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Growth, mineral absorption and yield of maize inoculated with microbe strains

Arturo Díaz Franco¹, Flor Elena Ortiz Cháirez¹, Mónica Guadalupe Lozano Contreras², Gerardo Armando Aguado Santacruz³ and y Oscar Arath Grageda Cabrera³

¹Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Rio Bravo Km 61 Carretera Matamoros-Reynosa, Rio Bravo, Tamaulipas, Mexico.

²Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Mocochoá, Km 25 Carretera Mérida-Motul, Yucatan, Mexico.

³Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Bajío Km 6.5 Carretera Celaya-San Miguel de Allende. Celaya, Guanajuato, Mexico.

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The agrobiological effectiveness of maize inoculation with microbial strains, from arid and semiarid regions of Northern Mexico ('3025W'), cultivated under irrigation conditions, was evaluated. We studied the impact of six experimental strains of arbuscular mycorrhizal fungi [3 (*Glomus mosseae*), 20 (*Gigaspora albida*), 32 (*G. mosseae*), 35 (*G. mosseae*), 39 (*G. mosseae*) and 55 (*Gigaspora albida*)], a consortium of *Pseudomonas* spp. (Bacteriano 2709) (Ba), arbuscular mycorrhizal INIFAP (*Rhizophagus intraradices*) (M), and fertilized control with 120 and 40 kg ha⁻¹ N and P, respectively (TC). The results in flowering stage indicated that greater chlorophyll index and foliar N were with M and TF; higher foliar P content was with strain 35; Fe with 39; and Zn was registered with Ba. Dry biomass production was increased with M, Ba and TC; and radical biomass were with 35 and M. Strains that exhibit the greatest colonization were 20, 32, 55 and M. Grain yield of TC exceeded 15.3% than M, at a time it was superior to the rest of microbe strains. The results showed that potential microbe inoculants expression in growth, nutrition and maize yield vary according to strain used.

Key words: Arbuscular mycorrhizal fungi, rhizobacteria, nutrition, *Zea mays*.

INTRODUCTION

The globalized market, climate change impact, population pressure and environmental degradation have led to reconsider the current status of agricultural production systems. The excessive utilization of agrochemicals has derived in an increased level of pollution, a decreased biodiversity in agricultural areas, agricultural systems degradation as well as in an increase of production costs. A sustainable agricultural production could replace traditional agriculture, even though it requires a greater awareness and understanding of the biological interactions within the agro-ecosystems (Boomsma and

Vyn, 2008; Plenchette et al., 2005). The microbial inoculants currently have a large both ecological and economic importance in agriculture. The relevance of the role performed by microorganisms has revealed a prominent increase within conservation and soil fertility with the persuasion of a new environmental awareness and the need for agricultural systems sustainable management (Hungria et al., 2010; Adesemoye and Kloepper, 2009). The preparation of bio-fertilizers or inoculants which shall be effective on plants and agro-ecology is of great importance, but particularly those

with economic viability. The arbuscular mycorrhizal fungi (AMF) and plant growth promoting bacteria (PGPB) are the most studied microorganisms. Different mechanisms are manifested by the AMF within the symbiotic activity that lead to an increased soil exploration by the hyphae, a reduction of adverse abiotic conditions for the plant, a plant hormones production stimulating plant growth, a facilitation of nutrients absorption, production of glomalin adhering soil particles, as well as an induction of a protective action against some soil pathogens (Smith and Read, 2008). The PGPB group can stimulate plants through phyto-hormone production, nitrogen fixation, P solubilization, or biocontrol of plant pathogens by siderophores or antifungal compounds, such as lytic enzymes (Glick et al., 1999; Rodríguez and Fraga, 1999; Vassey, 2003).

Many studies have been focused on becoming better acquainted with the effects derived from soil microorganisms, such as bio-fertilizers, used to increase production (Russo and Perkins, 2010; Xiang et al., 2012), either replace or reduce chemical fertilizer utilization (Adesemoye et al., 2009; Carpio et al., 2005), conferring tolerance against soil pathogens (Jetiyanon et al., 2003; Ferrera and Alarcón, 2008), for contaminated soils bioremediation (Franco et al., 2007), as well as to provide tolerance to other abiotic factors (Singh et al., 2011). Nevertheless, some strains benefit most to a given host compared to others, in addition to their functionality which could be altered under certain soil and climatic conditions; thus, the marked differences among species and even among strains is clearly revealed by such fact (Hungria et al., 2010; Montero et al., 2010; Rodríguez et al., 2004; Kironomos, 2003). Many inoculants are prepared from microbial strains either introduced or foreign, but an emphasis has been currently given to the use of native strains that can be reintroduced through their inoculation to crops with a higher adaptation and effectiveness ability in specific locations and climates (Ferrera and Alarcón, 2008; Hungria et al., 2010; Plenchette et al., 2005; Tchabi et al., 2010). It is important to understand the relation between the mycorrhizal forming fungi and production systems, aiming to have the selected strain integrated within the crop agronomic model (Plenchette et al., 2005). Consequently, the present study is aimed to evaluate microbe strains of national origin looking forward to become better acquainted their impact on growth, nutrition and yield of corn in the field.

MATERIALS AND METHODS

Experimental management

The study was conducted in clay soil, which pre-plant properties are described in Table 1, under irrigated conditions at the Rio Bravo Experimental Station, INIFAP, Rio Bravo, Tam. Determinations for soil analysis were: pH in 1:2 (soil-water); the organic matter was measured with potassium dichromate; the electric conductivity was

measured in a saturated paste; the inorganic nitrogen ($\text{NO}_3\text{-N}$) was measured through the salicylic acid method; the P available was obtained by the Olsen method; the K available was obtained by the atomic absorption method (Plenecassagne et al., 1999). To measure the initial AMF spores of the soil, extraction of spores was performed by a sieving and decanting procedure; subsequently, the spore counting was carried out in 100 g of soil (Gerdeman and Nicholson, 1963).

The maize hybrid seed used was Pioneer '3025W'. A total of six experimental AMF from INIFAP strains (General Teran Experimental Station, INIFAP) were evaluated from both the arid and semiarid regions of northern Mexico [3 (*Glomus mosseae*), 20 (*Gigaspora albida*), 32 (*G. mosseae*), 35 (*G. mosseae*), 39 (*G. mosseae*) and 55 (*Gigaspora albida*)], a consortium of PGPB [*Pseudomonas* spp. (Bacteriano 2709), Bajío Experimental Station, INIFAP, Celaya, Gto.] (Ba), arbuscular mycorrhizal INIFAP (*Rhizophagus intraradices*, General Teran Experimental Station, INIFAP, General Teran, N.L.) (M) and a fertilized control (FC), regional management, with 120 kg ha⁻¹ nitrogen and 40 kg ha⁻¹ phosphorus; half of the N was applied pre-plant and the other half before flowering (CERIB, 2011). The AMF with ≥ 60 spores g⁻¹ of soil were inoculated at a dose of 1 kg in 18 kg of seed (ha); the PGPB, with a 1×10^8 ufc g⁻¹ concentration, was inoculated at a 500 g dose in the same amount of seed (Díaz et al., 2011). The planting was established in February the 2nd, 2010 in a randomized complete block design with six replications. The experimental unit consisted of four 0.80 m width and 6 m long rows. A total of three gravity irrigations were applied with 10 cm at flowering and milky states, respectively; other agronomic practices were followed in accordance to local guidelines for maize (Rosales et al., 2005).

Evaluated variables

The *in situ* chlorophyll content was measured through the readings taken during the state of the seventh leaf and in flowering from the middle area (longitudinal and transverse) of 30 leaves, 7th and ear, respectively, by means of a Minolta SPAD-502® portable determiner. A leaf mineral content analysis for N, P, Fe and Zn was performed at flowering at the Soil-Plant Laboratory, Rio Bravo Experimental Station, INIFAP. Thus, N was quantified using the Kjeldahl method with rapid digestion; P was measured through the molybdo-ammonium metavanadate colorimetric method; Fe and Zn by a wet digestion with a nitric acid-perchloric mixture. The elements concentration was determined with an atomic absorption spectrophotometer (Plenecassagne et al., 1999). The foliar and radical dry biomass was estimated from five plants taken from the central rows of each plot.

A total of five plants in their physiological maturity were dug out at random (Díaz et al., 2011) from the central rows, which were removed with a shovel to extract the soil volume under the plant, aiming to determine the total colonization percentage. The roots were washed to eliminate the soil, cut into 1 cm pieces, having them mixed and taken 1 g subsamples, afterwards. The clearing technique was followed in order to determine colonization with 10% KOH and 0.03% trypan blue staining (Phillips and Hayman, 1970), mounted on slides to microscopically examine the segments and determine the fungal structures percentage, in accordance to the Giovannetti and Mosse (1980) method. The grain yield was quantified by harvesting and threshing the ears of the two central rows with grain moisture adjusted to 14%.

Statistical analysis

The effects derived from the treatments on the variables were determined by analysis of variance; the Tukey test ($p \leq 0.05$) was

Table 1. Soil characteristics determined prior to the experiment.

pH	M.O. (%)	NO ₃ -N (mg L ⁻¹)	P (mg L ⁻¹)	K (mg L ⁻¹)	Texture	AMF spores (100 g)
8.1	2	11.7	27.1	875	Silty-loam	1810

Table 2. SPAD chlorophyll index and maize leaf (3025W) nutritional content inoculated with microbial strains.

Strain	Chlorophyll (SPAD)		Foliar nutrients at flowering			
	7 th leaf	Flowering	N (%)	P (%)	Fe (mg L ⁻¹)	Zn (mg L ⁻¹)
3	39.8 ^b	44.4 ^b	1.99 ^b	0.212 ^{bc}	546 ^b	27.0 ^c
20	38.8 ^b	41.8 ^b	1.94 ^b	0.220 ^{bc}	401 ^b	31.3 ^{bc}
32	37.9 ^b	44.5 ^b	1.75 ^c	0.226 ^{abc}	472 ^b	28.6 ^{bc}
35	38.4 ^b	44.0 ^b	1.88 ^{bc}	0.256 ^a	522 ^b	39.0 ^{abc}
39	39.3 ^b	43.0 ^b	1.71 ^c	0.232 ^{ab}	1112 ^a	41.3 ^{ab}
55	39.0 ^b	43.3 ^b	1.30 ^d	0.198 ^c	571 ^b	32.6 ^{bc}
Mycorrhiza INIFAP	40.6 ^b	47.6 ^a	2.20 ^a	0.226 ^{abc}	393 ^b	39.0 ^{abc}
Bacteriano 2709	38.4 ^b	44.5 ^b	1.91 ^b	0.228 ^{abc}	422 ^b	54.6 ^a
140N-40P-00K	45.8 ^a	50.1 ^a	2.26 ^a	0.231 ^{ab}	463 ^b	31.3 ^{bc}
P>F	0.001	0.003	0.001	0.001	0.001	0.001

Value with the same letter do not differ in accordance to the Tukey test ($p < 0.05$).

used for means comparison. The statistical analysis was carried out with the Statgraphics Plus program (Manugistics, 1997).

RESULTS AND DISCUSSION

Nutritional status

Differences among treatments were revealed by the chlorophyll index recorded in both the seventh leaf and in flowering stages. The highest chlorophyll value in the seventh leaf stage ($p=0.001$) was observed in the fertilized control, compared with the rest of the microorganisms which had manifested themselves as statistically similar. The fertilized control and the mycorrhizal INIFAP were higher ($p=0.003$) during the flowering stage in chlorophyll index, with a 48.8 (average of both) than the rest of the microorganisms (Table 2). It has been observed that the photosynthetic pigments were increased by the sorghum inoculation with the *Rhizophagus intraradices* mycorrhizal fungus, as compared to uninoculated plants (Abdel and Mahaedin, 2000). A lack of significant differences was found by Díaz et al. (2008), in dry land conditions, in the SPAD index between the fertilized maize with 60-20-00 and the inoculated with *R. intraradices*.

Differences ($p=0.001$) among the treatments under evaluation were revealed by the N, P, Fe and Zn maize leaf content determinations (Table 2). Both the chemical fertilization and the mycorrhizal INIFAP were the treatments that recorded the highest N values. It has been indicated by different reports (Subramanian and Charest, 1999; Boomsma and Vyn, 2008; Boucher et al., 1999) that the mycorrhizal forming fungi are capable of

increasing the N acquisition by external hyphae. It was determined by Boucher et al. (1999) that, compared with *G. mosseae*, the N content in maize leaf was increased in plants colonized by the *Glomus versiforme* and *G. aggregatum* strains. By contrast, AMF strains evaluated by Tchabi et al. (2010) in white yam (*Dioscorea rotundata*) did not reveal any increase in N uptake; the highest P absorption was promoted by the strain 35. It has been noted by Miller (2000) that the mycorrhizal fungi mycelium transports the P from the soil to the plant immediately after connection with the developing root system. Several variations were also determined by Tchabi et al. (2010) for P concentrations in plants with different AMF strains. The 39 strain resulted outstanding for Fe content, which exceeded by more than twice as much the values obtained from most of the treatments. The inoculation with Bacteriano 2709 revealed the highest Zn content (Table 2). Considering that Fe deficiency is common among crops and that is a rather serious issue, particularly in calcareous soils, the 39 strain may represent a potential utilization in soils with a limited availability of such element. No response was revealed in other studies carried out by Tchabi et al. (2010) for neither Ca nor Mg content in plants throughout AMF strains inoculation from different origins. Quite the opposite, increases in Fe, Zn, Cu and Mn acquisition were reported by Liu et al. (2000) in maize inoculated with *R. intraradices*.

Biomass production

Microbial strains affected the maize production of dry biomass of foliar ($p=0.002$) and root ($p=0.002$). The

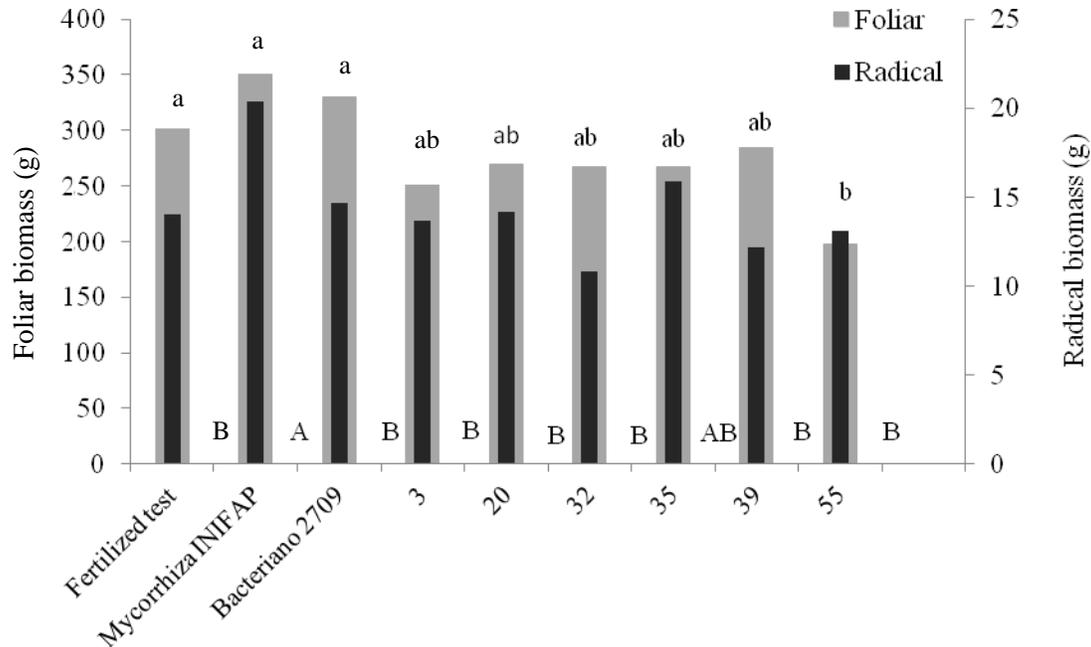


Figure 1. Weight ratio of foliar dry biomass (gray bar) and root (black bar) of maize (3025 W) with microbe strains inoculation. Bars with the same letters (lower case=foliar; uppercase=radical) do not differ in accordance to the Tukey test ($p < 0.05$).

treatments with the highest foliar biomass production were fertilized control, mycorrhizal INIFAP and Bacteriano 2709, with a 330 g average (Figure 1). Variations ranging from 16 to 24% were reported by Boucher et al. (1999) in greenhouse maize with regards to foliar biomass increase with four AMF species. The effectiveness of six AMF strains were evaluated by Rodríguez et al. (2004) in a study with tomato (*Lycopersicon esculentum*), grown in home screen, pointing out the response variability in plant height and dry biomass among strains.

Variations among the strains under evaluation were also revealed by the weight of the root dry biomass. The highest root biomass was registered with mycorrhizal INIFAP, followed by strain 35 (Figure 1). Likewise, the response to two AMF strains (*Glomus hoi-like* and *G. mosseae*) were evaluated by Montero et al. (2010) in field grown pepper (*Capsicum annuum*), determining remarkable differences in root dry biomass between both strains, but higher than the control; consequently, it was concluded that a higher soil volume is explored with an increase in root dry biomass, having both water and nutrients more easily absorbed, improving plant nutrition, thus.

Mycorrhizal colonization

Significant differences were observed ($p = 0.001$) in the mycorrhizal colonization, which ranged from 39.6 to 90.3%. High colonization percentages were revealed by

all of the AMF strains, with regards to the control and the Bacteriano 2709; particularly, those that had the highest colonization were 20, 32, 55 and mycorrhizal INIFAP, which showed similarities and averaged 87% (Figure 2). Colonization ranges in maize from 26 to 72% that depended on the AMF strain species were reported by Boucher et al. (1999). The difference in colonization ability among AMF strains has also been reported in other crops (Rodríguez et al., 2004; Tchabi et al., 2010; Montero et al., 2010). It was observed in the study of Tchabi et al. (2010) that all of the AMF strains were capable of colonizing, regardless of their geographical origin.

It is noteworthy that the Bacteriano 2709 fungal colonization exceeded the fertilized control (Figure 2). Such phenomenon of increase in AMF natural colonization through rhizobacteria inoculation, although not yet fully understood, was also reported with *A. brasilense* in husk tomato (*Physalis ixocarpa*) (Velasco et al., 2001).

Grain yield

The highest grain yield ($p = 0.001$) was observed in the fertilized control plots, represented by the model of irrigated maize in northern Tamaulipas. Then, and descending, the yield took three different ranges: The first by mycorrhizal INIFAP (9620 kg ha^{-1}); the second by the strains 35, 20, 39, 32 and Bacteriano 2709 (8300 kg ha^{-1} , average); the third formed by the strains 3 and 55 (7744

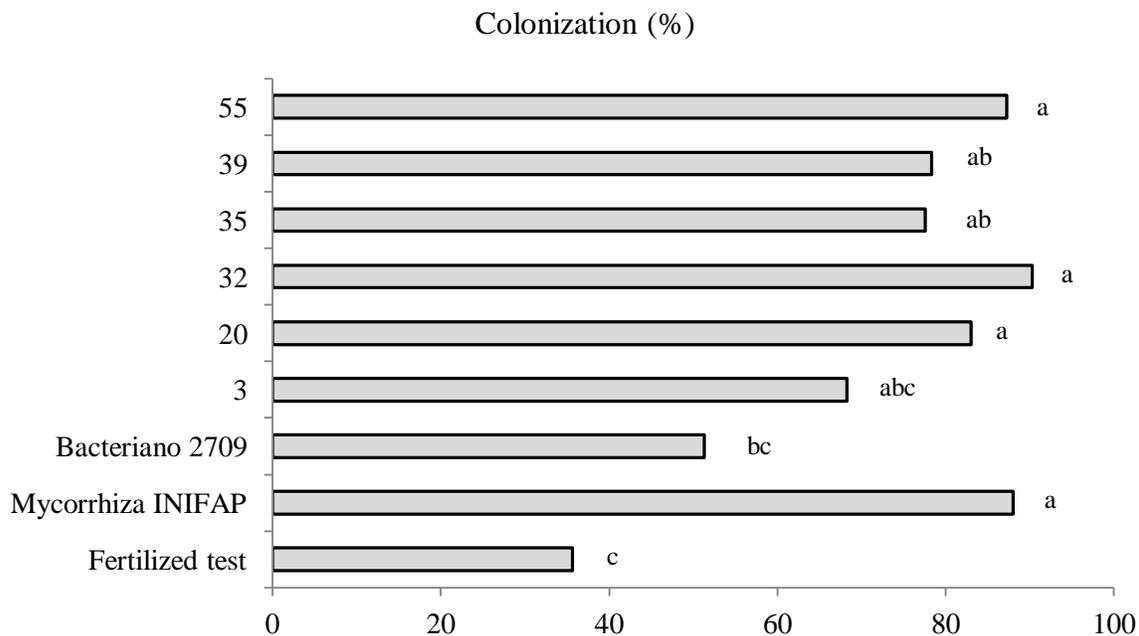


Figure 2. Mycorrhizal colonization in maize inoculated with different microbial strains. Bars with the same letter do not differ in accordance to the Tukey test ($p < 0.05$).

Table 3. Maize grain yield obtained with microbe strains inoculation.

Strain	Grain yield (kg ha^{-1})
3	7859 ^{c*}
20	8304 ^{bc}
32	8175 ^{bc}
35	8829 ^{bc}
39	8207 ^{bc}
55	7630 ^c
Mycorrhiza INIFAP	9620 ^{ab}
Bacteriano 2709	7986 ^{bc}
120N-40P-00K	11364 ^a
P>F	0.001

Value with the same letter do not differ in accordance to the Tukey test ($p < 0.05$).

kg ha^{-1} , average) (Table 3). Therefore it is concluded that the experimental AMF strains and Bacteriano 2709 did not exceed mycorrhizal INIFAP under this technical system of maize production. It is important to continue the search and identification of efficient strains in these agro-systems.

It is noteworthy, on the other hand, that the yield obtained in the study hereby are above the average annual production registered for northern Tamaulipas, which is 5.8 t ha^{-1} (SIAP, 2011). Despite the chemical fertilization result exceeded in 15.3% the yield obtained with mycorrhizal INIFAP, it is important to highlight the consequences, particularly of economic and environmental

impact, involved in fertilization practice. Studies have been focused to know the effects derived from soil microorganisms to replace or reduce chemical fertilization (Adesemoye and Kloepper, 2009; Carpio et al., 2005). Understandably, the need for and use of AMF inoculants based upon plants requiring efficient strains, particularly in situations of eroded soils, degraded, contaminated and with an inefficient mycorrhizal species (González et al., 2004). Yet another interesting aspect is to reach a better understanding of the interactions among microbial inoculants, chemical fertilization and plant, in order to reduce or replace the amount of fertilizers applied, reducing harmful effects in agro-ecology, thus. Such issue has been recently addressed in several studies (Adesemoye and Kloepper, 2009; Adesemoye et al., 2009; Hungria et al., 2010; Shaharoon et al., 2008).

It was revealed by the results of the present study that the expression of the potential microbe inoculants on growth, nutrition and yield of maize varies depending on the strain used. It was indicated by Tchabi et al. (2010) that the AMF effectiveness is more dependent of its capacity and intrinsic ability than of its geographical origin. Moreover, there may be a definite link between a given strain and a specific genotype (Khalil et al., 1994; Boomsma and Vyn, 2008).

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