Article

Inoculation of forage oats with arbuscular mycorrhizal fungi

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Abstract

Soil deterioration has limited the yield and production of forage species. Arbuscular mycorrhizal fungi (AMF) aid in the effective absorption of minerals from the soil. The objective was to evaluate the growth relationship of forage oats with the inoculation of arbuscular mycorrhizal fungi. The experiment was carried out in a greenhouse of the Technological Institute of the Valley of Oaxaca, a soil sampling and a pedological analysis were carried out. The substrate was a mixture of soil and sand (2:1), autoclaved, in two stages. Two commercial inoculants were used, to which a direct spore count was performed. The variety of oats used was Chihuahua. Inoculation was carried out by direct sowing, the dose was 5 g by inoculating an oat seed in individual pots. A completely randomized design with 11 treatments and four repetitions was used. The variables studied were plant height, leaf width, leaf length, stem diameter, leaf-to-stem-inflorescence ratio, yield and leaf area index. Normality tests, an analysis of variance and a Duncan mean test were performed. The variable height (26.5 and 25.9 cm) and the yield (8.2 and 6.8 t DM ha^{-1}) with inoculation of *Glomus cubense* and *Glomus fasciculatum* showed a significant statistical difference. It is concluded that there is an effect on growth and yield with inoculation of arbuscular mycorrhizal fungi.

Keywords: biofertilizer, *Glomus*, symbiosis, yield.

Reception date: February 2020
Acceptance date: May 2020

Introduction

Plants have developed strategies to cope with biotic and abiotic challenges. One of the most effective is the ability to establish symbiotic relationships with microorganisms (Morales *et al*., 2011). Mycorrhiza is a form of association of the roots with certain plants (Martínez, 2012). This association plays a very important role in the effective exploitation of the mineral resources of the soil and in the protection of the roots against pathogens. Mycorrhizae are essential for the survival of many plant taxa in various ecosystems (Ralde, 2000).

In agricultural production systems, the objective has been to achieve a high yield per unit area to satisfy food demand (Audelo and Irizar, 2012); however, this has caused a constant deterioration of the soil resource at the national level (Sánchez *et al*., 2008). To counteract the above, the indiscriminate use of chemical fertilizers has been resorted to and their application has caused environmental problems (Grageda and Cabrera, 2012). For this reason, the introduction of new fertilization options in the management of forage crops is studied (Dumont *et al*., 2005).

Arbuscular mycorrhizal fungi (AMF) have been reported in soybean, rice, alfalfa and sorghum crops, allowing greater exploitation of the root and absorption of nutrients. With the above, the use of agrochemicals is decreased without compromising yields (Morales, 2006). Studies have also been carried out for the establishment of *Panicum maximum* cv. Kikoni, who was inoculated with arbuscular mycorrhizal fungi and concluded that inoculation with biofertilizers had a significant effect ($p < 0.05$) on plant height and area dry matter yield (Calderón and González, 2007).

Oats (*Avena sativa* L.) is a species that adapts to different environmental conditions, it is mainly cultivated for the production of green forage, hay fodder, grain and balanced feed (Espitia *et al*., 2005). It is also used as forage for feeding grazing animals, hay or silage (Murillo *et al*., 2001). This grass produces good quality forage when other better-quality forage crops are scarce (Ávila *et al*., 2006). Avila and Salmeron (1999) point out that oats are grown mainly in seasonal conditions and their sowing represents 26% of the surface in Mexico.

In some regions of the country, it is a priority to produce forage oats for livestock feed, unfortunately the yield they obtain is low, producers' resort to the use of synthetic fertilizers to increase yield (Espitia *et al*., 2007). The use of AMF in forage crops is an interesting tool to increase growth and production by reducing the use of synthetic fertilizers (Smith *et al*., 1992). The objective of this work was to evaluate the growth of two varieties of forage oats (Turquesa and Chihuahua) inoculated with arbuscular mycorrhizal fungi.

Materials and methods

Experimental site

This study was carried out from March to June 2016 in a tunnel-type greenhouse, in the facilities of the Technological Institute of the Valley of Oaxaca, geographically located at 17° 01' 14'' north latitude and 96° 45' 56'' west longitude, at 1 563 masl.

Vegetal material

The Chihuahua variety that was derived from a cross between AB-177 and Putman 61 was used by hybridization and genealogical selection. At INIFAP, these seeds were purchased from producers in the Mixteca Alta Oaxaqueña.

Substrate preparation and chemical analysis

Soil sampling was carried out in accordance with NOM021SEMARNART. Following the methodology, the sampling units were defined, the sampling area was an area where the texture, color, slope, were homogeneous. The sampling procedure was zigzag and at a depth of 20 cm, starting on one side of the terrain and randomly choosing the starting point to define the sampling plane that would homogeneously cover the terrain. A total of 15 subsamples of 2 kg were obtained. Each subsample was mixed, subsequently divided into four equal parts and two were discarded, this procedure was repeated several times until it had a final weight of 1.5 kg.

The determinations for the soil-water chemical analysis were: pH with a potentiometer (Conductronic PC45 Ph ±0.01); organic matter with method d, electrical conductivity in saturated paste with conductivity meter (Conductronic PC45 mv ±0.01), in the well water the nutrient content was analyzed (N, K, Ca, P, Mn, Zn, Fe, Cu) by the method of atomic absorption spectrophotometry (Thermo scientific ICE 3000 series).

The substrate used was a mixture of two types of soil, sandy loam and sand, with a 2:1 ratio, which was sterilized in an autoclave (vertical autoclave 25 × 50 cm, 40 L, 110V), in two stages, the first it was 15 lb for two hours and the second 15 lb for 45 min.

Mycorrhizal inoculants

Two commercial inoculants were used, the first containing *Glomus fasciculatum* and the second *Glomus cubense*, both products were subjected to a direct counting process carried out in the microbiology laboratory of the University of the Sierra Juárez (UNSIJ). The two commercial inoculants contained 11 mycorrhizal fungal spores for every 100 g of substrate.

Inoculation of biofertilizer in oat plants

The inoculant dose was 5 g pot^{-1} (11 spores plant^{-1} of AMF on average for each strain studied). Inoculation of the seeds was performed. Inoculation was by seed coating. The experimental unit was a pot, where 5 oat seeds were sown. Within the treatments, mineral fertilizer was used to make the comparison with the AMF. Triple 17 (17-17-17) was used, the dose was 5 and 2.5 g was applied on the day of planting.

Experimental treatments

A completely randomized design with 11 treatments and four repetitions was used. Table 1 describes the treatments

Table 1. Description of treatments.

Treatment	Description
Control *Glomus fasciculatum*	A dose of 5 g plant^{-1} of sterilized fertilizer was applied
Control *Glomus cubense*	
Control	Without any application
Glomus fasciculatum	A dose of 5 g plant-1 of biofertilizer was applied.
Glomus cubense	A dose of 5 g plant^{-1} of biofertilizer was applied
Control + fertilizer at 100%	5 g of sterilized biofertilizer + 5 g of plant^{-1} of mineral fertilizer were applied
Control + fertilizer at 50%	5 g of sterilized biofertilizer + 2.5 g of plant^{-1} of mineral fertilizer were applied
Fertilizer at 100%	A dose of 5 g plant^{-1} of fertilizer was applied
Fertilizer at 50%	A dose of 2.5 g plant^{-1} of fertilizer was applied
Glomus fasciculatum + fertilizer at 100%	5 g of biofertilizer + 5 g of plant^{-1} of mineral fertilizer were applied
Glomus cubense + fertilizer at 50%	5 g of biofertilizer + 2.5 g of plant^{-1} of mineral fertilizer were applied

In each experimental unit two types of irrigation were applied in the first two weeks of sowing, daily irrigation was carried out with an atomizer (500 mL capacity) with purified water, in order not to affect the inoculation process, after these two weeks was watered with well water every three days until the end of the experiment the type of water used in irrigation was not considered as a study factor.

Variables evaluated

Plant height (cm), this variable was measured with a 100 cm metal ruler, with an accuracy of 1 mm, taking the distance from the base of the stem to the highest leaf tissue. Leaf length and width (cm), these variables were measured with a vernier (Mitutoyo). The inflorescence (cm) was measured with the 100 cm metal ruler, from the knot where the flag leaf was inserted to the paninule. The plants were harvested at 90 days, the cut was made at a height of approximately 5 cm above the soil surface; subsequently, the harvested forage was weighed in green, separated into its morphological components (stems, leaves, inflorescence), and subsequently dried in a forced air stove at 55 °C, for 72 h.

To determine the percentage of dry mass (DM), according to the formula: DM (%) = [DM of the sample (g)/fresh mass of the sample (g)] x 100. Then the yield (t ha^{-1}) of total dry matter (TDM), leaf (DMl), stem (DMs) and inflorescence (DMin). Leaf: stem ratio was determined by dividing the leaf yield by the stem. Foliar area index was obtained with a foliar integrator (LI-COR, LI-3100C USA). This last variable was recorded by destructive sampling of a 100 g subsample.

Statistical analysis

The data was analyzed using the SAS statistical program (SAS version 9.0). A normality test was carried out, but the assumption was not fulfilled. Transformations were carried out for three variables: the height, the transformation that best adjusted was the trigonometric cosine (ARCOS), the leaf length was square root (SQR) and in the Blade width was the arc sine (ARSIN) this for basic statistical measurements. An analysis of variance was applied making a comparison of means with the Duncan model (p= 0.05), a correlation test was used.

Results and discussion

The edaphological analysis showed that the soil had a pH of 6.34. The soil was determined to contain 84.5, 9.4, and 6% sand, clay, and silt, respectively, thus having a soil with a sandy loam texture. The electrical conductivity was 0.4 dS m^{-1} and 1.4% of organic matter. The water analysis obtained a pH of 6.7 and an electrical conductivity of 0.7 dS m^{-1}.

The summary of the analysis of variance (Table 2) shows that the variable plant height and yield showed a highly significant difference compared to the other variables studied.

Table 2. Analysis of variance of the studied oat variables.

Variable	Average squares	Error
AP	2 504**	186.98
LW	19.14 ns	73.07
LH	81.43 ns	54.96
DT	0.13 ns	7.47
INFL	3.11 ns	7.46
LAI	100.67 ns	264.86
H:T	0.04 ns	0.05
Yield	16.96 **	3.5

AP= plant height; LW= leaf width; LH= leaf length; DT= stem diameter; INFL= inflorescence; LAI= leaf area index; H: T= leaf: stem ratio. **= highly significant p< 0.05, ns p> 0.05 (Duncan; p= 0.05).

The results obtained in the mean test between the variables evaluated in the oat crop (Table 3), the best treatment at height was *Glomus fasciculatum* (26.5 cm), the treatment with the lowest value was the combination of *Glomus cubense* + 50% fertilizer (19.19 cm). The behavior of the height, during the experiment, was ascending until reaching its maximum points and this was considered when the inflorescence was homogeneous. The height of the plant is considered among those that most influences yield (Al-Mohammadi and Al-Zubi, 2011). In the yield the best treatment was *Glomus cubense* (8.26 t DM ha^{-1}), the lowest value was obtained with the *Glomus cubense* treatment + 50% fertilizer (3.09 t DM ha^{-1}). In the leaf-stem relationship and in the leaf area index, the treatments were statistically the same.

Table 3. Comparison of means of the variables measured in the oat crop.

Treatment	AP (cm)	Yield (t DM ha^{-1})	Rate H:T	LAI
Control *Glomus facsiculatum*	23.23 bc	4.91 bcd	0.74 a	51.46 a
Control *Glomus cubense*	24.82 ab	5.1 bcd	0.71 a	47.82 a
Control	21.95 dc	5.57 bc	0.73 a	46.27 a
Glomus fasciculatum	26.55 a	6.81 ab	0.76 a	47.2 a
Glomus cubense	25.55 a	8.26 a	0.61 a	43.67 a
Control + fertilizer at 100%	24.73 ab	4.42 cd	0.54 a	48 a
Control + fertilizer at 50%	21.62 dc	4.34 cd	0.56 a	47.82 a
Fertilizer at 100%	25.06 ab	4.16 cd	0.7 a	45.08 a
Fertilizer at 50%	20.63 de	4.04 cd	0.75 a	52.51 a
Glomus fasciculatum + fertilizer at 100%	21.01 de	4.22 cd	0.72 a	47.33 a
Glomus cubense + fertilizer at 50%	19.19 e	3.09 d	0.65 a	47.9 a

AP= plant height; H:T= leaf: stem ratio and LAI= leaf area index; abcde= treatments with the same letter are statistically the same (Duncan= 0.05).

Some authors mention that the response to inoculation varies depending on the degree of fertility and the availability of soil water, highlighting the importance that the strain to cultivate can acquire (Soroa *et al*., 2003). Other authors report that the inoculation of seeds with biofertilizers has a significant influence, on the height of the plant, they report 0.95 cm and for dry matter 12.6% (Sanchez *et al*., 2008). In this study, a height of 26.55 cm and a yield of 8.26 t DM ha^{-1} were obtained, this indicates that there was an effect on these variables when inoculating the seeds.

The effect of the application of biofertilizers on growth and yield in *Gerbera jamesonii* cv. Bolus, the results show that the treatments inoculated with AMF show a yield of up to 36% inoculated with *Glomus fasciculatum*, with the success of AMF being attributed to the extensive network of hyphae developed by mycorrhizae much higher than the control plants (Gillet, 2013). In the state of San Luis Potosi, productions between 10 t DM ha^{-1} have been obtained in sowings without having any type of fertilization and in sowings with inoculation up to 13 t DM ha^{-1} (Doehlert and Mc Mullen, 2008).

The results obtained in the test of means between the treatments with respect to the variables width and length of the leaf, stem diameter and inflorescence (Table 4) with a Duncan test (0.05), no significant statistical differences were found. In the length of the leaf the highest value was with the control treatment (16.26 cm), the lowest value was the control (15.08 cm), in the stem diameter the highest values were with the *Glomus cubense* control treatments and the control + 100% fertilizer (0.51 mm) and the lowest value was with 100% fertilizer (0.43 mm), in the inflorescence the highest values were recorded by the control + 100% fertilizer and control + 50% fertilizer treatments (9.68 cm) and the lowest value was with the *Glomus cubense* treatment + 50% fertilizer (8.4 cm).

Table 4. Comparison of means of the variables studied in the cultivation of oats.

Treatment	LW (cm)	LH (cm)	DT (mm)	Infl (cm)
Control *Glomus facsiculatum*	3.04 a	15.7 a	0.5 a	8.56 a
Control *Glomus cubense*	3.47 a	15.1 ab	0.51 a	9.06 a
Control	3.31 a	15.08 ab	0.49 ab	8.81 a
Glomus fasciculatum	3.33 a	15.25 ab	0.48 ab	8.93 a
Glomus cubense	3.51 a	15.18 ab	0.48 ab	8.68 a
Control + fertilizer at 100%	3.27 a	16.14 a	0.51 a	9.68 a
Control + fertilizer at 50%	3.18 a	16.26 a	0.45 ab	9.68 a
Fertilizer at 100%	3.74 a	15.34 ab	0.43b	8.56 a
Fertilizer at 50%	3.05 a	15.92 a	0.49 ab	8.62 a
Glomus fasciculatum + fertilizer at 100%	3.15 a	16.24 a	0.5 a	8.57 a
Glomus cubense + fertilizer at 50%	2.54 a	13.96 b	0.5 a	8.40 a

LW= leaf width; LH= leaf length. DT= stem diameter; Infl = Inflorescence. abcde= treatments with the same letter are statistically the same (Duncan; p= 0.05).

In another study conducted with rhizobacteria *Paenibacillus polymyxa* and *Bacillus megaterium* var. phosphaticum, inoculated to tomato plants, significantly improved different plant growth parameters, such as stem thickness, number of branches and leaf area (El-Yazeid and Abou-Aly, 2011).

A study was carried out on the effect of bioactive products on biofertilized bean plants (*Phaseolus vulgaris* L.), where Azofert® significantly stimulated leaf length compared to control treatment plants. It was also reported that the spraying of epibrasinolide (EBL) (5 µM) to bean seeds cv. Bronco significantly increased the length of the stems and roots, the number and leaf area of the leaves per plant, as well as the dry mass of the leaves (Martínez *et al*., 2016). However, these results differ from those reported in this study because no differences were found between inoculation with arbuscular mycorrhizal fungi with the control treatment.

Applied AMF help plants develop, adapt to adverse environmental conditions, including lack of soil moisture, high salinity, and extreme pH (Holland and Munkvold., 2001). Despite the different adverse conditions in which the beneficial microorganisms are subjected, they manage to survive by performing symbiosis with plants (Slafer, 2001), although the response of biofertilizers varies considerably, depending on the microorganisms (Peterson, 2005).

Conclusions

Inoculation with arbuscular mycorrhizal fungi in this forage oat production experiment under greenhouse conditions, showed a significant increase in the height variable (26.55 cm), highlighting the treatment with *Glomus fasciculatum* and the yield (8.26 t DM ha^{-1}) the treatment with *Glomus cubense*, inoculation with arbuscular mycorrhizal fungi in oat culture has an effect on its growth and yield.

Cited literature

Al-Mohammadi, F. y Al-Zubi, Y. 2011. Propiedades químicas del suelo y rendimiento del tomate según la influencia de diferentes niveles de agua de riego y fertilizante. J. Agric. Sci. Technol. 13(2):289-299.

Audelo, B. M. A. e Irizar, G. M. B. 2012. Diseño de un sistema para la desinfección de sustrato utilizado en la producción de micorrizas. México. Rev. Mex. Cienc. Agríc. 3(esp 4):624-635.

Ávila, M. M. R. y Salmerón, J. J. Z. 1999. Adopción de variedades de avena y su impacto en el Estado de Chihuahua. Folleto científico núm. 5. CESICH-CIRNOC-INIFAP-SAGAR. Ciudad Cuauhtémoc, Chihuahua, México. 38 p.

Ávila, M. M. R.; Gutiérrez, G. J. J.; Salmerón, Z. P.; Fernández, H. D. y Domínguez, D. 2006. Diagnóstico del sistema de producción de avena temporal en Chihuahua. Folleto técnico Núm. 22. CESICH-CIRNOC-INIFAP-SAGARPA. Ciudad Cuauhtémoc, Chihuahua, México. 43 p.

Calderón, M. y Gonzáles, P. J. 2007. Respuesta del pasto guinea (*Panicum maximum,cv. Kikoni*.) cultivado en el suelo Ferralítico Rojo lixiviado a la inoculación de hongos micorrízicos arbusculares. Cuba. Cul. Trop. 28(3):33-37.

Doehlert, D. C. y Mc.-Mullen, M. S. 2008. Medición de la densidad del grano de avena por desplazamiento de arena y análisis de componentes físicos del peso de prueba. Cereal Chem. 85(5):654-659.

Dumont, L. J. C.; Anrique, R. G. y Alomar, C. D. 2005. Efecto de dos sistemas de determinación de materia seca en la composición química y calidad del ensilaje directo de avena en diferentes estados fenológicos. Agric. Téc. 65(4): 388-396.

El-Yazeid, A. A. y Abou-Aly, H. E. 2011. Mejora del crecimiento, la productividad y la calidad de las plantas de tomate utilizando microorganismos solubilizadores de fosfato. Aust. J. Basic Appl. Sci. 5(7):371-379.

Espitia, R. E.; Villaseñor, M. E.; Tovar, G. R. y de la O, O. M. 2005. Momento óptimo de corte para rendimiento y calidad de variedades de avena forrajera. México. Mex. Cienc. Agríc. 3(4):771- 78.

Espitia, R. E.; Villaseñor, M. R. G.; Espino, J. H.; Salmerón, R. M. y González, I. L. 2007. Obsidiana, variedad de avena para la producción de grano y forraje en México. Chile. Agricultura Técnica. 33(1):95-98.

Gillet, M. 2013. Las gramíneas forrajeras: descripción, funcionamiento, aplicaciones al cultivo de la hierba. Zaragoza, España: Acribia Boock. 120 p.

Grageda, O. A. y Cabrera, A. D. 2012. Impacto de los biofertilizantes en la Agricultura. Rev. Mex. Cienc. Agríc. 3(6):1261-127.

Holland, J. B. y Munkvold, G. P. 2001. Relaciones genéticas de la resistencia a la oxidación de la corona, el rendimiento de grano, el peso de prueba y el peso de la semilla en avena. Crop Sci. 41(4):1041-1050.

Martínez, G. L.; Reyes, G. Y.; Falcón, R. A.; Nápoles, G. M. C. y Núñez, M. C. 2016. Efecto de productos bioactivos en plantas de frijol (*Phaseolus vulgaris* L.) biofertilizadas. Cuba. Cul. Trop. 37(3):165-171.

Martínez, S. J. 2012. Estrategias para aumentar la germinación y sobrevivencia de *Cenchrus ciliaris* L. y *Brachiaria brizantha* cv. Marandú. Tesis de Maestría. Oaxaca, México: Instituto Tecnológico del Valle de Oaxaca. 56-60 pp.

Morales, C. 2006. Escalado de la producción de *Anthurium andreanum* por métodos biotecnológicos. Informe final de proyecto de investigación, Código 051010, Instituto Nacional de Ciencias Agrícolas (INCA). 23 p.

Morales, C. A.; Calaña, N. J. M.; Corbera, G. J. y Rivera, E. R. 2011. Evaluación de sustratos y aplicación de hongos micorrízicos arbusculares en *Begonia* sp. Cuba. Cul.Trop. 32(32):17-22.

Murillo, A. B.; Escobar, H. A.; Fraga, M. H.; Pargas, L. R. 2001. Rendimiento de grano y forraje de líneas de triticale y centeno en Baja California Sur, México. México. Rev. Fitotec. Méx. 24(2):145-153.

NORMA Oficial Mexicana NOM-021-RECNAT-2000.

Peterson, D. M.; Wesenberg, D. M.; Burrup, D. E. y Erickson, C. A. 2005. Relaciones entre rasgos agronómicos y composición de granos en genotipos de avena producidos en diferentes ambientes. Sci. Soc. Am. 45(4):1249-1255.

Ralde, V. 2000. Producción de avena forrajera (*Avena sativa*) en cultivo hidropónico con diferentes densidades de siembra y frecuencias de riego. Tesis de grado La Paz –Bolivia. Universidad Mayor de San Andrés, Facultad de Agronomía. 65 p.

Sánchez, C. R.; Díaz, F. A.; Pecina, Q. V.; Garza, C. V. y Loera, G. J. 2008. *Glomus intraradices* y *Azospirillum brasilence* en trigo bajo dos regímenes de humedad en el suelo. Universidad y Ciencia. 24(3):239-245.

SAS. 2005. Statistical Analysis System. The SAS for Windows. V. 9.01. SAS Institute. Cary, NC, USA. 480 p.

Slafer, G. A. 2001. Productividad de cereales de estación fría: impacto del mejoramiento genético y características fisiológicas asociadas. *In*: Actas 21ª Reuniao da Comissao Brasileira de Pesquisa de Aveia. Lages, Brasil. 3-6 pp.

Smith, S. E.; Robson, A. D. y Abbott, L. K. 1992. La participación de las micorrizas en la evaluación de la eficiencia genéticamente dependiente de la absorción y uso de nutrientes. Plant and Soil. 146(1):169-179.

Soroa, M. R.; Cortés, S. L. y Hernández, A. 2003. Estudio del efecto de la aplicación de biofertilizantes sobre algunas variables de crecimiento y rendimiento en *Gerbera jamesonii* cv. Bolus. Cuba. Cul.Trop. 24(29):15-17.