased the shoot dry weight, nodulation, accumulated nitrogen and phosphorus as well as nitrate reductase activity in soybean plants. *G. mosseae* prevented *Pseudomonas syringae* from infecting soybean plants and improved plant growth, nitrogen and phosphorous accumulation and nitrate reductase activity (Shalaby and Hanna, 2000)

Recent literature suggests that the contribution of AM fungi to biological control takes many forms: i- Enhancement of plant nutrition, ii- Competition with the pathogen for resources and space, iii–Morphological

Plant changes, iv- Changes in biochemical compounds related to plant resistance response, v- Allevation of physical stresses and vi-changes in antagonstic and/or deleterious microbe populations in the mycorrhizosphere (Lindeman, 1994). The purpose of this study was to investigate the interactions between VA mycorrhizal fungus G. *mosseae*, and the pathogenic bacterium *Xanthomonas phaseoli*, in the presence or absence of *Rhizobium phaseoli* and on some physiological parameters of bean plants.

MATERIALS AND METHODS

Bean seeds (*Phaseolus vulgaris*) var Giza 3 obtained from Vegetables Croups Section, Agriculture Research Center, Giza, Egypt, were surface sterilized with 70% sodium hypochlorite for 3 min followed by washing with sterilized tap water (Asimi *et al.*, 1980) and sown (4 seeds per 20 cm-diameter pot) in clay loam soil which was autoclaved for 1 h at 1.5 atmosphereic pressure. *Rhizobium phaseoli* and *Xanthomonas phaseoli* strains, used for the inoculation of bean plants, were obtained from Sakha Agriculture Research Stataion. The bacterial cultures were grown to the stationary phase on 20-E medium (Werne *et al.*, 1975) at 27°C on a rotary Shaker (140 rpm). Where indicated plants were inoculated with *Rhizobium phaseoli*, 10 ml/pot, the unionculated pots received 10 ml of 20-E medium. Half of each batch was further inoculated with the pathogenic bacterium *Xanthomonas phaseoli*.

The treatments were as follow:-:

1- Control group free of *G. mosseae* and *Xanthomonas phaseoli* (G^-X^-)
2- No *G. mosseae* but inoculated with *Xanthomonas* (G^-X^+)
3- Inoculated only with *G. mosseae* (G^+X^-)
4- Inoculated with the *G. mosseae* and the *Xanthomonas* (G^+X^+)

The VA mycorrhizal inoculum consisted of 5 g of rhizosphere soil from the pots of maize plants that contain the spores of *Glomus mosseae* cultures. The samples were containing mycelia and some of the infected root fragments. The uninoculated plants were given filtered leaching from the inoculum soil using fine sieves. The filtrate contained common microorganisms but no inoculation of *G. mosseae* and *Xanthomonas phaseoli*,. An aqueous suspension in sterile distilled water containing approximately 2 x 10^7 bacterial cells ml^{-1} was prepared. The plants were inoculated by pouring 10 ml of the bacterial suspension on to the soil surface, 10 days post sowing. The pots were watered to fields capacity during the whole experimental period and left under ambient and experimental period conditions, protected by wiring against intruding animals or birds. After emergence, the seedlings were thinned to two uniform plants/pot. Plants were harvested 25 and 50 days after emergence (DAF). When harvested, part of the root system, each of replicate pots, was cleaned and stained

(Phillips and Hayman, 1970). The percentage of infected root length with mycorrhiza was measured (Giovannetti and Mosse, 1980). To detect the population of inoculated *Xanthomonas phaseoli*, rhizosphere soil was taken

from each experimental pot and diluted 10 times before inoculation on nutrient agar medium.

The number of colony forming units (CFU)/of Xanthomonas- phaseoli g soil was counted. The difference in count between inoculated and uninoculated soil represented the actual CFU of the inoculated soil. The total nitrogen and phosphorus contents of the plant shoot were determined colorimetrically as described by Raveh and Avnimelech (1979) and Murphy and Rily (1962), respectively. Nitrate reductase (NR) was extracted from a known amount of the fresh leaves with 0.025 M Tris-HCl buffer, pH 7.5 containing 0.025 M cysteine. The enzyme activity was assayed using the method described by Harper and Hageman (1972).

The experimental design was complete randomized block with eight replicates of each treatment to two sampling dates. Statistical analysis was carried out according to Senedecor and Cochran (1980) using LSD to compare the significance of the results.

RESULTS AND DISCUSSION

Clay-loam soil of (pH 8.21) was used . It contained 0.92% organic matter, 24% coarse sand, 22.5% fine sand, 31% silt and 39% clay. Cations and anions (mg per 100 g soil) include chlorides (0.33), sulphates (0.27), Calcium (0.24), Magnesium (0.25), Sodium (0.82) and Potassium (0.11).

Table 1 showed that, the shoots dry weight was usually higher in plants inoculated with *Rhizobium Phaseoli* than the non-inoculated ones. Infection with Mycorrhizal induced the highest dry weight gain, whereas Xanthamonas *phaseoli* infection induced the least dry weight gain. Coupling both organisms favored an intermediate gain. Under all conditions, the shoot dry weight increased with time.

The mycorrhizal treatment favoured the highest concentration of both total nitrogen and phosphorus content while the pathogen-treatment favored the least gain of either components (Table 1). The nitrogen or phosphorus content was higher in presence than in absence of the mycorrhizal fungus as well as at later stages of growth. Nitrate reductase activity (Table 1) of bean leaves behaved similarly though it was attenuated with progress of plant age. The CFU of *Xanthamonas phaseoli* in the rhizosphere (Table 1) decreased throughout the experimental period although the numbers-were consistently lower in the presence than in the absence of mycorrhizal *G. mosseae* but significantly increased in absence than presence of *R. phaseoli* and VA mycorrhizal fungi which stimulated bean plants to produce greater plant mass, nitrogen and phosphorus content as well as nodulation. These results support the contentions reported by Mc Allister *et al.*, (1995), Fathy *et al.* (2000) and Shalaby and Hanna (2000). The plants inoculated with mycorrhizal fungi and *R. Japonicum* showed a highly significant increase in growth compared to plants treated with either organisms separately. This indicated that there was a positive interaction between the two organisms for the welfare of the soybean plants. Growth increase due to dual inoculation of soybean plants with mycorrhizal fungi and *R. japonicum* was reported by

Pacovsky *et al.* (1986). The phosphorus and nitrogen content increased in soybean plants infected with mycorrhizal fungus. This is attributed to the large quantity of phosphours, made available to these plants by the symbiotic fungus, a response that was observed by Totta *et al.* (1996).

Xanthamonas phaseoli suppressed the growth of nodulating or non–nodulating bean plants, as indicated by dry weight, nitrogen and phosphorus contents as much treated plants. *Rizobium* was unable to overcome the deleterious effects of the pathogen. The reduction in nodules number, dry wight and efficiency of the nodules, in the presence of the pathogen, were partially alleviated by the presence of *Rhizobium.*

Coupling the mycorrhizal fungus with the pathogen almost completely countracted or prevented *Xanthamonas phaseoli* infection by killing the pathogenic bacteria or, at least, suppressing its rate of multiplication, as revealed in Table 1. The CFU was reduced 77% or more when VAM was added to the soil in the presence or absence of *Rhizobium.*

Nodulation of bean plants was similarity affected by inoculation with *G. mosseae* (Table 2). The number, dry weight and efficiency of nodules were significantly higher in the absence of the pathagenic bacterium whereas the latter also caused the least number, dry weight or efficiency. The percentage of root length of bean plants infected with VA mycorrhizal fungi was significantly increased when the soil was amended with R. *phaseoli.* The pathogen significantly decreased the root length infected with the mycrorhizal fungi in the absence of *Rhizobium.*

The results of this experiment also revealed that infection with *G. mosseae* seemed to protect the bean plants against the pathogen by increasing growth, nitrogen and phosphorus contents and nitrate reductase (NR) activity. Ocamp (1993) reported that VA mycorrhizal fungi were able to protect plants against the pathogenic microorganism. Also, Mc Allister *et al.* (1995) found that *G. mosseae* increased shoot dry weight of maize and lettuce plants when *Aspergellus niger* was inoculated two weeks after mycorrhiza, but the shoot dry weight and percentage of mycorrhizal colonization of the plant decreased when *A. niger* was inoculated at the same time or two weeks before, *G. mosseae.* In most studies, inoculation with VA mycorrhiza before exposure to the pathogen induced resistance that depended on the time that elapsed between inoculation and exposure to the pathogen (Trotta *et al.*, 1996 and Shalaby and Hanna, 2000).

Leaf NR activity in both nodulated and non-nodulated plants was highest at the initial harvest (25 DAE) followed by much lower activiy levels at later harvest (50 DAE). Similar results were reported by Carling *et al.* (1978). The increased activity of NR in the presence of R. *phaseoli* might be attributed to increase of nitrogen uptake. It might be recalled that Omokara and Ajakaiye (1989) found that NR activity was a rate-limiting enzyme and is substrate dependent. Shalaby and Hanna (2000) divided the presence of the mycorrhizal fungui, which suppressed the population density of the pathogenic bacterium in the rhizosphere of soybean plants. The mycorrhiza of the plant resistance to *P. syringae* was more prominently in the presence of *R. Japonicum*

Table (1): Shoot dry weight (SDW), total nitrogen content of shoot (TNS), total phosphorus content of shoot (TPS), nitrate reductase activity (NRA)/ VA mycorrhiza infection % and colony forming units (CFU) of *X.phaseoli* of beans infected with *G.mosseae* and /or *X. phaseoli* in presence or absence of *R. phaseoli*.

Treatment	SDμ (g plant $^{-1}$)		TNS (g plant Shoot^{-1})		TPS (g plant Shoot^{-1})		NRA (μM No2/g F.Wt h^{-1})		VAM infection %		CFUx100 g^{-1} Rhizo-spher	
	Day		Day		Day		Day		Day		Day	
	25	50	25	50	25	50	25	50	25	50	25	50
Without Rhizobium phaseoli												
G⁻X⁻	1.09 a	1.64ab	1.3b	29.6b	1.33 b	2.08c	1.45 c	0.99b	0.0	0.0	0.0	0.0
G⁻X⁺	0.99 a	1.18 b	8.9c	12.9c	1.21b	2.28c	1.89bc	1.31b	0.0	0.0	917a	492a
G⁺X⁻	1.38 a	2.02 a	19.4a	40.7a	3.44a	5.09a	3.59 a	2.53a	21.2a	39.1a	0.0	0.0
G⁺X⁺	1.21a	1.89 a	12.0a	31.2b	1.94b	4.00b	2.35 b	1.40b	19.1b	30.9b	505.1b	286b
With Rhizobium phaseoli												
G⁻X⁻	2.18b	3.41a	31.9c	51.2c	2.19c	2.99c	4.15c	2.71d	0.0	0.0	0.0	0.0
G⁻X⁺	1.94 bc	2.89b	25.5d	45.2d	2.02c	3.09c	4.40c	3.80c	0.0	0.0	898a	472a
G⁺X⁻	3.06a	3.86a	55.3a	89.1a	6.51a	9.10a	9.18a	7.70a	62.0a	84.0a	0.0	0.0
G⁺X⁺	1.71c	2.09b	36.4b	76.2b	4.96b	6.30b	7.9b	6.10b	44.5b	59.0b	219b	202b

Means with the same letter are not significantly different by Duncan's Multiple Range test.

G⁻X⁻ Control –free of myocorrhiza and pathogen.
G⁻X⁺ No mycorrhiza but inoculated with the pathogen (*Xanthomonos*)
G⁺X⁻ Inoculated only with the micorrhiza
G⁺X⁺ Inoculated with the microrrhiza and the pathogen (*Xanthomonos*)

Table (2): Nodules number (NN), nodules dry weight (NDW) and nodules efficiency (N E) of bean plants infected with *G. mosseae* and *X. phaseoli*

Treatment	Number of nodules		Nodules dry eight (NDW) mg plant		Efficiency (NE) mg N/mg NDW	
	Day		Day		Day	
	25	50	25	50	25	50
G⁻X⁻	45.5 b	51.2 bc	39.5 c	82.1 b	0.80 b	0.62 b
G⁻X⁺	31.4 c	47.1 c	36.3 c	74.2 b	1.40 a	0.61 b
G⁺X⁻	66.3 a	81.3 a	76.5 a	112.1 a	0.72 b	0.79 a
G⁺X⁺	32.4 c	59.3 b	49.3 b	101.2 a	0.73 b	0.75 ab

Means with the same letter are not significantly different by Duncan's Multiple Range test.

G⁻X⁻ Control –free of mycorrhiza and pathogen (*Xanthomonos*)
G⁻X⁺ No mycorrhioza but inoculated with the pathogen (*Xanthomonos*)
G⁺X⁻ Inoculated only with the micorrhiza
G⁺X⁺ Inoculated with the microrrhiza and the pathogen (*Xanthomonos*)

REFERENCES

Asimi, S.; V. Gianinazzl-Pearson and I. S. Gianazzl (1980). Influence of increasing soil phosphorus levels on interactions between vesicular-mycorrhyzae and Rhizobium in soybeans. Can. J. Bot., 58:2200.

Attia, M. (1994). Microbiological and ecological studies on mycorrhizal . Ph.D. *Thesis, Fac. Agric. Al Azhar Univ., Cairo, Egypt. P.132.*

Black, C. A.; D.D. Evans; L. E. Ensminger; J. L. White; F. E. Clark and R. C.Inauer (1965). "Method of soil Analysis". pp. 1149-1176. Am. Soc. Agron. Inc. madison , Wisconsin, USA.

Carling, D. E.; W. G. Riehle; M. F. Brown and D. R. Johnson (1978). Effect of vesicular-arbuscular mycorrhizal fungus on nitrate reductase and nitrogenase activities in nodulating and non-nodulating soybean. Phytopathology, 68:1590

Clavet, C.; J. Pinochet; I. A. Camprub and C. Femandez (1995). increased tolrance to the root lesion nematde Pratylenchus vulnus in mycorrhizal micropagated BA. 29-quince root stock. Mycorrhiza 5:253.

Fathy, T. Mikhaeel Ahmed, M. Shalaby and Mona, M. Hanna (2000). Dinitrogen fixation and nitrogen assimilation as influenced by dual (VA-Mycorrhizal Fungi-Rhizobium) inoculation in soybean plants. Annals Agric Sci., Ain Shams univ., Cairo. 45(1):67-77.

Giovannetti, M. and B. Mosse (1980). An evaluation of techniques for measur vesicular-arbuscular mycorhizai infection in roots. New Phytol. 84:489.

Harper, J. E. and R. H. Hageman (1972). Canopy and seasonal profiles of nitr reductase in soybean (Glycin max L. Merr.) Plant Physiol., 49:146.

Linderman, R. G. (1994): Role of VAM fungi in biocontrol In:"Mycorrhizae and Plant Health." F.L. Pfleger and R.G. Landerman (Ed.), pp. 1-25, APS Press, St. Paul, USA.

Liu, R. J. (1995). Effect of vesicular mycorrhizal fungi on Verticillium wilt of cotton. Mycorrhiza.. 5:293.

Mc Allister, C. B.; I. Garcia-Romera; J. Murtin; A. Godeas and J. A. Oceampo (1995). Interaction between *Aspergillus niger* and *Gloums mosseae. New Phyto., 129:309.*

Murphy, J. and J. P. Rily (1962). A modified single solution method for the determination of phosphate in nature waters. Anal. Chim. Acta., 27:31.

Ocampo, J. A. (1993). Influence of pesticide on vA mycorrhiza In:"Pesticide Interaction in Crop Production." J. Altman (Ed.), pp. 14-226, Boca Raton Florida: CRC Press.

Omokero, D.N. and C. O. Ajakalho (1989). Effect of soil applied herbicides on leaf nitrate reductase and crude protein in the leaf and seed of two cowpea cultivars. Plant and Soil., 116:141.

Pacovsky, R. S.; E. A. Paul and G. J. Benthlenfalavy (1986). Response of mycorrhizal and phosphorus-fertilized soybean to nodulation by Bradyrhizobium or ammonium nitrate. Crop Sci., 26:145

Phillips, J. M. and D. S. Hayman (1970). Improved procedure fo clearing roots and staining parasitic and vescular-arbuscular mycorrhizobium or ammonium nitrate. Crop Sci., 116:141

Raveh, A. and Y. Avnimelech (1979). Total nitrogen analysis in water, soil and plant material with persulphate oxidation. Water Resaerch., 13:911.

Shalaby, A. M. and Mona M. Hanna (2000). Interactions between VA Mycorrhizal fungus *Glomus mossage*, *Bradyrhizobium Japonicum* and Pseudomonas syringae in soybean plants. Egyptian J. of Microbiology, 35(2):199-208

Snedecor, G. W. and W. J. Cochran (1980):" Statistical Methods " 7th ed ., p. 534. Iowa State University Press.

Soliman, S.; I. A. El-Ghandour and A. Kharda-Abbady (1996). Effects of nitrogen and phosphours supply and of *Rhizobium* and VAM fungus inoculants on dinitrogen fixation in soybean folia. Microbiologica, 4(2):197-200.

Totta, A.; G. C. Varese; I. E. Cnov; I. A. Fuscon; S. Sampo and G. Berta (1996). Interaction between the soil borne root pathogen phytophthora nicotianae avr. Parasitca and the arbuscular mycorrhizal fungus Glomus mosseae in tomato plants. Plant and Soil, 185:199.

Udalyan, K.; S.Manian; T. Muthukumar and S.Greep (1995). Biostatic of fumigation and pesticide drenches on an endomycorrhizal Rhizobium-legume tripartite association under field condition. Soil. Fertil. .Biol Soils, 20:275.

Werne, D.; J. Wilckson and E. Zimmermann (1975). Adsorpition and selection of rhizobia with ion-exchange paper. Arch. Microobiol.. 105:27.

دراسات على العلاقة التداخلية لفطر الميكوريزا جلومـــس موزيـــا والريزوبيـــوم فاصولاى والاكسانوموناس فاصولاى على نبات الفاصوليا

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تناولت الدراسة العلاقة التداخلية بين فطر الميكوريزا جلومـــس موزيـــا والريزوبيـــوم فاصولاى وتأثيرها على البكتريا الممرضة (الاكسانوموناس فاصولاى) وعلـــى مقاومـــة نبـــات الفاصوليا للمرض. وقد اجرى هذا البحث فى صوبة كلية الزراعة جامعة المنيا سنة ٢٠٠٢

أوضحت الدراسة أن العلاقة التداخلية أدت الى زيادة كلا من الوزن الجـــاف للمجمـــوع الخضرى وتكوين العقد فى نبات الفاصوليا وأن وجود فطر الميكوريزا ربما أنه قد منـــع أو قلـــل الأصابة بالبكتريا الممرضة مما نتج عن ذلك تحسين فى نمو النبات وزيـــادة تراكـــم النـــتروجين والفوسفور وزيادة نشاط انزيم اختزال النترات. كما أن وجود فطرالميكوريزا ربما أدى الى نقـــص كثافة البكتريا الممرضة فى منطقة ريزوسفير النبات مما نتج عنه مقاومة النبات للبكتريا الممرضـــة وخاصة فى وجود بكتريا ريزوبيم فاصولاى.