STUDIES OF THE INTERACTION BETWEEN VA MYCORRHIZAL FUNGUS *G glamus 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 phosphorus 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 noclous effects on rhizobial growth, nodulation and N2 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 *Glamus 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 phosphorus 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
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Plant changes, iv- Changes in biochemical compounds related to plant resistance response, v- Alleviation of physical stresses and vi-changes in antagonistic 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 Crops 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 atmospheric pressure. Rhizobium phaseoli and Xanthomonas phaseoli strains, used for the inoculation of bean plants, were obtained from Sakha Agriculture Research Station. The bacterial cultures were grown to the stationary phase on 20-E medium (Werner et al., 1975) at 27°C on a rotary Shaker (140 rpm). Where indicated plants were inoculated with Rhizobium phaseoli, 10 ml/pot, the uninoculated 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 10⁷ 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 Riley (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 Xanthomonas 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 favored 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 Xanthomonas phaseoli in the rhizosphere (Table 1) decreased throughout the experimental period although the numbers were consistently lower in the presence than in 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 phosphorus, made available to these plants by the symbiotic fungus, a response that was observed by Totta et al. (1996).

*Xanthomonas* *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 weight 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 curtailed or prevented *Xanthomonas* *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 similarly affected by inoculation with *G. mosseae* (Table 2). The number, dry weight and efficiency of nodules were significantly higher in the absence of the pathogenic bacterium whereas the latter also caused the least number, dry weight or efficiency. The percentage of root length in 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 mycorrhizal 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. Ocampo (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 *Aspergillus 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 (Totta 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 activity 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 Ajakayie (1989) found that NR activity was a rate-limiting enzyme and is substrate dependent. Shalaby and Hanna (2000) divided the presence of the mycorrhizal fungus, 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. jepsonicum*.
### 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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SDW (g plant⁻¹)</th>
<th>TNS (g plant⁻¹)</th>
<th>TPS (g plant Shoot⁻¹)</th>
<th>NRA (μM NO₃/g F.Wt h⁻¹)</th>
<th>VAM infection %</th>
<th>CFU x 10⁶ g⁻¹ Rhizosphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>G X</td>
<td>1.09 a</td>
<td>1.64ab</td>
<td>1.3b</td>
<td>2.96b</td>
<td>1.33 b</td>
<td>2.05c</td>
</tr>
<tr>
<td>G X'</td>
<td>0.99 a</td>
<td>1.18 b</td>
<td>8.9c</td>
<td>12.9c</td>
<td>1.21 b</td>
<td>2.21c</td>
</tr>
<tr>
<td>G X''</td>
<td>1.38 a</td>
<td>2.02 a</td>
<td>19.4a</td>
<td>40.7a</td>
<td>3.44a</td>
<td>5.09a</td>
</tr>
<tr>
<td>G X'''</td>
<td>1.21 a</td>
<td>1.8a</td>
<td>12.0a</td>
<td>31.2b</td>
<td>1.94 b</td>
<td>4.00b</td>
</tr>
<tr>
<td><strong>Without Rhizobium phaseoli</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G X</td>
<td>2.1b</td>
<td>3.4a</td>
<td>31.9c</td>
<td>51.2c</td>
<td>2.19c</td>
<td>2.99c</td>
</tr>
<tr>
<td>G X'</td>
<td>1.94 bc</td>
<td>2.89b</td>
<td>25.5d</td>
<td>45.2d</td>
<td>2.02c</td>
<td>3.09c</td>
</tr>
<tr>
<td>G X''</td>
<td>3.0a</td>
<td>3.86a</td>
<td>55.3a</td>
<td>89.1a</td>
<td>6.51a</td>
<td>9.10a</td>
</tr>
<tr>
<td>G X'''</td>
<td>1.11 c</td>
<td>2.09b</td>
<td>39.4b</td>
<td>76.2b</td>
<td>4.95b</td>
<td>6.30b</td>
</tr>
<tr>
<td><strong>With Rhizobium phaseoli</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

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

- G X: Control - free of mycorrhiza and pathogen.
- G X': No mycorrhiza but inoculated with the pathogen (Xanthomonos)
- G X'': Inoculated only with the micorrhiza
- G X'''': Inoculated with the micorrhiza and the pathogen (Xanthomonos)

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Nodules</th>
<th>Nodules dry weight (NDW) mg plant</th>
<th>Efficiency (NE) mg N/mg NDW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 Day</td>
<td>50 Day</td>
<td>25 Day</td>
</tr>
<tr>
<td>G X</td>
<td>45.5 b</td>
<td>51.2 bc</td>
<td>39.5 c</td>
</tr>
<tr>
<td>G X'</td>
<td>31.4 c</td>
<td>47.1 c</td>
<td>36.3 c</td>
</tr>
<tr>
<td>G X''</td>
<td>66.3 a</td>
<td>81.3 a</td>
<td>76.5 a</td>
</tr>
<tr>
<td>G X'''</td>
<td>52.4 c</td>
<td>59.3 b</td>
<td>49.3 b</td>
</tr>
</tbody>
</table>

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 mycorrhiza but inoculated with the pathogen (Xanthomonos)
- G X'': Inoculated only with the micorrhiza
- G X''': Inoculated with the micorrhiza and the pathogen (Xanthomonos)
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REFERENCES


دراسات على العلاقة التداخلية للفطر الميكوريات ناجم عن الروكبة موزييا والريزوبيوم

فاصوليا والأكساتوموسات فاصوليا على نبات الفاصوليا

سامية فرحات أحمد

قسم الميكروبيولوجيا الزراعية- كلية الزراعة- جامعة المنها

تناولت الدراسة العلاقة التداخلية بين فطر الميكوريات ناجم عن الروكبة موزييا والريزوبيوم

فاصوليا وتأثيرها على البكتيريا الممرض (الأكساتوموسات فاصوليا) وعلى موقعية نبات

الفاصوليا الممرض. وقد أجري هذا البحث في مستشفى كليه الزراعة جامعة المنها في

2002.

وقد أوضح الدراسة أن العلاقة التداخلية أدت إلى زيادة كثافة البكتيريا الممرض من

الخصائص وتكوين المucus في نبات الفاصوليا وأن وجود فطر الميكوريات ربما مهدد

بمتعة أو أقل في الأنسجة بالبكتيريا الممرضة مما نتج عن ذلك تحسين في نمو النباتات وزيادة تراكم السكريات والفوتوغروس زيادة نشاط النيازك التناقل. كما أن وجود فطر الميكوريات ربما أدى إلى تقلص

كثافة البكتيريا الممرضة في منطقة ريزوسفر النبات مما نتج عنه مقاومة النباتات للبكتيريا الممرضة.

وهي خاصة في وجود بكتيريا ريزوسفر فاصوليا.