

# ENDOMYCORRHIZAL INOCULATION EFFECT ON BEANS (*Phaseolus vulgaris* L.), OAT (*Avena sativa* L.), AND WHEAT (*Triticum aestivum* L.) GROWTH CULTIVATED IN TWO SOIL TYPES UNDER GREENHOUSE CONDITIONS

Abdul Khalil GARDEZI

Programa de Hidrociencias. Instituto de Recursos Naturales. Colegio de Postgraduados.  
Montecillo, Texcoco, Estado de Mexico. 56230, Mexico

Sergio R. MARQUEZ-BERBER

Departamento de Fitotecnia. Universidad Autónoma Chapingo  
Chapingo, Estado de México. 56230, Mexico

Benjamín FIGUEROA-SANDOVAL

Programa de Hidrociencias. Instituto de Recursos Naturales. Colegio de Postgraduados.  
Montecillo, Texcoco, Estado de Mexico. 56230, Mexico

Adolfo EXEBIO-GARCÍA

Programa de Hidrociencias. Instituto de Recursos Naturales. Colegio de Postgraduados.  
Montecillo, Texcoco, Estado de Mexico. 56230, Mexico

Ulises LARQUÉ-SAAVEDRA

Área de Estadística e Investigación de Operaciones. Universidad Autónoma Metropolitana-Azcapotzalco.  
Mexico, D.F. Mexico

and

Miguel ESCALONA-MAURICE

Programa de Desarrollo Rural. Colegio de Postgraduados.  
Montecillo, Texcoco, Estado de Mexico. 56230, Mexico

## ABSTRACT

The objective of this research was to evaluate the effect of inoculation with *Glomus intraradices* on oat, beans, and wheat. The study was done under greenhouse conditions at the Montecillo Campus of the Postgraduate College, Mexico. Two soil types from San Luis Potosí State were used, one was red (Xerosol), and the other one was grey (Litosol). With and without *Glomus intraradices* inoculation. Three bean cultivars: Pinto Saltillo, Bayo comercial and Flor de Mayo; Chihuahua (oat variety); and Tlaxcala wheat genotype were planted. The experimental design was factorial complete randomized block and three replications. The result showed that bean yield (average 3.7 g plant<sup>-1</sup>), pod number and dry weight, leaf area, plant height, stem diameter, and aerial part dry weight were positively affected by the inoculation with *Glomus intraradices*, but not by soil type. A similar trend was observed in root length, volume and dry weight, and in the nodule number. In relation with the species studied, *Phaseolus vulgaris* varieties had higher values than wheat and oats in growth and yield variables evaluated. It is concluded that endomycorrhiza inoculation (*Glomus intraradices*) gave better growth and yield, especially in beans. The soil types studied did not affect significantly plant responses in this study.

**Keywords:** *Glomus intraradices*, yield, nitrogen fixation, genotypes, and growth.

## 1. INTRODUCTION

Farmers are facing new challenges every day. Input prices are constantly increasing. Fertilizers are a good example of those increments [13]. Moreover, those chemicals cause widespread and sometimes irreversible environmental degradation. Sometimes the impoverished soils make them increasingly dependent on chemicals [4]. The society is demanding sustainable and affordable food production. It puts pressure on producers to maintain their cost of production as low as possible.

Endomycorrhizas are a low cost option to improve plant nutrition. Several researchers consider that they are the most important organisms on earth interacting in agro environments. More than 80% of all terrestrial plants, among them most of horticultural and crop plants have a symbiotic relationship with these fungi. The stimulation of plant growth can be attributed mainly to the improvement of phosphorus nutrition [1, 8, 9, 15].

Currently, these fungi are classified in the *Glomeromycota* Division, particularly in the *Glomerales* Order. There are about 200 species described [1].

Mycorrhizal (*Glomus mosseae*) inoculation increased root, shoot, and total dry weight, compared to the control in wild emmer (*Triticum turgidum* subsp. *dicoccoides*). This species may be used as a source of mycorrhizal dependency in wheat breeding [17].

Common bean is the most important legume crop in Mexico. Almost 2 million hectares were planted in 2010 in this country. Oats and wheat are key cereals with high planted area (About 800 thousand, and 700 thousand hectares respectively in 2010). They are cultivated in the Mexican central highlands and in the northwest.

The objective of this study was to investigate the effect of *Glomus intraradices*, an arbuscular mycorrhizal fungus, on growth and yield of beans, oats and wheat in two different soils under greenhouse conditions.

## 2. MATERIALS AND METHODS

The study was done under greenhouse conditions at the Postgraduate College, Montecillo Campus, State of Mexico, in the spring and summer of 2009. Two soil types were used. One red (xerosol) and another grey (litosol) with the characteristics shown on Table 1.

The seeds were sterilized with 1% sodium hypochlorite during 4 minutes, and hydrated on filter paper in petri dishes for 48 hours. The seeds were sown in polyethylene bags that had been filled with 2 kg of two soil types. The treatments were: planting in red soil or grey one. Both soil types were collected at the San Luis Potosi State, north Mexico. Grey soil is medium alkaline, the red one is neutral. Both have medium amounts of inorganic nitrogen and a low one of phosphorus. The contents of iron, manganese, and zinc are adequate. Grey soil is deficient in copper, but the red one has sufficient quantity [3].

The soil organic matter was determined using the Walkey and Black method, for phosphorus, Olsen was used. Interchangeable bases were measured utilizing ammonium acetate pH 7:1 Normal (CH<sub>3</sub>COONH<sub>4</sub>), and micronutrients with DTPA (from dietilen-triamino-pentaacetic acid).

**Table 1** Soil analysis for the two types.

Soil	SP	EC	pH	OM	N inorg	P	
		dS m <sup>-1</sup>	1:02	%	mg kg <sup>-1</sup>		
Xerosol (red)	28	0.54	8.3	1.78	35.27	0.85	
Litosol (grey)	34.7	0.65	7.07	2.05	28.86	1.07	
Soil	K	Ca	Mg	Fe	Cu	Mn	Zn
	mg kg <sup>-1</sup>						
Xerosol (red)	477.04	6413.24	110.0	12.33	0.11	19.18	1.90
Litosol (grey)	453.58	2249.79	209.6	21.96	2.81	47.96	2.28

Key: SP=Saturation point, EC=Electric conductivity, pH=Hydrogen potential, OM= Organic matter, N inorg= Inorganic nitrogen.

Three bean genotypes (Pinto Saltillo, Bayo comercial and Flor de Mayo), one for oats (Chihuahua), and one for wheat (Tlaxcala) were planted. The seed was provided by the

National Institute for Agriculture, Livestock and Forestry Research.

The inoculation was done during the planting, mixing 5 g of sand with sorghum roots with 85 % colonization of *Glomus intraradices* and 1050 spores per 100 g of inert material. Two levels of *Glomus* were applied, with and without *Glomus*.

The variables evaluated were plant height (PH, cm), stem diameter (SD, mm), biomass dry weight (BDW, g), grain dry weight (GDW, g), leaf area (LA, cm<sup>2</sup>), root length (RL, cm), root volume (RV cm<sup>3</sup>), root dry weight (RDW, g), pod number (PN), and nodule number (NN).

A factorial arrangement with 20 treatments (5x2x2) was used with a completely randomized block design using three replications. An analysis of variance for all variables registered was done and a Tukey mean comparison test for the significant variables.

## 3. RESULTS AND DISCUSSION

There are significant differences among treatments for all the variables recorded, due to the positive effect of inoculation with *Glomus intraradices*. Also highly significant differences were found among cultivars for all the variables studied. The soils used in this experiment did not provide any significant difference. Significant interactions were recorded between cultivars and inoculation with *Glomus intraradices* for plant height, grain dry weight and leaf area, and among cultivars and soils for grain dry weight (Table 2).

Yield, root and shoot growth were superior to those of plants without inoculation (Table 3). This is an indication of a positive effect of mycorrhiza on plant growth originated by better mineral nutrient absorption required by the plant [1, 2]. Gardezi *et al.* [6, 9] also found this beneficial effect in *Leucaena leucocephala* associated with endomycorrhizal and with *Rhizobium*.

Positive responses to the inoculation with mycorrhiza were also found in a number of species, including those studied in this experiment [16], and in beans [2].

For the studied variables, the behavior was similar to that found by Gardezi *et al.* [7, 8, 9, 10, and 11]. The plants inoculated with endomycorrhiza fungi (*Glomus intraradices*) were taller than those not inoculated.

**Table 2.** Analysis of variance mean squares of the variables and treatments studied in oat (*Avena sativa* L.), common bean (*Phaseolus vulgaris*) and wheat (*Triticum aestivum* L.).

Source of variation	DF	Plant height	Stem diameter	Biomass dry weight	Grain dry weight
Treatments	19	4750.1219**	0.0380**	27.0074**	30.1439**
Cultivars (C)	4	17690.5167**	0.1447**	86.3889**	125.7250**
Soils (S)	1	322.0167	0.0002	0.0327	3.2667
C*S	4	283.6833	0.0022	1.3156	6.0583**
Inoculation ( <i>Glomus</i> , I)	1	7063.3500**	0.0721**	86.4000**	17.0667**
C*I	4	1560.2667*	0.0078	7.3404	5.1083*
S*I	1	400.4167	0.0209	4.2667	0.2667
C*S*I	4	1082.1667	0.0027	10.5654	1.1417
ERROR	40	626.1333	0.0092	4.3912	1.4000

**Table 2.** Continuation

Source of variation	DF	Leaf area	Root length	Root volume	Root dry weight
Treatments	19	241198.3250**	112.1866**	25.9650**	0.5703*
Cultivars (C)	4	959932.9000**	296.0375**	88.5428**	1.7596**
Soils (S)	1	21470.4170	31.5375	2.2815	0.3271
C*S	4	16395.5000	21.7042	7.8786	0.2757
Inoculation (Glomus, I)	1	357744.8170**	392.7042**	32.4135**	1.0481*
C*I	4	51125.2330*	49.2458	7.7281	0.2611
S*I	1	936.1500	7.7042	7.2802	0.0770
C*S*I	4	23200.5670	57.9125	8.6906	0.0494
ERROR	40	18116.2500	24.6375	3.8043	0.2540

\* Significant at 5% level of the treatment, main effect or interaction.

\*\* Significant at 1% level of the treatment, main effect or interaction.

**Table 3.** Honest significant difference of the effect of *Glomus intraradices* on oat (*Avena sativa* L.), common bean (*Phaseolus vulgaris*) and wheat (*Triticum aestivum* L.).

<i>Glomus intraradices</i>	Plant height (cm)	Stem diameter (cm)	Biomass dry weight (g)	Grain dry weight (g)	Leaf area (cm <sup>2</sup> )	Pod number
Inoculated	100,50a	0,34a	6,94a	4,10a	566,5a	1,25a
Non inoculated	78,80b	0,27b	4,54b	3,03b	412,07b	1,06b

<i>Glomus intraradices</i>	Pod dry weight (g)	Root length (cm)	Root volume (cm <sup>3</sup> )	Root dry weight (g)	Nodule number
Inoculated	0,97a	27,17a	5,89a	1,12a	1,97a
Non inoculated	0,61b	22,05b	4,42b	0,86b	1,64b

Means with the same letter in each column are not significantly different (Tukey  $\alpha = 0.05$ ).

A better vegetative growth of oat, bean and wheat plants inoculated with *Glomus intraradices* was the result of an enhanced root growth ( $p \leq 0.05$ ) expressed as greater root length (23.2%<sup>1</sup>), greater root volume (33.3%), and higher root dry weight (30.2%); as well as a larger photosynthetic apparatus ( $p \leq 0.05$ ), expressed in biomass dry weight (52.8%) and leaf area (37.5%).

Plants were taller with inoculation (27.5%), and with a thicker stem diameter (25.9%, Table 3)

Bean dry grain yield was also higher (35.3%) in plants inoculated with *Glomus intraradices*. This was a result of a higher number of pods per plant (18%), and especially heavier pods (59%). The increase in pod number and pod weight can be attributed to better root and shoot growth that provided a greater quantity of photosynthetic compounds and mineral nutrients, as well as a better nitrogen fixation because the number of nodules increased 20% in plants inoculated with endomycorrhizae.

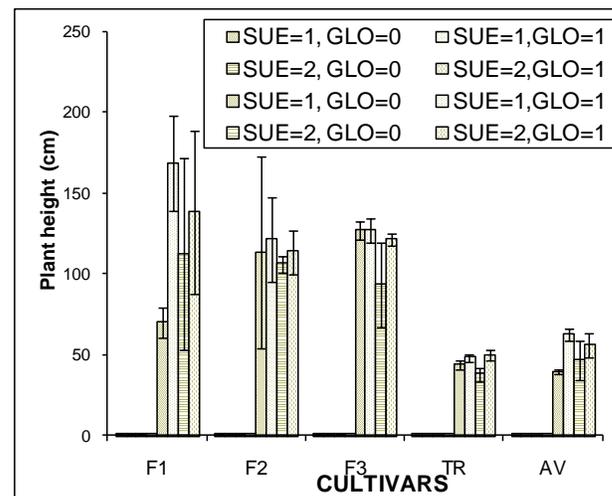
<sup>1</sup> Increase rate are referred to the values found in oat, bean and wheat plants inoculated with mycorrhiza compared to those without inoculation.

In other experiments, *Glomus intraradices* has increased the yields. In most of the locations where trials were conducted, there was an increase in yields in comparison with the chemically fertilized control, of up to 60% in maize, 85% in wheat, 25% in oats, 74% in barley, 36% in common bean and 111% in orange trees. It is convenient to promote the transfer of this technology to farmers for obtaining higher yields and for the development of a more sustainable agriculture [14].

The beneficial effect of mycorrhiza was also documented in wheat [17] originating greater growth and yield. Legumes had the same positive results, especially when they combine mycorrhiza and bacteria fixing nitrogen [7, 8, 9, 10, and 11].

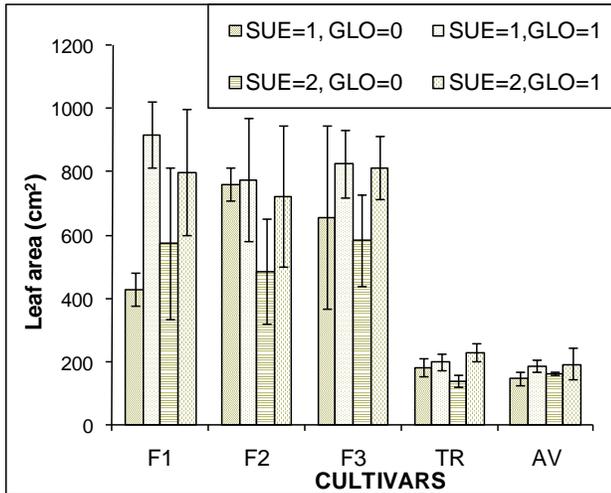
The varieties of bean do not show differences in plant height at harvest (Figure 1,  $p \leq 0.05$ ). The Pinto Saltillo bean cultivar inoculated with *Glomus* showed the greater plant height. The lowest one corresponded to wheat and oats without inoculation. Both oats and wheat responded less to the inoculation with *Glomus intraradices* and to litosol soil type.

The diameter of the stem also did not show significant differences among bean varieties, with major values in Pinto Saltillo bean cultivar and lesser diameter in wheat and oats without inoculation ( $p \leq 0.05$ ).



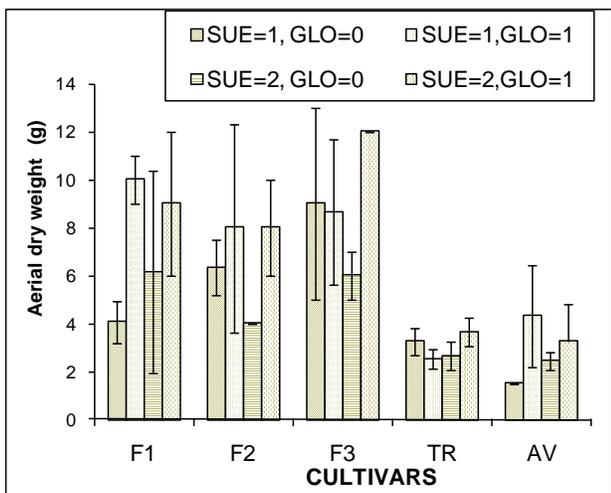
**Figure 1.** Effect of inoculation with *Glomus intraradices* on two soil types in plant height of three cultivars common bean (*Phaseolus vulgaris*), one of oat (*Avena sativa* L.), and another one of wheat (*Triticum aestivum* L.).

Key: Cultivars: F1: Pinto Saltillo bean, F2: Commercial Bayo bean, Flor de Mayo bean, TR: wheat, AV: oat. Soil type: Sue1= Litosol, Sue2= Xerosol. Glo0= Noninoculated, Glo1= Inoculated with *Glomus intraradices*. The vertical lines indicate standard error.

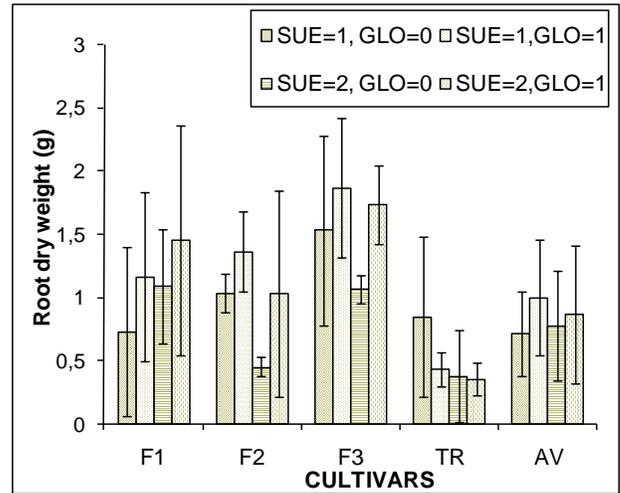


**Figure 2.** Effect of inoculation with *Glomus intraradices* on three cultivars in two soil types on leaf area of three cultivars common bean (*Phaseolus vulgaris*), one of oat (*Avena sativa* L.), and another one of wheat (*Triticum aestivum* L.).  
Key: Cultivars: F1: Pinto Saltillo bean, F2: Commercial Bayo bean, F3: Flor de Mayo bean, TR: wheat, AV: oat. Soil type: Sue1= Litosol, Sue2= Xerosol. Glo0= Noninoculated, Glo1= Inoculated with *Glomus intraradices*. The vertical lines indicate standard error.

The three varieties of bean developed a leaf area with significant differences ( $p \leq 0.05$ ). The greater leaf area (917 cm<sup>2</sup>) occurred in Pinto Saltillo bean cultivar grown in litosol inoculated with *Glomus intraradices*, followed by Flor de Mayo in both soil types and inoculated. Also a significant interaction was observed between the bean cultivars, oats, and wheat and the inoculation with *Glomus intraradices* ( $p \leq 0.05$ ). The lowest leaf area corresponded to wheat and oats (Figure 2,  $p \leq 0.05$ ).



**Figure 3.** Effect of inoculation with *Glomus intraradices* on three cultivars in two soil types on aerial dry weight of three cultivars common bean (*Phaseolus vulgaris*), one of oat (*Avena sativa* L.), and another one of wheat (*Triticum aestivum* L.).  
Key: Cultivars: F1: Pinto Saltillo bean, F2: Commercial Bayo bean, F3: Flor de Mayo bean, TR: wheat, AV: oat. Soil type: Sue1= Litosol, Sue2= Xerosol. Glo0= Noninoculated, Glo1= Inoculated with *Glomus intraradices*. The vertical lines indicate standard error.



**Figure 4.** Effect of inoculation with *Glomus intraradices* on three cultivars in two soil types on root dry weight of three cultivars common bean (*Phaseolus vulgaris*), one of oat (*Avena sativa* L.), and another one of wheat (*Triticum aestivum* L.).  
Key: Cultivars: F1: Pinto Saltillo bean, F2: Commercial Bayo bean, F3: Flor de Mayo bean, TR: wheat, AV: oat. Soil type: Sue1= Litosol, Sue2= Xerosol. Glo0= Noninoculated, Glo1= Inoculated with *Glomus intraradices*. The vertical lines indicate standard error.

In the case of the root system, the length, volume, and dry weight had similar development in the bean cultivars. The same happened between oats and wheat (Figure 4).

For the dry weight of the aerial part it was not found significant differences between varieties of bean (Figure 3,  $p \leq 0.05$ ). The Flor de Mayo and Pinto Saltillo bean cultivars had the highest weight. Oats without inoculation in both soil types produced the lowest foliage.

Flor de Mayo bean cultivar had more nodules than Pinto Saltillo, but similar to Bayo Comercial. The Flor de Mayo bean cultivar in both types of soils and inoculated with *Glomus intraradices* presented the greatest number of nodules, followed by Bayo Comercial in xerosol without inoculation. Gardezi et al. [5] found a great genetic diversity in nitrogen fixation of 48 varieties of bean with six types of growth habits.

The number and weight of pods were similar among the varieties of beans. The Bayo cultivars of bean had a grain yield average greater than Pinto Saltillo, but similar to Flor de Mayo ( $p \leq 0.05$ ). The treatments with a higher yield were Bayo and Flor de Mayo planted in xerosol and inoculated with *Glomus intraradices*. The yields of oats and wheat could not be evaluated.

The inoculation with *Glomus intraradices* improved root and shoot growth and also had a beneficial effect on the biological nitrogen fixation, possible joint with a higher absorption of nutrients [12], contributing to higher yield in beans, coinciding with other studies [2].

#### 4. CONCLUSIONS

Mycorrhizal inoculation and nitrogen fixation provided higher bean root and shoot growth and therefore better yields. Previous evidence with legumes showed that they have

benefited with this symbiosis because the treatments with this fungus produces the highest values for all evaluated variables. In oats and wheat, inoculation with *Glomus intraradices* leads also to superior root and shoot growth. The soil types did not limited the effect of mycorrhiza on beans, oats and wheat.

## 5. REFERENCES

- [1] Alarcón, A. 2008. Los hongos micorrízicos arbusculares como biotecnología en la propagación y manejo de plantas en viveros. **Agroproductividad** 1(1): 19-23
- [2] Aryal, U. K., H. L. Xu, and M. Fujita. 2003. Rhizobia and AM fungal inoculation improve growth and nutrient uptake of bean plants under organic fertilization. **J Sustainable Agric.** 21(3): 27-39.
- [3] Castellanos, J. Z., J. X. Uvalle B. y A. Aguilar S. 2000. **Manual de interpretación de suelos y aguas.** Colección INCAPA. México. 226 p.
- [4] Clermont-Dauphin, C. and Meynard, J. M. 1997. L'emploi des pesticides et des engrais en Agriculture. Pages 175–186 in P. Stengel and S. Gelin, eds. **Sols: interfaces fragiles.** INRA, Paris.
- [5] Gardezi, A.K., R. Ferrera-Cerrato, J. Kohashi, E.M. Engleman y M. Larqué-Saavedra. 1990. Potencial diferentes variedades de *Phaseolus vulgaris* de alta eficiencia en la fijación de nitrógeno en asociación con *Rhizobium leguminosarum* Biovar *Phaseoli*. **Agrociencia** 1(4): 25-39.
- [6] Gardezi, A. K., I. D. Barceló-Quintal, V.M. Cetina-Alcalá, A. L. Bussy, M. U. Larqué-Saavedra, E. M. Saenz, A. Exebio-García, J. Pérez-Nieto, and M. A. Borja-Salin. 2005. Phytoremediation by *Leucaena leucocephala* in association with arbuscular endomycorrhiza and *Rhizobium* in soil polluted by Cr. In Callaos *et al.* **Proceedings of the 9<sup>th</sup> World Multiconference on Systemics, Cybernetics and Informatics.** Orlando, Florida. U.S.A. VII: 289-298.
- [7] Gardezi, A. K., E. Ojeda-Trejo, E. Mejia-Saenz, A. Exebio-García, U. Larque-Saavedra, S. Márquez-Berber, y V.M. Cetina –Alcalá. 2007. Effect of arbuscular endomycorrhizas associated with cow manure in *Leucaena leucocephala*. In Callaos *et al.* **Proceedings of the 11<sup>th</sup> World Multiconference on Systemics, Cybernetics and Informatics.** Orlando, Florida. U.S.A. IV: 113-118.
- [8] Gardezi, A. K., A. Exebio-García, E. Ojeda-Trejo, E. Mejia-Saenz, L. Tijerina-Chavez, M. Delgadillo-Piñon, U. Larqué-Saavedra, C. Villanueva-Verduzco, and H. Gardezi. 2008a. Growth response of *Leucaena leucocephala* associated with arbuscular endomycorrhizae to applications of organic matter in an irrigated soil with sewage water. In Callaos *et al.* **Proceedings of the 12<sup>th</sup> World Multiconference on Systemics, Cybernetics and Informatics.** Orlando, Florida. U.S.A. IV: 153-158.
- [9] Gardezi, A. K., E. Ojeda-Trejo, and S. R. Márquez-Berber. 2008b. Respuesta a la inoculación de *Glomus intraradix*, materia orgánica y dosis de fertilización fosfatada en el crecimiento de mezquite (*Prosopis* sp.). **Agroproductividad** 1(1): 24-28.
- [10] Gardezi, A. K., I. D. Barceló, A. Exebio-García, E. Mejía-Saenz, U. Larqué-Saavedra, S. R. Márquez-Berber, C. Villanueva-Verduzco, H. Gardezi, and D. Talavera-Magaña. 2009. Cu<sup>2+</sup> bioaccumulation by *Leucaena leucocephala* in symbiosis with *Glomus* spp. and *Rhizobium* in copper-containing soil. In Callaos *et al.* **Proceedings of the 13<sup>th</sup> World Multiconference on Systemics, Cybernetics and Informatics.** Orlando, Florida. U.S.A. II: 17-22.
- [11] Gardezi, A. K., B. Figueroa-Sandoval, A. Exebio-García, S. R. Márquez-Berber, M. U. Larqué-Saavedra, E. Mejía-Saenz, D. Talavera-Magaña, M. E. Delgadillo-Piñon, C. Villanueva-Verduzco, and H. Gardezi. 2010. Effect of *Glomus intraradices* associated with different genotypes of *Phaseolus vulgaris* (common bean) in two soil types. In Callaos *et al.* **Proceedings of the 14<sup>th</sup> World Multiconference on Systemics, Cybernetics and Informatics.** Orlando, Florida. U.S.A. II:5-10.
- [12] George, E.; V. Romheld y H. Marschner. 1994. Contribution of mycorrhizal fungi to micronutrient uptake by plants. In J.A. Manthey, D.E. Crowley y D.G. Lewis (eds.). **Biochemistry of metal micronutrients in the rhizosphere.** London. pp. 93-109.
- [13] Huang, W. 2009. Factors Contributing to the Recent Increase in U.S. Fertilizer Prices, 2002-08. **Outlook AR-33.** A Report from the Economic Research Service. USDA. <http://www.ers.usda.gov/Publications/AR33/AR33.pdf>
- [14] Irizar Garza, M. B.; Vargas Vázquez, P.; Garza García, D.; Tut y Couoh, C.; Rojas Martínez, I.; Trujillo Campos, A.; García Silva, R.; Aguirre Montoya, D.; Martínez González, J. C.; Alvarado Mendoza, S.; Grageda Cabrera, O.; Valero Garza, J.; Aguirre Medina, J. F. 2003. Respuesta de cultivos agrícolas a los biofertilizantes en la Región Central de México. **Agricultura Técnica en México** Vol. 29 No. 2 pp. 213-225.
- [15] Plenchette, C., C. Clermont-Dauphin, J. M. Meynard, and J. A. Fortin. 2005. Managing arbuscular mycorrhizal fungi in cropping systems. **Can J Plant Sci.** 85:31-40.
- [16] Tawaraya, K. 2003. Arbuscular mycorrhizal dependency of different plant species and cultivars. **Soil Science and Plant Nutrition** 49 (5): 655-668.
- [17] Yücel, C.; Özkan, H.; Ortaş, İ.; Yağbasanlar, T. 2009. Screening of wild emmer wheat accessions (*Triticum turgidum* subsp. *dicoccoides*) for mycorrhizal dependency. **Turkish Journal of Agriculture and Forestry** 33 (5): 513-523.