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Effects of arbuscular mycorrhizal fungi and *Rhizobium* on ion content and root characteristics of green bean and maize under intercropping

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ABSTRACT

In order to evaluate arbuscular mycorrhizal fungi and rhizobium bacteria effects on leaf nitrogen (N) and phosphorus (P) concentration and root characteristics of green bean and maize under intercropping, experiment was carried out in the research field of College of Agriculture, Payame Noor University of Azna, Lorestan, Iran. In experiment, sandy loam soil with pH 7.3 and EC 0.49 dS m^{-1} was used.The treatments comprised three cropping systems (sole cropping of green bean and maize, and intercropping), and four inoculations (control, arbuscular mycorrhizal fungi, rhizobium and mix of arbuscular mycorrhizal fungi and rhizobium). The results showed that inoculation with rhizobium improved length, diameter, volume and area of green bean root.The highest of green bean N, P concentration and root dry mass were observed in sole culture of green bean inoculated with arbuscular mycorrhizal fungi. Moreover, root length, diameter, volume and area of maize increased by arbuscular mycorrhizal fungi, and total concentration of N and P enhanced with use of rhizobium in sole cropping. Although the usage of *Rhizobium* and AMF can be affected on increasing the root growth and nutrient uptake of crops, application of bacterium and fungi combination at the same time would not be suitable. Overall, intercropping of maize with green bean caused to increase of leaf N and P concentrations and root growth of maize.

Key words: inoculation; AMfungi; rhizobacteria; intercropping; root length; root area; root dry mass; phosphorus; nitrogen

IZVLEČEK

UČINKI ARBUSKULARNE MIKORIZE IN BAKTERIJ IZ RODU *Rhizobium* NA VSEBNOST N in P TER LASTNOSTI KORENIN V MEDSETVENEM POSEVKU KORUZE IN FIŽOLA

Z namenom ovrednotenja učinkov arbuskularnih mikoriznih gliv in bakterij iz rodu *Rhizobium* na listno vsebnost dušika (N) in fosforja (P) in na lastnosti korenin navadnega fižola in koruze v medsetvi je bil narejen poljski poskus na raziskovalnem polju College of Agriculture, Payame Noor University of Azna, Lorestan, Iran. Tla v poskusu so bila ilovnato-peščena s pH 7.3 in EC 0.49 dS m^{-1}. Obravnavanja so obsegala tri setvene sisteme (čista setev fižola in koruze in medsetve) in štiri inokulacije (kontrola, arbuskularne mikorizne glive, rizobium in mešanica arbuskularnih mikoriznih gliv in rizobium). Rezultati so pokazali, da je inokulacija z rizobijem izboljšala dolžino, premer, volumen in površino korenin fižola. Največja vsebnost N, P in največja suha masa korenin fižola sta bili izmerjeni v čistem posevku fižola inokuliranem z arbuskularnimi mikoriznimi glivami. Dolžina korenin, premer, volumen in površina korenin koruze so se povečali pri inokulaciji z arbuskularnimi mikoriznimi glivami, a vsebnost celukopnega N in P se je povečala le pri čistem posevku koruze in inokulaciji z rizobijem. Čeprav uporaba rizobijuma in arbuskularnih mikoriznih gliv lahko poveča rast korenin in privzem hranil poljščin pa njihova hkratna uporaba ni vedno primerna. Na osnovi raziskave lahko zaključimo, da medsetev fižola v koruzo povzroči povečanje listne vsebnosti N in P koruze in poveča rast njenih korenin.

Ključne besede: inokulacija; arbuskularne mikorizne glive; rizobakterije; medsetev; dolžina korenin; površina korenin; suha masa korenin; P; N

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1 INTRODUCTION

Fertilizer of nitrogen (N) and phosphorus (P) are crucial to the growth of all plants. However, uncontrolled use of chemical and pesticides can destroy environment. In addition, the prices of these chemicals are high. Accordingly, application of plant growth promoting rhizobacteria and symbiotic arbuscular mycorrhizal fungi (AMF) can perform the goals of sustainable agriculture.The AMF symbiosis is an association between the roots of higher plants and soil fungi that promotes plant development, especially under sub-optimal growth conditions (Koltai et al.,2010). Previous studies suggested that AMF can promote plant N uptake and improve plant N nutrition (Corkidi et al., 2002). The successful association between plants and AMF is a strategy to improve the nutritional status of both, which reduces the use of fertilizers especially P.These fungi increase the surface area of roots and help in absorbing some diffusion-limited nutrients such as Zn, Fe and Cu (Almagrabi and Abdelmoneim, 2012). During the formation of arbuscular mycorrhizae, fungal hyphae enter the rhizodermal, exodermal and cortical cell layers of the roots, reaching the inner cortex, where the functional units, the arbuscules, develop. The fungi also form hyphae outside of the plant, extending the root-soil interface to facilitate the uptake of nutrients such as phosphates and water (Kistner and Parniske, 2002). Moreover, the beneficial plant-microbe interactions in the rhizosphere are the primary determinants of plant health and soil fertility (Klyuchnikov and Kozherin, 1990). *Rhizobium* species can exist as free-living soil saprophytes or as N_2 fixing endo-symbionts of legume host plants that is within the root nodules of host legumes or in close association with the plant roots (Shamseldin et al., 2008). Rhizobium, root-colonizing bacteria are known to influence plant growth by various direct or indirect mechanisms. Plant growth promoting bacteria are reported to influence the growth, yield, and nutrient uptake by an array of mechanisms. Some bacterial strains directly regulate plant physiology by mimicking synthesis of plant hormones, whereas others increase mineral and nitrogen availability in the soil as a way to augment growth (Yasmin et al., 2007).

Many intercropping systems have proved to be better than sole crops in terms of yield (Zhang et al., 2007) because intercropping makes better use of one or more agricultural resources both in time and in space (Rodrigo et al., 2001). Intercropping maize and various legumes has been investigated with species such as cowpea (*Vigna unguiculata* L.) and various species of bean (Dahmardeh et al., 2009; Armstrong et al., 2008; Contreras-Govea et al., 2009). In intercropping, absorption of N, P and K is more than pure cultures (Kuo and Jellum, 2002). Plant nutrient uptake can be improved by intercropping (Li et al., 2003). Nitrogen (N) transfer from the N_2 fixing legume to the maize and other species has also been reported (He et al., 2009). According to the above, the objective of this paper was to investigate the interactiveeffects of AMF, *Rhizobium* inoculation and intercropping on the leaf N and P concentrations, and root characteristics of green bean and maize.

2 MATERIALS AND METHODS

Field experiment was conducted in spring of 2013 at the research farm of College of Agriculture, Payame Noor University of Azna (PNU), Lorestan, Iran (latitude 38°05′ N, longitude 46°17′ E, and 1360 m above sea level). The climate of area is cold-dry and the average annual rainfall is 160 mm. Soil of experiment was a sandy loam-type; with pH and EC were 7.3 and 0.49 dS m^{-1}, respectively. The mean values of soil total N, available P and C contentswere 0.70 %, 15 mg kg^{-1} and 7.8 g $kg^{-}1$, respectively. The study was laid out a factorial based on randomized complete block with three cropping systems (sole cropping of green bean and maize, and intercropping), and four inoculations (control, AMF, *Rhizobium* and mix of AMF and *Rhizobium*). Seeds of maize (*Zea mays* 'S.C. 704') and green bean (*Phaseolus vulgaris*‘Derakhshan’) were obtained from the Khoram Abad Agricultural Jihad Institute. The pattern of intercropping was a replacement series.The experimental plots consisted of 7 rows with 50 cm distance between rows and 20 cm between plants in the row. Fertilizers providing 15 kg of N (KNO_3) per hectare was applied at sowing time. The mycorrhizal inoculum contained soil, plant roots and fragments of *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe (obtained from Turan Biotech Co), and symbiovar *trifolii* of mesorhizobium bacteria (*Rhizobium leguminosarum* (Frank 1879) Frank 1889) was obtained from the Mehr Asia Biotechnology Company (MABCo.). Before inoculation for more adhesion of inoculums, the seeds surface was mixed with 15 % sugar completely for 2 hours (Shariati et al., 2015). Seeds were washed with distilled water then inoculation was performed by a suspension of *Rhizobium* at the dose of 500 g per 100 kg^{-1} seed in the darkness at 20 - 26 °C. Finally, seeds were dried in the shade for 2 h. For AMF treatment, the amount of 15 g of mycorrhizal inoculum was placed 3 cm below of each seed at 2^{nd}April 2013. Irrigation was carried out as required to keep the soil water content near field capacity and weeds were controlled by hand. Plants were harvested and measured at flowering stage. To measure root characteristics, including root length, root diameter, root volume, root area and root dry mass, sampling was done at flowering stage. Root sampling was based on root profile observations and soil sample analysis. For each plant sample, a block of soil (66 cm length × 17 cm width × 20 cm depth) was extracted from the center region surrounding the plant. Each 20 cm layer of soil was placed into nylon netting bags, and sampling was done to 200 cm. Roots were washed and collected after the soil was passed through a 0.5 mm sieve using a hose and nozzle attachment. Root length (cm) and root area were obtained according to Newman (1966) and Bohn (1979) methods, respectively. Root volume was calculated by difference of initial water volume with second water volume after putting it in.

At flowering stage, five plants of each treatment were collected randomly. All samples were heat-treated at 105 ˚C for 30 min, dried at 70 ˚C and then ground into fine powder (passed through a 2 mm mesh screen). Nitrogen was analyzed by a micro-Kjeldahl procedure after digestion with H_2SO_4-H_2O_2 (Nelson and Sommers, 1973).Total P of leaves was estimated after digestion in di-acid mixture 9:1 ratio (HNO_3:$HClO_4$) using the standard methods described by AOAC (1970). Experimental factors were determined from analysis of variance (ANOVA) using the generalized linear model (GLM) procedure in SAS (SAS Institute, Cary, NC, USA). The mean values were compared by Duncan's test at 0.05 level of probability.

3 RESULTS AND DISCUSSION

3.1 Green bean traits

3.1.1 Nitrogen content

Analysis of variance indicated that the effects of cropping system, *Rhizobium* and interaction of cropping system × *Rhizobium* and AMF × *Rhizobium* were considerable on nitrogen content (Table 1).The results showed that application of rhizobium and AMF under different cropping systems increased nitrogen content of green bean. The maximum nitrogen content was recorded in sole culture of bean with AMF by 86.59 % and the minimum was observed in intercropping without inoculation by 49.4 % (Table 1). Nitrogen is an essential constituent of proteins, nucleic acids, some carbohydrates, lipids, and many metabolic intermediates involved in synthesis and transfer of energy molecules (Davis, 1980). Optimum growth of leguminous plants is usually dependent on symbiotic relationships with AMF and N_2-fixing bacteria (Xavier and Germida, 2003). AMF infection of plant roots usually stimulates plant growth through effects on nutrient uptake, nodulation, nitrogen fixation, and water supply (Redecker et al.,1997).

3.1.2 Phosphorus content

Leaf phosphorus content was significantly ($P \leq 0.01$) affected by cropping system, *Rhizobium* and interaction of cropping system × AMF and AMF × *Rhizobium* (Table 1).The highest phosphorus content was achieved in sole culture of bean with AMF; in contrast, the lowest was obtained in sole culture without inoculation (Table 1). Phosphorus plays a fundamental role in the very large number of enzymic reactions that depend on phosphorylation. Phosphorus is essential for cell division and development of meristem tissue (Vessey, 2003). AMF has strong mycelia, which expand the area of roots available for absorption of nutrients, especially phosphorus (Ortas, 2012). Fungi showed high solubilization of P with reduction in the pH of the medium. The reduction in the soil pH, increase in available P and organic carbon was greater in inoculated by fungi as compared to non-inoculated which may be attributed to ability of such microorganisms to excrete organic acids, thereby decrease the pH and increase the concentration of phosphorus in soil by mechanisms involving chelation and exchange reactions (Reyes et al., 2006).There are many reports stating that P absorption and availability would be increased in mycorrhizally inoculated plants (Martin et al., 2012; Grace et al., 2009). AMF colonization can significantly promote plant P uptake from the soil, so that other functions are often inextricably linked with the improvement of P nutrition status (Cozzolino et al., 2010).

3.1.3 Root characteristics

Presented results in Table 1 clearly show that root dry mass, length, diameter, volume, area of green bean was noticeably influenced by cropping system, AMF and interaction of cropping system × *Rhizobium* and AMF × *Rhizobium* ($P \leq 0.01$).The effect of rhizobium was remarkable on root length, diameter and area. Moreover, the interaction of cropping system × AMF had considerable effect only on root volume and area (Table 1).

The maximum root dry mass of green bean was obtained in sole culture of bean with AMF treatment, while the minimum was produced under sole culture and none-inoculation (Table 1). AMF increased the surface area of roots and thus helped in absorbing some diffusion-limited nutrients. Based on the mean comparison result the highest root length, diameter, volume and area were recorded in mono-cropping inoculated with rhizobium; however, the lowest were observed in intercropping with use of rhizobium and AMF combination (Table 1). *Rhizobium* increases plant growth by various ways such as production of plant growth hormones, vitamins, siderophores, by solubilisation of insoluble phosphates, induction of systemic disease resistance and enhancement in stress resistance (Hussain et al., 2009). Zahir et al.,(2004) found that the inoculation by rhizobium increased root elongation and root dry mass in wheat. Induction of longer roots with increased number of root hairs and root laterals is a growth response attributed to IAA production by other bacteria. Reductionof root length, diameter, volume and area by use of dual rhizobium and AMF may be due to antagonistic activity of rhizobial inoculation (Gachande and Khansole, 2011).

Table 1: Effects of cropping system, *Rhizobium* and AMF on nitrogen and phosphorus content, and root characteristics of green bean. Means are average values of three replicates ± standard errors. Within the column, different letters indicate statistically significant difference between treatments.

Cropping system	Inoculation	Nitrogen content (%)	phosphorus content (ppm)	Root dry mass (g)	Root length (cm)	Root diameter (mm)	Root volume (mm^3)	Root area (cm^2)
Mono cropping	Control	59.9±1.02de	16.3±0.84d	3.25±0.77e	10±1.38c	6.01±0.37b	16.6±1.1b	53.4±1.28b
	AMF	86.5±3.05a	31.6±1.03a	8.61±0.96a	11.2±1.48b	5.67±0.23e	12±1.03d	38.7±1.58d
	Rhizobium	77.5±1.8ab	30.5±1.49ab	6.73±1.03b	15.8±1.03a	7.13±0.52a	19.1±1.06a	61.7±1.25a
	AMF+*Rhizobium*	62±1.93cd	27.6±1.25ab	5.83±0.68bc	11.9±1.93b	6.18±0.65b	14.1±1.126c	46.0±1.43c
Intercropping	Control	49.4±1.13e	22.1±0.87c	5.22±0.93c	9.04±1.37d	5.38±0.58d	11.6±1.26d	36.3±1.79e
	AMF	54.7±2.9de	16.5±0.63d	4.78±0.88cd	8.67±1.71d	5.2±0.44d	11.1±0.93d	35.6±1.07e
	Rhizobium	73.2±1.16bc	26.8±1.59b	5.1±0.63cd	9.5±1.67cd	5.52±0.58cd	11±0.96d	36.7±1.28de
	AMF+*Rhizobium*	74.4±1.29b	18.1±0.78cd	3.85±0.72de	5.94±1.44e	4.36±0.52e	6.67±0.61e	22.2±1.56f
Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range tests.								
Analysis of variance								
Cropping system (C)		**	**	**	**	**	**	**
R (*Rhizobium*)		**	**	ns	**	**	ns	**
AMF (Arbuscular Mycorrhizal)		ns	ns	**	**	**	**	**
C×R		**	ns	**	**	**	**	**
C×AMF		ns	**	ns	ns	ns	**	**
R×AMF		**	**	**	**	**	**	**
C×R×AMF		**	**	**	*	ns	**	**

ns= non-significant, * significant in 5 %, ** significant in 1 %

3.2 Maize traits

3.2.1 Nitrogen content

The statistical analysis of the data revealed that the effects of cropping system and interactive effects of AMF × *Rhizobium* were remarkable on nitrogen content of maize plants (Table 2). The maximum nitrogen content was recorded in sole culture of maize with rhizobium and sole culture with AMF by almost 80 %. In contrast, the lowest belonged to mono-cropping of maize with combination of AMF and rhizobium by 65 % (Table 2). Mirzai et al., (2010) reported that the effect of the inoculation of a mixture of several free-living rhizobacteria have enhanced nitrogen accumulation of plants. Das and Saha (2005) indicated that the effect of non symbiotic N_2 fixing bacteria (*Azotobacter* and *Azospirillum*) were found significantly improved inorganic and organic nitrogen content in rhizosphere.

As an average, intercropping enhanced nitrogen content of maize in comparison with sole culture. Nitrogen transfer from the N_2-fixing legume to the maize and other species has also been reported (He et al., 2009), reducing the need for N fertilizer. Li et al., (2003) showed that plant nutrient uptake can be improved by intercropping. Growing plant species with differing root architecture in the same field also can increase nutrient use efficiency. Therefore, intercropping may be an important strategy to use N efficiently and to reduce the risks of N leaching.

3.2.2 Phosphorus content

Presented results in Table 2 clearly show that phosphorus content of maize was considerably influenced by cropping system, *Rhizobium*, AMF and interaction of cropping system × *Rhizobium* and AMF × *Rhizobium*. Inoculated maize plants with rhizobium under sole culture and also inoculated with AMF under intercropping had the highest of phosphorus content among other treatments. The lowest of phosphorus content was found under sole culture by 9.28 ppm (Table 2). Plant growth promoting rhizobacteria stimulate plant growth directly either by synthesizing hormones such as indole-3-acetic acid or by promoting nutrition, for example, by phosphate solubilization or more generally by accelerating mineralization processes (Kannahi and Kowsalya, 2013; Baniaghil et al., 2013).

Abdel-Fattah and Mohamedin (2000) demonstrated that phosphorus and nitrogen contents in shoots and roots of AMF sorghum plants were significantly greater than those of non-inoculated with AMF plants. Over 90 % of plants will engage in AMF symbiosis, which mainly improves the nutrient uptake of phosphorus, and several other nutrients (Bonfante, 2003). As a result of the increased uptake of P, plants inoculated with mycorrhizae frequently produce higher yields than do those without AMF (Martin et al., 2012). Maize is more competitive than green bean for phosphorus, so phosphorus supply for green bean in intercropping was less than its sole crop.

3.2.3 Root characteristics

Root dry mass was significantly ($P \leq 0.01$) affected by cropping system, *Rhizobium* and interaction of cropping system × AMF, cropping system × *Rhizobium* and AMF × *Rhizobium*. Effects of all treatments were noticeable on root length, diameter, volume and area (Table 2). The greatest root dry mass was recorded in intercropping without inoculation by 39.47 g. The treatment of intercropping with AMF had the maximum root length, diameter, volume and area. However, the least of root dry mass, length, diameter, volume and area were observed in sole culture of maize (Table 2).

Li et al., (2006) showed that roots of maize had not only penetrated deeper than those of the faba bean but had also spread under the faba bean strip in a maize and faba bean intercropping system. Adikuet al. (2001) indicated that although the roots of maize and cowpea had extended into the rhizospheres of each other, the encroachment on part of maize was much greater. In the maize and green bean intercropping that we studied, interspecific interactions resulted in higher N and P uptake in maize but lower in green bean. Such interactions were defined as asymmetric interspecific facilitation between the intercropped species by Li et al. (2006), who suggested that asymmetric interspecific facilitation results from greater lateral deployment of roots and increased root length of one crop, and that compatible spatial root distribution of the intercropped species contributes to the symmetric interspecific facilitation observed in faba bean and maize intercropping. Results of our experiment demonstrated that intercropping favored lateral spread of maize roots possibly the main reason for the superiority of maize over green bean in terms of root growth, N and P uptake.

Table 2: Effects of cropping system, *Rhizobium* and AMF on nitrogen and phosphorus content, and root characteristics of maize. Means are average values of three replicates ± standard errors. Within the column, different letters indicate statistically significant difference between treatments.

Cropping system	Inoculation	Nitrogen content (%)	phosphorus content (ppm)	Root dry mass (g)	Root length (cm)	Root diameter (mm)	Root volume (mm^3)	Root area (cm^2)
Mono cropping	Control	69±2.3c	9.28±1.3c	11.8±1.49e	58.8±2.35e	13.7±0.25e	20.1±2.16h	121±2.45e
	AMF	80.6±3.33a	14±1.67c	29.9±2.11b	121±3.59b	19.3±0.47b	97.5±3.21d	385±4.98b
	Rhizobium	82.2±2.37a	16.3±1.35a	25.8±2.03c	72.6±2.9d	15.2±0.19d	68±2.93e	249±3.59c
	AMF+*Rhizobium*	65±3.48c	15±0.94ab	22±1.01d	77.4±2.51d	15.7±0.58d	25±1.02g	155±3.59d
Intercropping	Control	79±3.36a	15.6±1.36ab	39.4±2.57a	104±3.55c	18.2±0.29c	49.3±3.283f	253±4.68c
	AMF	75.1±2.36c	16±1.13ab	31.3±2.18b	161±2.93a	22.7±0.76a	152±3.42a	554±5.22a
	Rhizobium	77.3±2.55bc	15.2±1.25ab	24.8±3.51cd	105±1.94c	19.3±0.87b	111±4.08c	358±4.59b
	AMF+*Rhizobium*	78±2.56b	14.9±1.03ab	25.4±2.42c	88.8±2.31c	16.9±0.42c	132±3.97b	383±4.32b

Means within columns followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range tests.

Analysis of variance		Nitrogen content	phosphorus content	Root dry mass	Root length	Root diameter	Root volume	Root area
Cropping system (C)		*	**	**	**	**	**	**
R (*Rhizobium*)		ns	**	**	**	**	**	**
AMF (Arbuscular Mycorrhizal)		ns	*	ns	**	**	**	**
C×R		ns	**	**	**	**	**	**
C×AMF		ns	ns	**	**	**	**	**
R×AMF		**	**	**	**	**	**	**
C×R×AMF		**	*	**	**	ns	**	**

ns= non-significant, * significant in 5 %, ** significant in 1 %

4 CONCLUSION

Plants grown from seeds dressed with rhizobium showed high root length, diameter, volume and area for green bean, and N and P content for maize over the control. However, N and P content of green bean and root characteristics of maize in soil inoculated with AMF improved compared to none-inoculated. Although the usage of rhizobium and AMF can be affected on increasing the root growthand nutrient uptake of crops, application of bacterium and fungi combination at the same time would not be suitable. This study showed that maize and green bean intercropping could increase root growth of maize. As an average, compared with mono-cropped, the N and P content of green bean were decreased by intercropping; however, for maize were intensified. In fact, green bean as a legume can help nutrient uptake such as N and P of maize slightly. Similar trend was observed in root characteristics of green bean and maize. Therefore, intercropping of maize and green bean caused to increase of leaf N and P concentrations and root growth of maize.

5 REFERENCES

Abdel-Fattah, G. M., Mohamedin, A. H. (2000). Interactions between a vesicular-arbuscular mycorrhizal fungus (*Glomus intraradices*) and *Streptomyces coelicolor* and their effects on sorghum plants grown in soil amended with chitin of brawn scales. *Biology and Fertility of Soils,32*, 401-409. doi:10.1007/s003740000269

Adiku, S. G. K., Ozier-Lafontaine, H., Bajazet, T. (2001). Patterns of root growth and water uptake of a maize–cowpea mixture grown under greenhouse conditions. *Plant and Soil, 235,*85-94. doi:10.1023/A:1011847214706

Almagrabi, O. A., Abdelmoneim, T. S. (2012). Using of Arbuscular mycorrhizal fungi to reduce the deficiency effect of phosphorous fertilization on maize plants (*Zea mays* L.). *Life Science, 9,* 1648-1654.

AOAC, 1970. Official Methods Analysis, 10th ed. Association of Official Agricultural Chemistry, Washington, DC.

Armstrong, K. L., Albrecht, K. A., Lauer, J. G., Riday, H. (2008). Intercropping corn with lablab bean, velvet bean, and scarlet runner bean for forage. *Crop Science, 48*, 371-379. doi:10.2135/cropsci2007.04.0244

Baniaghil, N., Arzanesh, M. H., Ghorbanli, M., Shahbazi, M. (2013). The effect of plant growth promoting rhizobacteria on growth parameters, antioxidant enzymes and microelements of canola under salt stress. *Journal of Applied Environmental and Biological Sciences, 3,* 17-27.

Bohn, W. (1979). Methods of studying root systems. Ecological Studies 33:188. Springer-Verlag, Berlin. doi:10.1007/978-3-642-67282-8

Bonfante, P. (2003). Plants, mycorrhizal fungi and endobacteria: a dialog among cells and genomes. *Biological Bulletin, 204*, 215-220. doi:10.2307/1543562

Contreras-Govea, F. E., Muck, R. E., Armstrong, K. L., Albrecht, K. A. (2009). Nutritive value of corn silage in mixture with climbing beans. *Animal Feed Science and Technology, 150,* 1-8. doi:10.1016/j.anifeedsci.2008.07.001

Corkidi, L., Rowland, D. L., Johnson, N. C., Allen, E. B. (2002). Nitrogen fertilization alters the functioning of arbuscular mycorrhizas at two semiarid grasslands. *Plant and Soil, 240,* 299-310. doi:10.1023/A:1015792204633

Cozzolino, V., Pigna, M., Di Meo, V., Caporale, A. G., Violante, A. (2010). Effects of arbuscular mycorrhizal inoculation and phosphorus supply on the growth of *Lactuca sativa* L. and arsenic and phosphorus availability in an arsenic polluted soil under non-sterile conditions. *Applied Soil Ecology, 45,* 262-268. doi:10.1016/j.apsoil.2010.05.001

Dahmardeh, M., Ghanbari A., Syasar B., Ramroudi, M. (2009). Effect of intercropping maize (*Zea mays* L.) with cowpea (*Vigna unguiculata* L.) on green forage yield and quality evaluation. *Asian Journal of Plant Sciences, 8,* 235-239. doi:10.3923/ajps.2009.235.239

Das, A. C., Saha, D. (2005). Non symbiotic nitrogen fixing bacteria influencing mineral and hydrolysable organic nitrogen in rhizosphere soil of rice (*Oryza sativa*). *Indian Journal of Agricultural Sciences*, *75(5)*, 265-269.

Davis, L.C. (1980). Limiting factors in nitrogen fixation. *What's New in Plant Physiology,11*, 41-45.

Gachande, B. D., Khansole, G. S. (2011). Morphological, cultural and biochemical characteristics of *Rhizobium japonicum* syn. and *Brady Rhizobium japonicum* of soybean. *Bioscience Discovery, 2*, 1-4.

Grace, E. J., Cotsaftis, O., Tester, M., Smith, F. A., Smith, S. E. (2009). Arbuscular mycorrhizal inhibition of growth in barely cannot be attributed to extent of colonisation, fungal phosphorus uptake or effects on expression of plant phosphate transport genes. *New Phytologist, 181*, 938-949. doi:10.1111/j.1469-8137.2008.02720.x

He, X., Xu, M., Qiu, G. Y., Zhou, J. (2009). Use of 15N stable isotope to quantify nitrogen transfer between mycorrhizal plants. *Journal of Plant Ecology, 2*, 107-118. doi:10.1093/jpe/rtp015

Hussain, M. B., Mehboob, I., Zahir, Z. A., Naveed, M., Asghar, H. N. (2009). Potential of *Rhizobium* spp. For improving growth and yield of rice (*Oryza sativa* L.). *Journal Soil and Environment, 28*, 49-55.

Kannahi, M., Kowsalya, M. (2013). Efficiency of plant growth promoting rhizobacteria for the enhancement of Vigna mungo growth. *Journal of Chemical* and *Pharmaceutical Research, 5,* 46-52.

Kistner, C., Parniske, M. (2002). Evolution of signal transduction in intracellular symbiosis. *Trends in Plant Science,7,* 511-518. doi:10.1016/S1360-1385(02)02356-7

Koltai, H., Gadkar, V., Kapulnik, Y. (2010). Biochemical and practical views of Arbuscular Mycorrhizal fungus-host association in horticultural crops. *Horticultural Reviews, 36*, 257-287.

Kuo, S., Jellum, E. J. (2002). Influence of winter cover crop and residue management on soil nitrogen availability and corn yield. *Agronomy Journal, 94*, 501-508. doi:10.2134/agronj2002.5010

Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, A., Smith, S. E. (2006). Root distribution and interactions between intercropped species. *Oecologia,147*, 280-290. doi:10.1007/s00442-005-0256-4

Li, L., Zhang, F., Li, X., Christie, P., Sun, J., Yang, S., Tang, C. (2003). Interspecific facilitation of nutrient uptake by intercropped maize and faba bean. *Nutrient Cycling in Agroecosystems, 65*, 61-71. doi:10.1023/A:1021885032241

Martin, S. L., Mooney, S. J., Dickinson, A. J., West, H. M. (2012). The effects of simultaneous root colonisation by three Glomus species on soil pore characteristics. *Soil Biology and Biochemistry, 49*, 167-173. doi:10.1016/j.soilbio.2012.02.036

Mirzai, A., Vazan, S., Naseri, R. (2010). Response of yield and yield components of safflower (*Carthamus tinctorius* L.) to seed inoculation with Azotobacter and Azospirillum and different nitrogen levels under dry land condition. *World Applied Science Journal, 11(10),* 1287-1291.

Nelson, D. W., Sommers, L. E. (1973). Determination of total nitrogen in plant material. *Agronomy Journal, 65,* 109-112. doi:10.2134/agronj1973.00021962006500010033x

Newman, E. I. (1966). A metod of estimating the total length of root in a sample. *Journal Applied Ecology, 3*, 139-145. doi:10.2307/2401670

Ortas I. (2012). The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions. *Field Crops Research, 125*, 35-48. doi:10.1016/j.fcr.2011.08.005

Redecker, D., Vonbereswordtwallrabe, P., Beck, D. P., Werner, D. (1997). Influence of inoculation with arbuscular mycorrhizal fungi on stable isotopes of nitrogen in *Phaseolus vulgaris*. *Biology and Fertility of Soils, 24*, 344-346. doi:10.1007/s003740050255

Reyes, I., Valery, A., Valduz, Z. (2006). Phosphate-solubilizing microorganisms isolated from rhizospheric and bulk soils of colonizer plants at an abandoned rock phosphate mine. *Plant and Soil,287,* 69-75. doi:10.1007/s11104-006-9061-z

Rodrigo, V. H. L., Stirling, C. M., Teklehaimanot, Z., Nugawela, A. (2001). Intercropping with banana to improve fractional interception and radiation-use efficiency of immature rubber plantations. *Field Crops Research, 69*, 237-249. doi:10.1016/S0378-4290(00)00147-7

Russell, E. W. (1973). *The individual nutrients needed by plants*. pp.31-48. In E.W. Russel (Ed.). Soil conditions and plant growth, 10th edition. Longman, London and New York.

Shamseldin, A., Sadowsky, M. J., El-Saadani, M., Chung, S. A. (2008). Molecular biodiversity and identification of free living *Rhizobium* strains from diverse Egyptian soils as assessed by direct isolation without trap hosts. *American-Eurasian Journal of Agricultural and Environmental Sciences, 4*, 541-549.

Shariati, J., Weisany, W., Torabian, S. (2015). Effect of azetobacter and arbescular mycorrhizal on growth of safflower (*Carthamus tinctorious* L.) at different irrigation regimes. *Electronic Journal of Polish Agricultural Universities, 18(4)*, #01.

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, *255*, 571-586. doi:10.1023/A:1026037216893

Xavier, L. J. C., Germida, J. J. (2003). Selective interactions between arbuscular mycorrhizal fungi and *Rhizobium leguminosarum* bv. *viceae* enhance pea yield and nutrition. *Biology and Fertility of Soils, 37*, 261-267.

Yasmin, F., Othman, R., Saad, M. S., Sijam, K. (2007). Screening for beneficial properties of Rhizobacteria isolated from sweet potato rhizosphere. *Journal of Biotechnology*, *6*, 49-52. doi:10.3923/biotech.2007.49.52

Zahir, Z. A., Arshad, A., Frankenberger, W. T. (2004). Plant growth promoting rhizobacteria: Application and prospectives in agriculture. *Advances in Agronomy, 81*, 97-168. doi:10.1016/S0065-2113(03)81003-9

Zhang, L., Van Der Werf, W., Zhang, S., Li, B., Spiertz, J. H. J. (2007). Growth, yield and quality of wheat and cotton in relay strip intercropping systems. *Field Crops Research,103*, 178-188. doi:10.1016/j.fcr.2007.06.002