EFFECT OF *Glomus intraradices* ASSOCIATED WITH DIFFERENT GENOTYPES OF *Phaseolus vulgaris* (COMMON BEAN) IN TWO SOIL TYPES

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SUMMARY

The objective of this research was to evaluate three bean seed varieties (*Phaseolus vulgaris*) Bayo Mecentral, Flor de Durazno and Bayomex. Sewage water is used for irrigation, which creates problems and opportunities. It is an option to reduce stress on limited fresh water and help to meet the nutrient requirement of crop, but also produces contamination. In the Irrigation District 028, Tulancingo, Hidalgo, Mexico, forage for cattle has been irrigated with residual water for several years. To evaluate the level of contamination of two plots, one hectare was irrigated with sewage water and another with underground water. Soil was collected for a greenhouse experiment with different genotypes of common beans (*Phaseolus vulgaris*) to observe its growth, inoculating their seed with *Glomus intraradices* in two soil types. The experiment used a completely randomized block design in a factorial arrangement (3x2x2). In soil the contamination of Cu, Cr, and Ni are bellow the established limits for contaminants. Pb was not found. A comparison of soil irrigated with clean water or sewage water indicated that Cu is nearly twice as concentrated in soil irrigated by contaminated water; Ni is slightly greater, Cr is more abundant. In all the registered variables, the plant inoculated with *Glomus intraradices* showed significantly greater values ($p \leq 0.01$) than the plants without inoculation. Irrigation with residual water generally had no significant effect, even when there were higher numerical values in growth and in yield of the bean plants, except for the stem diameter, with significant differences ($p \leq 0.05$).

Key words: Edible legumes, Arbuscular endomycorrhiza, Soil contamination, Residual water.

INTRODUCTION

Beans are the most important edible legume in Mexico; they were planted in 2008 on 1.6 million hectares, producing almost 1.1 million tons (Table 1). The grain is a key source of protein [19] and its consumption complements the profile of essential amino acids of maize. In Hidalgo its cultivation is primordial, since it occupied the second place in importance, after corn during 2008. More than 45 thousand hectares were planted mainly under rainfall conditions during spring and summer. There was a production of 31,294 tons with an average yield of 729 kg ha^{-1} (Table 1).

Table 1. Area, production, and yield of beans in Mexico and state of Hidalgo during 2008.

REGION	Area (ha)			Production (ton)	Yield (ton ha^{-1})
	planted	harvested	lost	harvested	harvested
HIDALGO Irrigated AW 08	630	630		1,249	1.983
Mexico Irrigated AW 08	**121,468**	**116,693**	**4,775**	**188,779**	**1.618**
HIDALGO Rainfall AW 08	4,68	4,68		3,153	0.674
Mexico Rainfall AW 08	**137,996**	**135,026**	**2,97**	**118,733**	**0.879**
HIDALGO Rainfall SS 08	33,428	31,011	2,417	12,479	0.402
Mexico Rainfall SS 08	**1,291,189**	**1,178,670**	**112,519**	**685,59**	**0.582**
HIDALGO Irrigated SS 08	6,616	6,606	10	14,414	2.182
Mexico Irrigated SS 08	**80,349**	**75,303**	**5,045**	**129,622**	**1.721**
HIDALGO Total	45,354	42,926	2,427	31,294	0.729
MEXICO TOTAL	**1,631,002**	**1,505,693**	**125,309**	**1,122,724**	**0.746**

Key: AW= Autumn-Winter Season, SS= Spring-Summer Season.
Source: SIAP, 2009.

Marked differences in bean yields obtained under dissimilar conditions are found at the national level and in the State of Hidalgo. The yield under irrigation during the spring-summer season is about three times of those under rainfall conditions (Table 1).

A great genetic diversity is found in common beans with different growth habits and nitrogen fixing capacity [5]. These results are very important because of the high costs of nitrogen fertilizers [12].

Another option to increase the nutrition of plants is inoculation with endomycorrhiza that some people consider as the most important terrestrial organisms in the interaction with agroecosystems. Most of the cultivated plants have a symbiotic association with these fungi that are involved in mineral nutrition, pathogen control and drought tolerance. The growth stimulation promotes an increase in phosphorus nutrition. [17].

Guzmán and Ferrera [11] reported that endomycorrhiza belong to the Zygomycetes from the Endogonaceae family, *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystic* and *Scutellospora* genus. They are characterized by the production of hyphae, vesicles and arbuscules in the root parenchyma.

Glomus intraradices arbuscular endomycorrhiza fungi offer potential benefits for host plants. It develops in root systems in all soil types. It is used for biofertilizations as inoculants [8].

These fungi play a fundamental role in crops and natural ecosystems nutrient absorption, related to the ability to influence mineral nutrition, specially the nutrients of low mobility such as P, Zn, and Cu are important for plant development [10].

Glomus intraradices has augmented the aerial and root growth of *Leucaena leucocephala*; both in noncontaminated soils [7, 8, 9], and in contaminated soils [8], suggesting its possible use in bioremediation of heavy metals in such a soils [7].

In beans mycorrhizas can increase growth and yield in low P availability soils, specially in the presence of *Glomus mosseae* [14]. The formation of the cited fungi is in function of P soil concentration, the host development stage, and the nitrogen relative quantity, because fertilization with this element can retard the colonization of mycorrhiza [20]. If the mycorrhiza present in the soil are efficient, there can be a lack of response to the mycorrhizal inoculation [18].

The double inoculation of *Rhizobium,* and of endomycorrhiza (*Glomus* spp.) increases both bean growth and yield, especially when organic fertilizer is used [1]. Bermúdez *et al*. [2] found an increase dry matter production of beans associated with *Glomus manihotis* fertililized with phosphate rock, and an increase in N, P, Ca, K, Mg, Mn, Fe, Zn, and Cu absorption.

The utilization of *Glomus intraradices* as a bio fertilizer has shown increases of 22.1% in bean yields, which is superior to that found in barley (20.7%), corn (11.5%), and sorghum (10.8%) in validation trials [13]. Novella *et al*. [16] also have reported that beans and corn yield increase when inoculated with a combination of *Rhizobium* and mycorrhiza.

MATERIALS AND METHODS:

Two plots of one hectare each, with pasture, were located in the 028 Irrigation District, Tulancingo, Hidalgo, Mexico; one was irrigated with residual water and the other with clean water. Twenty soil samples were taken in 25 x 20 m plots at three depths (0-5, 5-10 y 10-40 cm). For the soil analysis, the 20 samples were homogenized to obtain 3 samples for each plot. The rest of the soil (about 500 kg) was prepared for the greenhouse experiment.

The study lasted 110 days from planting to harvest and was conducted under greenhouse conditions in the Colegio de Postgraduados, Montecillo Campus, State of Mexico, in the spring of 2008.

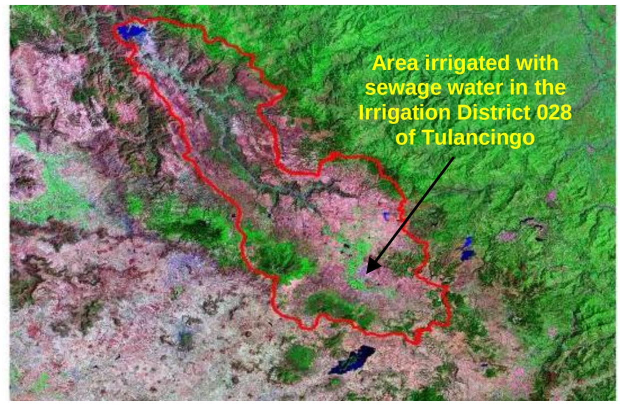

Figure 1. Tulancingo Region, Hidalgo State, Irrigation District 028.

Three bean genotypes were planted. The seed was provided by the National Institute for Agriculture, Livestock and Forestry Research. Bayo Mecentral is a semi-indeterminate variety of an intermediate cycle (110-115 days) with high yield potential, resistant to anthracnose, and rust tolerant. Flor de Durazno and Bayomex are determinate varieties of shorter cycle (95 to 105 days) and lower yield potential, but they are recommended for irrigated conditions. They are resistant to anthracnose and rust, and tolerant to bacterial diseases [4].

The seeds were sterilized with sodium hypochlorite and hydrated on filter paper in petri dishes. The seeds were sown in polyethylene bags that had been filled with 3 kg of two soil types with the following characteristics. The average characteristics of the soil irrigated with residual water were: texture clay loam, pH 8.6, electric conductivity (EC) 1.9 Dms^{-1}, organic matter (OM) 3.3, total nitrogen (TN) 0.14 %, phosphorus (P) 170 $mg*kg^{-1}$, copper (Cu) 0.6896 $mg*kg^{-1}$, chromium (Cr) 0.0248 $mg*kg^{-1}$, lead (Pb) 0.0 $mg*kg^{-1}$, nickel (Ni) 0.1014 $mg*kg^{-1}$. For the soil irrigated with clean water they were as follows: Texture clay loam, pH 7.7, EC 0.6 Dms^{-1}, OM 2.0, TN 0.05 %, P 30 $mg*kg^{-1}$, Cu 0.3592 $mg*kg^{-1}$, Cr 0.0316 $mg*kg^{-1}$, Pb 0.0 $mg*kg^{-1}$, Ni 0.0828 $mg*kg^{-1}$.

The inoculation was done 15 days after planting, mixing 5 g of sand with alfalfa roots with 80 % colonization of *Glomus intraradices*. 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^2), root length (RL, cm), root volume (RV cm^3), root dry weight (RDW, g), pod number (PN), and nodule number (NN).
A factorial arrangement with 12 treatments (3x2x2) was used with a completely randomized block design using four replications. An analysis of variance for all variables registered was done and a Tukey mean comparison test for the significant variables.

RESULTS AND DISCUSSION:

The soils are shallow (40 cm) underlain by tepetate (fragipan), the soil irrigated with residual water is saturated with water most of the time.

Table 2 Soil analysis for the three depths (0-5, 5-10, 10-40 cm)

Soil sample	pH 1:2	EC (dms^{-1}) 1:2	O.M. % Walkley Black	TN %	P mg/kg	Cu ppm	Cr ppm	Pb ppm	Ni ppm
1.- Soil depth 0-5 Residual water	8.6	1.80	4.5	0.14	170	0.6896	0.0248	00	0.1014
2.- Soil depth 5-10 Residual water	8.7	1.90	3.3	0.10	155	0.5103	0.0223	00	0.994
3.- Soil depth 10-40 Residual water	8.4	1.98	2.2	0.07	140	0.4734	0.0257	00	0.0958
4.- Soil depth 0-5 Clean water	7.7	0.60	2.0	0.05	30	0.3592	0.0316	00	0.0828
5.- Soil depth 5-10 Clean water	7.9	0.46	2.0	0.06	20	0.3811	0.0369	00	0.0793
6.- Soil depth 10-40 Clean water	8.0	0.50	1.63	0.06	20	0.3644	0.0265	00	0.0766

Table 2 shows the results of the soil sample analysis for the three mixed samples for each depth for each plot (residual and clean water). The soil texture for the soil irrigated with residual water is clay loam in the first five centimeter and clay to the 40 cm. The soil irrigated with clean water is clay in all the profile [3]. The chemical characteristics of the soil (pH, EC, OM, TN and P) have higher values in the soil irrigated with residual water. The pH is alkaline in both plots; however, it increases with depth in both soils, except for sample 3. The EC increased in the plot with residual water (1.98 DMS^{-1}) and increases with depth, this contrasts with the plot irrigated with residual water where the EC decrease with depth. The TN is greater in the irrigated soil with residual water at all depths with differences up to 0.09%, with the principal accumulation in the upper horizon for irrigation with residual water. The OM is up to 2.87 % greater than in residual water, the principal accumulation is in the upper layer, the horizon of 10-40 cm has levels similar to those of clean soils. With respect to phosphorus there is a noticeable difference among the two soils, up to 6.5 times greater. The amount of phosphorus is from 140 to 170 mg Kg^{-1} and soil irrigated with residual water; in contrast the soil irrigated with clean water had only 20-30 mg Kg^{-1}

In general there is no contamination of heavy metals, but the soil analysis shows the presence of Cu, Cr and Ni in low concentration. Pb is absent in the soil. The levels of the soils irrigated with residual water, showed differences between residual and clean water, Cu is almost the double, Ni is slightly higher, but Cr is more abundant in soils irrigated with clean water. The metals have a higher concentration in the upper five centimeters and less with depth.

With respect to the characteristics of the soil, there is an increase in pH, EC, TN, % OM and P (table 2) which would probably continue increasing with time. The farmer actually considers that irrigation with residual water is beneficial because of the elevated yields of pasture. However, with time the increase of organic matter and pH could have toxic effects on grassland due to high pH and inadequate balance of nutrients.

Table 3. Analysis of variance mean squares of the variables and treatments studied in common bean (*Phaseolus vulgaris*).

Source of variation	Degrees of freedom	Plant height		Stem diameter		Biomass Dry weigth		Grain Dry weigth		Leaf Area	
Treatments	11	3095,3693	**	0,0565	**	8,1155	**	15,9198	**	118049,7480	**
Variety	2	2594,2500	**	0,0067		1,0019		1,2224		30294,3960	*
Soil	1	0,0208		0,0188	*	0,7008		5,7546		792,1880	
Var*Soil	2	377,3333		0,0057		0,8902		2,4711		23992,5630	
Glomus	1	23986,0208	**	0,5526	**	80,0833	**	151,7985	**	1119046,6880	**
Var*Glo	2	1871,5833	**	0,0069		2,2152		1,6970		21497,3130	
Soil*Glo	1	247,5208		0,0063		0,0208		2,0750		4900,5210	
Var*Soil*Glo	2	64,5833		0,0026		0,1252		2,3548		11119,6460	
Error	36	268,7986		0,0044		0,7533		1,9740		8650,8820	
Variation coeficient		18,1036		16,0087		17,9886		29,9941		19,9744	

Table 3. Continuation

Source of variation	Degrees of freedom	Root lenght		Root volume		Root Dry weigth		Pod number		Nodule number	
Treatments	11	207,8693	**	30,5620	**	0,1474		0,4001	**	2,0567	**
Variety	2	10,5625		1,4740		0,0158		0,2816		2,7320	**
Soil	1	4,6875		1,8802		0,0204		0,0023		0,0346	
Var*Soil	2	86,3125		2,9740		0,0376		0,0626		0,2538	
Glomus	1	1837,6875	**	302,5052	**	0,8086	**	2,9623	**	12,8498	**
Var*Glo	2	104,6875		6,5990		0,0457		0,2628		1,3275	
Soil*Glo	1	0,1875		1,1719		0,2283		0,1562		0,1773	
Var*Soil*Glo	2	20,4375		4,2656		0,1843		0,0334		0,4677	
Error	36	41,9514		3,0747		0,0801		0,1022		0,4724	
Variation coeficient		20,8515		23,8095		47,6132		17,8395		22,7121	

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

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

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

Plants inoculated with *Glomus intraradices* showed significantly higher values than noninoculated, in all registered variables ($p \leq 0.05$). 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 lecocephala* associated with endomycorrhizal and with *Rhizobium.*

Table 4. Effect of soils irrigated with clean and residual water on shoot and root growth, and yield of common beans (*Phaseolus vulgaris*).

Soil	Plant height (cm)	Stem diameter (cm)	Dry weight aerial part (g)	Leaf area (cm2)	Grain (g)
Contaminated	90,58a	0,44a	4,95a	469,7a	6,46a
Clean	90,54a	0,39b	4,70b	461,6a	4,33a

Soil	Root length (cm)	Root volume (cm3)	Dry weight root (g)	Pod number (ln)	Nodule number (ln)
Contaminated	31,38a	7,56a	0,62a	1,79a	3,05a
Clean	30,75a	7,16a	0,57a	1,78a	2,99a

Means with the same letter in each column are not significantly different (Tukey α= 0.05)

A better vegetative growth of bean plants inoculated with *Glomus intraradices* was the result of an enhanced root growth ($p \leq 0.05$) expressed as greater root length (150%[1]), greater root volume (204%), and higher root dry weight (157%); as well as a larger photosynthetic apparatus ($p \leq 0.05$), expressed in dry weight of aerial parts (173%) and leaf area (198%, Table 5).

Table 5. Honest significant difference of the effect of Glomus intraradices of the factorial experiment in three bean varieties (*Phaseolus vulgaris*) at 110 days after planting in ten variables evaluated.

Glomus intraradices	Plant height (cm)	Stem diameter (cm)	Dry weigth aerial part (g)	Leaf area (cm2)	Grain dry weight (g)
Inoculated	112,9a	0,52a	6,12a	618,3a	6,46a
No inoculated	68,2b	0,31b	3,53b	312,7b	2,90b

Glomus intraradices	Root lenght (cm)	Root volume (cm3)	Dry weigth root (g)	Pod number (ln)	Nodule number (ln)
Inoculated	37,3a	9,88a	0,72a	2,04a	3,54a
No inoculated	24,9b	4,85b	0,46b	1,54b	2,51b

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

The height of bean plants (*Phaseolus vulgaris*) showed clearly the beneficial effects of *Glomus intraradices* in the two soil types used. Bayo Mecentral (indeterminate type) had the largest shoots when inoculated with endomycorrhiza fungi regardless soil contamination. A similar trend was found in the two other varieties (Figure 2). Previous research found the same behavior in other species of legumes, *Glomus intraradices* played a key role promoting vegetative growth and heavy metals biological control [6, 9].

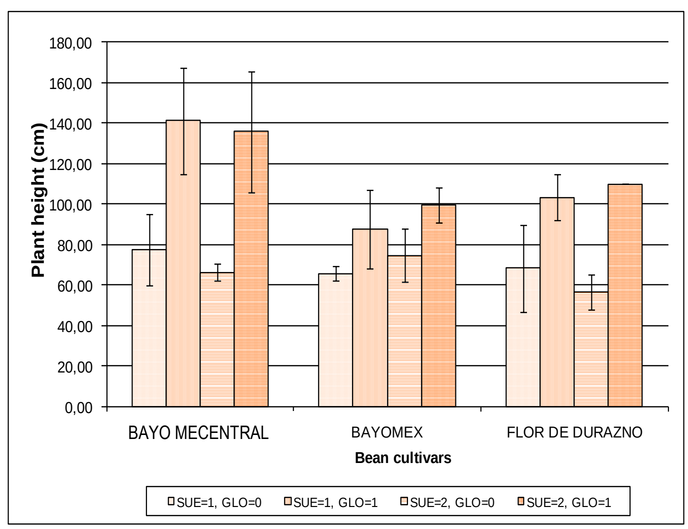

Figure 2. Effect of inoculation with *Glomus intraradices* on three cultivars in two soil types in plant height of common bean (*Phaseolus vulgaris*).

Soil type: Sue1= Contaminated soil, Sue2= Clean soil. Glo0= Noninoculated, Glo1= Inoculated with *Glomus intraradices.* The vertical lines indicate standard error.

[1] Percentages are referred to the values found in bean plants inoculated with mycorrhiza related to those without inoculation.

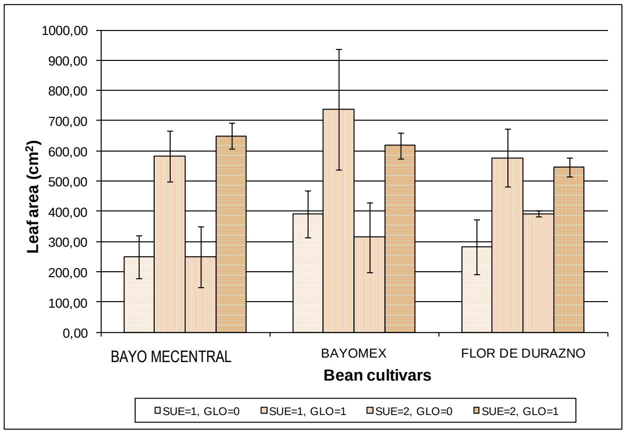

Figure 3. Effect of inoculation with *Glomus intraradices* on three cultivars in two soil types on leaf area of common bean (*Phaseolus vulgaris*).
Soil type: Sue1= Contaminated soil, Sue2= Clean soil. Glo0= Noninoculated, Glo1= Inoculated with *Glomus intraradices.* The vertical lines indicate standard error.

In a similar manner to that found by Irizar *et al*. [13] the inoculation with mycorrhiza increased the yield of grain, that can be related with a better nitrogen fixation, since the inoculation with mycorrhiza produce more nodules, explained by a greater development of the photosynthetic apparatus, that can transfer additional carbohydrate to nitrogen fixing bacteria, that come from soil native strains. It is known that the combination of both symbioses is beneficial for yield [15, 16].

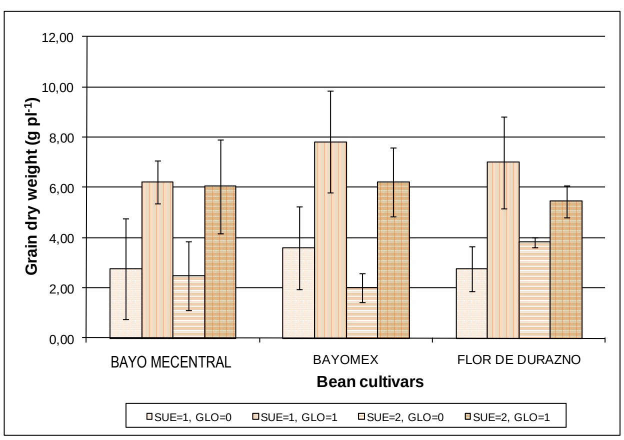

Figure 4. Effect of inoculation with *Glomus intraradices* on three cultivars in two soil types on grain dry weight of common bean (*Phaseolus vulgaris*).
Soil type: Sue1= Contaminated soil, Sue2= Clean soil. Glo0= Noninoculated, Glo1= Inoculated with *Glomus intraradices.* The vertical lines indicate standard error.

Leaf area was also higher in plants inoculated with *Glomus intraradices.* Bayomex had the maximum leaf area, but Bayo Mecentral presented the largest increase in leaf area (247%[2]) due to inoculation. The minimum increase was found in Flor de Durazno (167%).
Grain yield (dry weight, Figure 4) can be explained by leaf area, because they follow the same pattern and higher photosynthetic surface is related with superior dry matter accumulation. Bayomex as a commercial variety had the highest yield when inoculated with mycorrhiza, especially on contaminated soils, that have more organic matter and nutrients. [1] found a similar relationship. Bayo Mecentral had the highest yield increment due to mycorrhizal inoculation.

CONCLUSIONS

Mycorrhizal inoculation provides higher bean growth and yield. Irrigation with contaminated water also has a positive but no significant effect. 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 general terms, irrigation with residual waters is beneficial because they have high yields. The top soil horizon (0-5 cm) of these soils has higher organic matter, nitrogen, phosphorus and copper content. However, in the long run, accumulation of organic matter, nutrients and heavy metals may cause toxic effects.

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