Mycorrhizal fungi Associationswith Rhizobiumjaponicumfor Soybean (*Glycine max* L)onGrowthand Root colonization

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Abstract: Inoculation with *Rhizobium japonicum* significantly increased the dry matter yield of aerial and ground parts of soybean plants, where the mean values were 30.50 and 16.05% for shoot and root in case of super-phosphate, while these values increased to 33.1and 17.40%, respectively when rock-P was applied. The results showed that*R. japonicum* increased the yield by 49%, 20%, 56% and 27.50%of shoot and root compared to uninoculated treatments in the presence of super-phosphate and rock-P, respectively.Data also showed that single inoculation with mycorrhizae in the presence of such P-sources increased dry weight of shoot by about, 41.81 and 47.41% over the uninoculated when super-phosphate and rock-P, respectively. The maximum yield was recorded with combined inoculation (*Glomus mosseae* + *R. japonicum*) even in the presence of super or rock-phosphate. It is obvious that, 60 days after inoculation in the presence of P-sources and Fe-levels, application of iron and treatment of *R. japonicum* culture significantly increased nodule dry weights by 139% and 255%, respectively. Inoculation with *R. japonicum, G. mosseae* and combined inoculation were significantly increased P-uptake in soybean shoots about 42.7, 49.7 and 91.80% in case of super-P, as compared with 81.3, 89.2 and 189.7% with rock-P dressing.Such clear difference can be attributed to the concentration of P% in each unit in the rock-p;13.8 comparing with 8.6 in superphosphate (of ca 5.2 difference) as well as the presence of many nutrients in the rock-p that support the rhizobial strain and Soybean cultivar growth.

Introduction

Legumes have the capacity of derive a considerable proportion of their N requirements from the atmosphere through symbiosis with Rhizobium-Plant roots are exposed to a range of soil microorganisms with which they form a variety of interactions.Soybean enters into one type of symbiotic association with the bacteria of the genus *Rhizobium* and into another with mycorrhizal (arbuscular mycorrhizae ; AM) fungi that improve nutrition of plants. Some interactions are beneficial whereas some are detrimental for the plant. The roots of many plants are infected by certain fungi (mycorrhizal association) that help them acquire phosphate from the soil (Smith & Read 1997, Smith et al. 2003)and other nutrients. In return for its services, the fungus is provided with a carbon source, derived from plant photosynthesis (photosynthesates), for biosynthesis and energy metabolism. In arbuscular mycorrhizae, arbuscules are the major site of nutrient exchange and mycorrhiza-specific plant phosphate transporters are exclusively localized at the arbuscular interface (Harrison et al. 2002).Mycorrhiza and Rhizobia are natural mini-fertilizer factories that are an economical and safer source of plant nutrition than synthetic chemical fertilizers, which contribute

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substantially to environmental pollution. These associations can improve soil fertility and increase agricultural production and therefore have great potential as a supplementary, renewable, and environment friendly source of plant nutrients (sustaible agriculture). By providing essential nutrients, these microbes help both their host plants and the world's agricultural systems (Marx, 2004)). Newman and Reddell (1987) have reported that from 202 species of legumes, 90% had been found to form mycorrhizal symbiosis. Arbuscular mycorrhizal (AM) association enhances legume growth and nitrogen fixation by improved phosphorus (P) nutrition. Nitrogen and phosphorus concentration and nodule formation increase with the mycorrhizal inoculum in the soil (Azcon & El-Atrash, 1997). Both interactions are extreme in terms of host specificity. Whereas nodulation is almost exclusively restricted to legumes and requires the organogenesis of a root nodule (Kuster et al., 2007).The objective of this work was to evaluate of these symbionts on each other in a pot experiment and Mycorrhizal Associations with rhizobial inoculation on growth as well asmacronutrients content and Fe uptake by plants grown in a loamy clay soil, and N_2-fixation by plant

Keywords:*G. mosseae***,**Soybean (*Glycine max* L *), Rhizobium japonicum*, Nitrogen, Phosphorus, Plant

Material and methods Soil

A soil with a loamy clay texture was passed through a 2 mm mesh sieve, mixed thoroughly and autoclaved (110 °C, 1 h, twice at 48 h intervals) to remove indigenous AM propagules.

Soil properties were 29% sand, 35% silt, 36% clay, 2.1% organic matter, Electrical conductivity (ECe 2.1 dS.m); 3.2 mg/kg P (Olsen et al., 1954) available Fe 4.3 ppm. The cation exchange capacity (CEC) was 22.62 Cmol.kg^{-1} (Mg, Ca,Na and K were 0.5, 2.2, 21.6 and 3.02 Cmol.kg^{-1}respectively).

Rhizobium culture

Rhizobium strain (*Rhizobium japonicum*) was isolated from soil in (pure the root nodules). These nodules were thoroughly washed with tap water and surface sterilized with 95% ethanol, finally washed with series of sterile distilled water and then crushed to form solution and subjected for Gram staining. Then the extract was observed under a high-power microscope with oil immersion and also streaked on yeast extract manitol agar (YEMA) medium (Subba Rao, 1977; 1999) for isolation. This isolate was identified according to Bergey and Holt (1994). The number of rhizobium cells per gram inoculant ranged from 30-35 x10^8 Gum Arabic was used as sticker to ensure viable rhizobia per seed, before sowing.

Arbuscular-mycorrhizal preparations

Arbuscular-mycorrhizal (AM) species belonging to the genus *Glomus* was used in this study. Mycorrhizal inoculum (*Glomus mosseae*) consisted of a mixture of rhizospheric soil from trap cultures (*Sorghum sp*.) containing spores, hyphae and mycorrhizal root fragments, prepared from Faculty of Agriculture, Ain Shams University of Cairo.

The staining method of Phillips and Hayman (1970) was used for preparing root samples for microscopic observations. The gridlines intersect method of Giovannetti and Mosse (1980) used to estimate the mycorrhizae infection percentages using the following equation:

Percentage of AM colonization = (Root length colonized /Root length observed) ×100.

Pot experiment

Pot experiment was carried out at the green house. Plastic pots, 120 cm deep and 50cm in diameter with holes in their bottom, were filled with 5 kg of Sterilized soil.four Seeds of soybean were treated by 0.05% NaOCl solutionand planted in each pot. About fifty grams of inoculum *Glomusmosseae* (colonized root segments) were placed 2 cm below theseeds. four weeks after planting, plants were thinned to two plant per pot. all pots were irrigated with tap water every three days to keep the soil at 70% of its field capacity by regular weighing of pots. The pots were divided into two main groups, one of which has received P in the form of single superphosphate (8.6%P) at rate of 22mg P/kg soil; the other received a fine rock-phosphate (13.8%P) at the same rate, every main group was treated with two levels of iron separately, 0 and 6mg Fe/kg Soil in the form of ($FeSO_4$-$7H_20$). All pots received 12mg N/kg soil added as calcium ammonium nitrate.

Plant assay

At harvesting (60 days after planting), samples of plants were dried, ground and dry weight of plants (shoots and roots) were recorded. A 0.5 g of dried plant materials was digested in H_2SO_4-H_2O_2 mixture according to Lowther (1980).Representative samples of shoots were ground for N analysis by micro-kjeldahl method. Phosphorus content in plant material were analyzed according to John (1970) and Black (1965).

Statistical analysis

The experiment was arranged in four treatments with completely randomized block design: (1) soybean was not inoculated with AM fungus and rhizobia (2) soybean was inoculated with AMF, but not rhizobia (3 soybean was inoculated with rhizobia, but not AMF and (4) soybean was inoculated with both AMF and rhizobia. Each treatment had 4replicates, with the total of 16pots (2 seedlings/pot).Data were subjected to analysis of variance using the ANOVA procedures according Snedecor and Cochran (1972), Differences among means of treatments analysis of variance treatment were distinguished by the L.S.D$_{0.05}$

Results and Discussion

Shoot and root dry weights

Effect of inoculation with *G. mosseae* and/or *R. japonicum* on shoot and root dry weights are presented in Table 1.-showed that inoculation with *R. japonicum* significantly increased the dry matter yield of aerial and ground parts of soybean plants~~,~~ with mean values of ca30.50 and 16.05% for shoot and root in case of super-phosphate, while it was 33.1and 17.40% when rock-P was applied.Also, the results showed notably that*R. japonicum* increased the yield by 49%, 20%, 56% and 27.50%of aerial and ground parts compared to uninoculated treatmentsin the presence of P-sources (super-phosphate and rock-P),respectively. These results areagreement with those obtained by(E1-Ghandour et al. 1996)who found thatinoculating faba-bean with*Rhizobium*bv increased the yield and the quality of grain compared to uninoculated treatments.Also, data showed that single inoculation with mycorrhizae in the presence of P-sources increased dry weight of shoot by about, 41.81 and 47.41% over the uninoculated when super and rock-P amended, respectively.This is in line with (Smith et al. 2003) who reported that roots of many plants are infected by certain fungi (mycorrhizal association) to help them acquire phosphate from the soil, the fungus is provided with a carbon source, derived from plant photosynthesis, for biosynthesis and energy metabolism.

In arbuscular mycorrhizae, arbuscules are the major site of nutrient exchange and mycorrhiza-specific plant phosphate transporters are exclusively localized at the arbuscular interface (Harrison et al. 2002). The maximum yield was recorded with combined inoculation (*G. mosseae* + *R. japonicum*) even in the presence of super or rock-phosphate. This may be due to synergistic beneficial effect between the two symbionts on plant growth (Marx, 2004). In both interactions, the two partners engage in a complex molecular conversation that allows the microbes to infect the plant cells and then entice the cells to undergo the developmental changes necessary for establishing the symbioses(Geetanjali and Neera, 2007). Concerning the effect of Fe-application, data show that increasing Fe-level lead to increase in different plant parts in the presence of P-sources, but the increase was not significant.

As mentioned previouslybyPapastylianou (1993) the obtained results indicated that in peanut, the moderate chlorosis of the plants not treated with Fe did not significantly affect the final yield. But On the other hand (Shweta et al, 2017) show that application of iron in the form of $FeSO_4$-7$H_2$0 either in soil or as aerial spray enhance the rate of photosynthesis, which results in the production significant increase in dry matter yield of chickpeas when plants inoculated with *Rhizobium*under super- P application.From the results mentioned above,increasing shootwere 52.3, 41.7 and 76.4% in case of super-P; 53.6, 47 and 88.5% when rock-P was applied for inoculation with *R. japonicum, G. mosseae* and combined inoculation respectively.(El-Ghandour et al.,1996)reported that Fe-deficiency may have effect on nodule initiation and effect on host plant growth.Regardless of inoculation and Fe treatments~~,~~. The dry weight of shoots and roots was higher in the treatmentof rock than super-P althoughincreases were not significant, whereas the meanweight of shoots were 33.3 when rock-P was applied while it was 30.78 in case of super phosphate. These results are concordantly supported by the findings ofShweta et al (2017).

Mycorrhizal infection, Plant growth and nodule development

Concerning inoculation, the data in the table (2) indicated that, the~~,~~ studied inoculants resulted in significant increase in nodule No. and dry weight. As compared with the other treatments, the effectiveness of the inoculants was of the following order: dual inoculation with *R. japonicum* and *G. mosseae* were significantly greater than those of plants inoculated with *R. japonicum* only, at 60days after inoculation in the presence of P-sources and Fe-levels.It is obvious from the data given in table 2 that application of iron andtreatment of *R. japonicum* culture significantly increased nodule dry weights by 139% and 255%, respectively as affected by P-source.The gained results were the same as those obtained by(Allah et al, 2018)who explained that in alkaline soil and low(or high) P-deficient, rock-phosphate enhanced nodulation and nitrogen fixation of mycorrhizal plants. Also nodulated roots can enhance the production of root exudates which may affect the growth of soil microflora(Qin et al, 2020)

Table 3 showed that in the presence of rock-phosphate mycorrhizal parameters were found to be more beneficial than when superphosphate was used Mycorrhizal infection was observed with single inoculation by VAM 52% and 55%, but the most pronounced results were obtained in case of a combined inoculation 64% and, 77% in case of super and rock-P and Fe-levels, respectively. These results are explained by (Fairchild and Miller 1990; Tabassum et al, 2016)

Nutrient concentration, N and P contents

Nutrient P-uptake in shoots differed depending on treatments. However, inoculation with *R. japonicum, G. mosseae* and combined inoculation were significantly increased P-uptake in soybean shoots (Table4). In the present study, we hypothesized that increases the uptake ca 42.7, 49.7 and 91.80% in case of super-P, as compared with 81.3, 89.2 and 189.7% with rock-P dressing.P in the form of single superphosphate (8.6% P) at rate of 22mg P/kg soil; the other received a fine rock-phosphate (13.8% P) at the same rate

These findings are agreement with (Sabannavar and Lakshman 2009)reported that the mycorrhizal~~.~~ inoculation can thus help in effective utilization of rock phosphate by changing it into available forms, that result could be due to a more efficient uptake of chemically dissociated P-ions by fungal hyphae as well as the the increase of the surface area of the roots. Which is later taken up by the plants for their better growth and development. Results indicated that N-content of the plants followed the order: *R. japonicum, G. mosseae*>*R. japonicumG. mosseae*> uninoculated, and used of *R. japonicum* bacteria behaved as mycorrhiza helper bacteria promoting higher colonization rate and spore number of mycorrhizal inoculum which helps in solubilization of the mineral phosphate and contribute to the biogeochemical P cycling, thus promoting a sustainable nutrient supply to the crop for higher yield. Also(Sabannavar and Lakshman, 2009) reported that, mycorrhizal inoculation is able to increase N-concentration in plant shoots by one or more mechanism such as, indirect enhancement of symbiotic N_2-fixation as a consequence of increased P-supply by mycorrhiza~~l~~ or direct uptake of N compounds by AMF hyphae.Also, data showed that the addition of P-sourcesand, Fe; which is a main constituent in nitrogenase,increased N-content compared to control.

It is clear from the results obtained in Table 4 that, in the presence of iron application super or rock-P were 642.8 and 634.0mg/ plant, while in iron absent treatments were 525.65 and 511 mg/plant, respectively. (E1-Ghandour et al. 1992) reported that iron deficiency, generally, decreases nodule formation, leghaemoglobin production. This leading to low nitrogen concentration in the shoots of some legumes. Chien et al. (1993) reported that N-uptake was much lower in the nonnodulating soybean than in the nodulating soybean at the 100 mg P/kg rate when P was added in the form of rock- phosphate.

Conclusion

In Conclusions, The overall results show that the mycorrhizal inoculation can thus help in effective utilization of super or rock-P phosphate by changing it into available forms, which is later taken up by the plants for their better growth and development.Its impact on soil microflora may show that rhizobia alone or combined with mycorrhiza and conjugated with the proper amount of mineral nitrogen, iron and other co-factors is the practice to be adopted in agriculture soil.

Table 1. Effect of inoculation with *G. mosseae* and/or *R. japonicum* on dry weights of Plant (g) under P-sources and Fe-Levels

Treatment	Shoot			Root		
	-Ferrous	+ Ferrous	Mean	-Ferrous	+ Ferrous	Mean
ddition ofASuperphosphate						
Control	19.3	21.6	**20.45**	12.8	13.9	**13.35**
-Gm+Rh	28.1	32.9	**30.50**	14.7	17.4	**16.05**
+Gm-Rh	27.4	30.6	**29.00**	13.9	16.5	**15.2**
+Gm+Rh	34.8	38.1	**36.45**	17.9	20.6	**19.25**
Mean	**27.4**	**30.78**		**14.83**	**17.10**	
ddition ofArock-phosphate						
Control	19.8	22.6	**21.20**	12.2	15.1	**13.65**
-Gm+Rh	31.5	34.7	**33.1**	15.0	19.8	**17.40**
+Gm-Rh	29.3	33.2	**31.25**	14.8	18.2	**16.50**
+Gm+Rh	39.5	42.6	**41.05**	20.4	22.1	**21.25**
Mean	**30.03**	**33.30**		**15.60**	**18.80**	
$LSD_{0.05}$						
Fe-rates		**2.87**			**1.35**	
Inoculations		**6.03**			**1.06**	
P-sources		**2.06**			**N.S**	
Fe × P x Inoculations		**N.S**			**N.S**	

Table 2. Effect of inoculation with *G. mosseae* and/or *R. japonicum* on nodule number and dry weights of Nodule under P-sources and Fe-levels

Treatment	Nodule Number per plant			Nodule dry wt per plant		
	- Ferrous	+ Ferrous	Mean	- Ferrous	+ Ferrous	Mean
Addition of Superphosphate						
Control	10.6	14.8	**12.7**	55.24	70.30	**62.77**
-Gm+Rh	41.4	47.3	**44.35**	119.54	168.10	**143.82**
+Gm-Rh	19.6	27.8	**23.7**	98.20	118.90	**108.55**
+Gm+Rh	47.8	53.9	**50.86**	230.40	271.85	**251.13**
Mean	**29.85**	**35.95**		**125.85**	**157.29**	
Addition of rock-phosphate						
Control	15.23	17.74	**16.49**	68.10	74.50	**71.30**
-Gm+Rh	47.3	53.9	**50.6**	238	264.3	**251.15**
+Gm-Rh	25.3	33.6	**29.45**	140.5	155.9	**148.20**
+Gm+Rh	58.9	67.5	**63.2**	278.3	325.6	**301.95**
Mean	**36.68**	**43.19**		**181.23**	**205.08**	
LSD$_{0.05}$						
Fe-rates		**3.87**			**18.74**	
Inoculations		**8.21**			**22.92**	
P-sources		**5. 98**			**23.50**	
Fe × P x Inoculations		**10.54**			**N.S**	

Table 3. Effect of inoculation with *G. mosseae and/or R. japonicum* on mycorrhizal root infection under P-sources and Fe-levels

	Treatment	*Mycorrhizal* infection %	
- Ferrous		+ Ferrous	Mean
		Addition of Superphosphate	
Control	12	16	**14**
-Gm+Rh	19	25	**22**
+Gm-Rh	45	52	**48.5**
+Gm+Rh	60	64	**62**
Mean	**34**	**39.25**	
		Addition of rock-phosphate	
Control	21	23	**22**
-Gm+Rh	24	31	**27.50**
+Gm-Rh	45	55	**50**
+Gm+Rh	69	77	**73**
Mean	**39.75**	**46.50**	
LSD $_{0.05}$			
Fe-rates		2.9	
Inoculations		3.8	
P-sources		2.7	
Fe × P x Inoculations		N.S	

Table 4.Effect of inoculation with *G. mosseae and/or R. japonicum* on P and N Uptake in shoots under P-sources and Fe-levels.

Treatment	P mg/pot			N mg/pot		
	- Ferrous	+ Ferrous	Mean	- Ferrous	+ Ferrous	Mean
Addition of Superphosphate						
Control	41.5	50.3	**45.9**	287	394	**340.5**
-Gm+Rh	60.6	70.4	**65.5**	562.6	693	**627.8**
+Gm-Rh	64.3	73.1	**68.7**	468	595	**531.5**
+Gm+Rh	83.8	92.3	**88.05**	785	889	**837**
Mean	**62.55**	**71.53**		**525.65**	**642.8**	
Addition of rock-phosphate						
Control	25.7	33.6	**29.65**	301	370.4	**335.7**
-Gm+Rh	47.8	59.7	**53.75**	531.6	655.2	**593.4**
+Gm-Rh	49.4	62.8	**56.1**	472.5	606.4	**539.45**
+Gm+Rh	79.3	92.5	**85.9**	738.9	903.8	**821.35**
Mean	**50.55**	**62.15**		**511.0**	**634.0**	
$LSD_{0.05}$						
Fe-rates		7.59			92.4	
Inoculations		10.77			96.87	
P-sources		3.98			N.S	
Fe × P x Inoculations		15.06			108.12	

المستخلص: أدى التلقيح بـ *R. japonicum.* إلى زيادة معنوية في الوزن الجاف للأجزاء الهوائية والأرضية لنبات فولالصويا،حيث كانت القيمة المتوسطة 30.50 و 16.05٪ للمجموع الخضري والمجموع الجذري في حالة السوبر فوسفات، بينما كانت 33.1 و 17.40٪ عند استخدام الفوسفات الصخري. أظهرت النتائج أن بكتريا الرايزوبيوم زادت المحصول بنسبة 49٪ و 20٪ و 56٪ و 27.50٪ من الأجزاء الهوائية والأرضية مقارنة بالمعاملات غير الملقحة في وجود مصادر الفوسفور (سوبر فوسفات والفوسفات الصخري) على التوالي. أظهرت البيانات أيضًا أن التلقيح الفردي بالميكورايزا في وجود مصادر الفوسفور زاد الوزن الجاف لنباتات فولالصويا بحوالي 41.81 و 47.41٪ مقارنة بالغير ملقحة عند استخدام السوبر فوسفات والفوسفات الصخري، على التوالي. تم تسجيل أقصى إنتاجية بالتلقيح المشترك (*Glomus mosseae +R. japonicum*) حتى في وجود السوبر أو الفوسفات الصخري. من الواضح أنه بعد 60 يومًا من التلقيح في وجود مصادرPومستوياتFe، أدى استخدام الحديد واستخدام *R.japonicum* إلى زيادة كبيرة في أوزان العقد الجذرية الجافة بنسبة 139٪ و 255٪ على التوالي. أدى التلقيح بالبكتريا العقدية *R.japonicum* والميكورايزا *G.mosseae*والتلقيح المشترك لكليهما إلى زيادة معنوية في امتصاص الفوسفور في براعم فولالصويا بحوالي 42.7 و 49.7 و 91.80٪ في حالة السوبر فوسفات مقارنة بـ 81.3 و 89.2 و 189.7٪ مع الفوسفات الصخري.

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