PLANT GROWTH PROMOTING BACTERIA AND MYCORRHIZAL ON VARIETIES OF *Sorghum* spp. GERMINATION UNDER STRESSING ABIOTIC CONDITIONS †

[BACTERIAS PROMOTORAS DEL CRECIMIENTO VEGETAL Y MICORRIZAS SOBRE LA GERMINACION EN VARIEDADES DE *Sorghum* spp. BAJO CONDICIONES DE ESTRESES ABIÓTICOS]

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SUMMARY

Background. Mexico currently ranks first in *Sorghum* production. Notwithstanding, soils in a productive area of Sonora are too poor in organic matter with saline intrusion and high temperatures. Farmers apply synthetic fertilizers but the indiscriminate use increase salinity. Under dry and arid zones, plant growth promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) signifies a substitute to obtain nutrients. **Objective.** New and productive varieties of *Sorghum* in Sonora desert (Silo Miel 370 VCB (370), Sorgo Silo Miel 315 VC (315), Sorgo Silo Miel II (II) and Camino 526 Croplan winfield (526)) were evaluated inoculated with a species PGPB and AMF. **Methodology.** Under salinity, temperature regime (night/ day) in germination stage, tests were carried out considering the inoculation with PGPB and AMF; growth variables were measured like a percentage and rate, plant height, root length, and produced biomass (fresh and dry matter). **Results.** The cultivar 526 was the most outstanding with the PGPB and AMF evaluated; at the seedling stage, there were significant differences between the treatments for plant height, root length, fresh and dry weight, considering NaCl levels and temperature regimes. **Implication.** Studies of the association of PGPB and AMF with the *Sorghum* cv. 526 are recommended under field conditions. **Conclusion.** This study is the first step to obtain an ideal variety of *Sorghum*, which is a principal crop in dry arid and desert zones of México. This kind of study promotes beneficial microorganisms as a resource to increase the development and productivity of plants.
Keywords: temperature; salinity; basic crops.

RESUMEN

Antecedentes. Actualmente, México ocupa el primer lugar en producción de sorgo. No obstante, los suelos en un área productiva de Sonora son demasiado pobres en materia orgánica con intrusión salina y altas temperaturas. Los agricultores aplican fertilizantes sintéticos, pero el uso indiscriminado aumenta la salinidad. En zonas áridas, las bacterias promotoras del crecimiento vegetal (PGPB) y los hongos micorrícicos arbusculares (AMF) representan un sustituto para obtener nutrientes en los cultivos. **Objetivo.** Se evaluaron las variedades nuevas y productivas del desierto de Sonora (Silo Miel 370 VCB (370), Sorgo Silo Miel 315 VC (315), Sorgo Silo Miel II (II) y Camino 526 Croplan winfield (526)) al inocularse con una especie de PGPB y AMF. **Metodología.** Bajo condiciones de salinidad, un régimen de temperatura (noche / día) en la etapa de germinación, se realizaron pruebas considerando la inoculación con la PGPB y

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el AMF; las variables de crecimiento se midieron como porcentaje y tasa de germinación, altura de la plántula, longitud de la raíz y biomasa producida (materia fresca y seca). **Resultados.** El cultivar 526 fue el más sobresaliente con la PGPB y el AMF evaluados; en la etapa de plántula existieron diferencias significativas entre los tratamientos para la altura de la planta, longitud de la raíz, el peso fresco y seco considerado los niveles de NaCl y los regímenes de temperatura. **Implicaciones.** Estudios de la asociación de PGPB y AMF con el sorgo var. 526 se recomiendan en condiciones de campo. **Conclusión.** Este estudio es el primer paso para obtener una variedad ideal de sorgo, que es un cultivo principal en zonas secas, áridas y desérticas de México. Este tipo de estudio promueve microorganismos benéficos como un recurso para aumentar el desarrollo y la productividad de las plantas.
Palabras clave: temperatura; salinidad; cultivo básico.

INTRODUCTION

Sorghum (*Sorghum* bicolor) is one principal cereal crop in many countries; it contains high levels of minerals and vitamins, i.e. those of group B are listed thiamine, riboflavin, and niacin (López-Ortíz *et al.* 2011). This richness makes it an important antioxidant food. While it is also rich in iron, calcium, phosphorus, zinc and insoluble fiber (de Morais *et al*. 2017). *Sorghum* (subsp. bicolor) belongs to the family Gramineae and it is sowed in countries of America (Mexico, Argentina, and the USA), Africa (where the *Sorghum* is originated), Asia and Oceania (Ethiopia, Sudan; Chine; Australia, respectively). *Sorghum* is a protein-rich cereal and stands out for containing a lower caloric intake, with fewer carbohydrates and fats. Besides, it is a gluten-free food, so it is optimal for celiac people. Besides, it has antidiarrheal and homeostatic properties. Currently, new markets for *Sorghum* are developed producing novel functional foods. Mexico currently ranks first in *Sorghum* production with 11 million tons per year. Likewise, our country has reached the world record in yield of *Sorghum* of up to 17 tons per hectare, reflecting that this crop is one of the most productive crops.

Sonora and Baja California Peninsula are some of the aridest states of Mexico, with 80 mm average annual precipitation (Rueda *et al*. 2004). The main problems facing agriculture are a declining supply of water and a decrease in the quality of the water. While the total world population today demands proportionally more and more food, that total is expected to exceed 9,000 million by 2050. In the agricultural sector, many adverse factors reduce productivity, i.e. water extraction in excess, use of inappropriate fertilizers, which have caused increases in the salinity of agriculture soil (SAGAR 1981; Salim 1989; Shalaby *et al*. 1993; Sangakkara *et al*. 1995; Abdullah *et al*. 2001; Rashid *et al*. 1999). In this sense in dry arid and desert zones, Novo production alternatives are developing concerning the selection and evaluation of salt-tolerant plants that already are adapted to salty areas (Flowers and Yao 1995; Flowers 2004).

Studies with a salt-tolerant plant have been carried out in an agronomic, physiological, biochemical, and molecular sense. However, in recent years, the number of works that address tolerance to salinity from a germinations stage, point of view has increased considerably, to search responses under different conditions, because the variability is extensive (Abdus *et al*. 1999; Acosta-Motos *et al*. 2017; Miranda *et al*. 2017; Chen *et al*. 2018; Yan *et al*. 2018; Dilnur *et al*. 2019; Hernández 2019; Zhong *et al*. 2019). However, although there are many *Sorghum* varieties with a high viable seed, the germination and establishment are poor under the soils of the Sonora region (Zhang *et al*. 2019). In this sense, farmers due the climatic conditions are favorable to produce *Sorghum*, they invest in agro-inputs and technology, so in the Mexican Sonora state, the *Sorghum* has a wide distribution along with the central parts of Sonora state (Zhang *et al*. 2019).

Moreover, the productivity of *Sorghum* is limited by the absence of accessible nitrogen (de Morais *et al*. 2017; SAGAR 1981). This situation disturbs its development and reproduction possible (Juarez and Harrison 2018). Traditionally, growers apply artificial fertilizers to recompense for soil nitrogen deficit. However, unselective use of these nourishments forces the increasing salinity and severely injuries soil microorganism's structure and configuration (Jefferies 1977; Kapulnik *et al*. 1981; Banwari and Rao 1990; Nahid and Gomah 1991).

Few studies on plant growth-promoting bacteria (PGPB) have been published of *Sorghum* spp (Akhavan *et al*. 1991; Isopi *et al*. 1995; Luigi *et al*. 1998; Compant *et al*. 2010), but not in association with mycorrhizas diversity within the rhizosphere. Concerning varieties sowed in the Sonora desert, the studies are null. So is very important to increase the number of known salt-tolerant, nitrogen-fixing bacteria and arbuscular mycorrhizal (Hamdi 1999; Whipps 2000).

On the other hand, several principal stressing factors as a temperature (Adams *et al*. 2001;

Aksouh *et al*. 2006; Luo 2011), and salinity (Pichu 2010; Kroes and Supit 2011) has been proved in glicophytes, which regulate some stages of the plants (germination, pre-flowering, flowering and mature physiologic) and interact within the soil interface (Ungar 1995; Laynez-Garsaball *et al*. 2007). The osmotic and matrix potential in saline soils decreases the temperature that is effective for seed germination (Hegarty 1978). The adverse factor such as salinity interacting with another abiotic factor such as temperature, determine high germination percentages (Khan and Ungar 1998; Rivers and Weber 1971; Philipupiya and Ungar 1984; Keiffer and Ungar 1995; Khan and Ungar 1996; Khan and Ungar 1998; Gulzar et al. 2000); it is worth mentioning that, the ability of plants to counteract adverse factors is conditioned by multiple biochemical and physiological pathways that help to better capture cellular water; physiological protection of chloroplasts, maintaining a homeostasis of the ions. Plants to detoxify radicals under salinity conditions activate to essential routes such as the synthesis of osmotically active metabolites, specific proteins and certain free radical elimination enzymes that maintain a balance and flow of ions and water; they also favor and contribute a function in the elimination of oxygen radicals or chaperones; some species accumulate methylated metabolites, whose function is osmoprotectant and eliminate free radicals. Photorespiration is a physiological phenomenon which it is altered under abiotic conditions (Asish and Anath 2005). In the case of halophyte seeds, they can be viable for prolonged periods when they are in hypersaline conditions, allowing them to germinate when the conditions of salinity in the soil and water are reduced (Khan *et al*. 1976; Woodell 1985; Khan and Ungar 1997; Khan and Ungar 1999).

Notwithstanding the foregoing, it is important to indicate that among salinity tolerant plants, there is variability to respond positively and negatively to adverse conditions varying conditions such as temperature and salinity (Khan and Ungar 1997; Gul and Weber 1999). The present study defines the influence of the inoculation with the PGPB (*Bacillus amyloliquefaciens*) and the AMF (*Glomus intraradices*) under different conditions of salinity and discontinuous temperature regimes on the seed germination of varieties of *Sorghum bicolor*.

MATERIALS AND METHODS

Effect on germination and seedling growth of four varieties of *Sorghum* spp. by inoculants in NaCl conditions

Seeds of four varieties of *Sorghum* spp. were collected, from production areas of Sonora, Northwest of Mexico. The collected varieties were Silo Miel 370 VCB (370), Sorgo Silo Miel 315 VC (315), Sorgo Silo Miel II (II) and Camino 526 CROPLAN WINFIELD (526). Largest and health seeds were carefully chosen and with no observable destruction. Seeds of each variety were disinfected in sodium hypochlorite (3% active chlorine) for 10 s, and washed with sterilized and distilled water and dried with sterile paper. The halo bacterium *B. amyloliquefaciens* (Accession number KJ433614), on N-free OAB agar medium according to (Renganathan *et al*. 2019), with three different concentrations of NaCl (0.25 M) was replicated. Later, at a concentration of 108 cells.mL-1, seeds were inoculated with bacteria treatments according to (Carrillo *et al*. 1998). At the same time and according to (Strullu and Romand 1986; Declerck *et al*. 1998; Bécard and Piché 1992; Bécard and Fortin 1988), and using *Allium schoenoprasum* was reproduced *Glomus intraradices*. The inoculum obtained was from a sample of AM 120 Granite Seed Company (Lehi, UT 84043). Furthermore, seeds were treated with the AMF-*G. intraradices* according to (Rueda-Puente *et al*. 2018), using a concentration of 120 propagules.cm^3. Into the sterilized Petri dishes, germination tests were performed, each Petri dish with a cotton layer substrate (150 x 15 mm) covering the bottom of the dish. Dishes were moistened with uniform NaCl solution (0, 0.06, or 0.12 M). All seeds varieties considering its treatment and separated form were done inside a growth chamber at 27°C ± 0.5°C and 35% ± 1% RH, with continuous white light. Every four days, 20 mL of the appropriate solution was added to each dish. When the radicle was at least 2 mm long from seeds, were considered germinated. Final percentage germination was measured after 25 days; also the number of germinated seeds was recorded daily (germination rate). Using the next formula, the germination rate was calculated (Maguire 1962):

$$M=n1/t1 + n2/t2 +\ldots n25/t25$$

where n1, n2, … n25 are the numbers of germinated seeds at times t1, t2, … t25 in days. The hierarchic experiment of a randomized design included three factors (varieties, inoculation, and salinity), with five replicates of 50 seeds. The first factor (variety) had four levels (varieties: 370, 315, II and 526); three bioinnoculations: without inoculation, inoculation with mycorrhiza arbuscular (*Glomus intraradices*) and inoculation with the bacterium *Bacillus amyloliquefaciens* were the second factor; and the three concentrations of NaCl (0, 0.06, and 0.12 M) were the third factor. The three

studied factors combination turned out 36 treatments. Arcsine transformation was realized to obtain percentage germination (56), with three-way analyses of variance (ANOVA). Germination rates were obtained by sums of germinations per day and were not transformed before analysis. Duncan's multiple range test at P = 0.05 was carried out to obtain significant differences between means of treatments. Data were analyzed using the Statistical Analysis System (Sokal and James 1962). Seedling growth of four varieties under all conditions was measured by dry and fresh weights on 30 days after inoculation (dai); other variables calculated were root length and height and dry weight. Colony-forming units (CFU) was a measurement in the root system from seedlings considering Carrillo *et al*. (Bashan *et al*. 2007). In all variables least significant differences (LSD) were obtained by Duncan's Multiple Range Test at P = 0.05. All statistical analysis was done with SAS (2004).

Estimation of *Bacillus amyloliquefaciens*, and *Glomus intraradices*, and NaCl on germination and seedling growth of the superior variety 526 of *Sorghum* spp

As a second experiment, variety 526 was carried out to evaluate the effect of temperature on germination alternating temperature regimes of 5-15 (night-day), 10-20, 15-25, 20-30, and 25-35°C, based on a 24-hr cycle of 12 hr (Growth chambers with Sylvania cool white fluorescent lamps, 25 μmol m-2 s-1, 400-750 nM). Seeds were submitted at the same treatment (disinfected and inoculated with PGPB and AMF treatments under NaCl solutions (0, 0.06, and 0.12 M) as it was indicated in the first experiment. Variables as percentage germination, germination rates, root length, height, and dry and fresh weights were evaluated as previously cited in the first phase. All data were analyzed using the Statistical Analysis System (Maguire 1962; SAS 2004). A hierarchic experiment of randomized was designed with three factors (inoculation, temperature regime, and salinity); same three levels as a 1st experiment were analyzed: a) without inoculants, with inoculant PGPB (*B. amyloliquefaciens*), and AMF (*Glomus intraradices*); three concentrations of NaCl (0, 0.06, and 0.12 M), and c: five temperature regimes (5-15, 10-20, 15-25, 20-30, and 25-35 °C, based on a 24-hr cycle of 12 hr).

RESULTS

Germination and seedling growth of four *Sorghum* varieties

The germination percentage was significantly modified by biotreatments studied in this experiment (*B. amyloliquefaciens* and *Glomus intraradices*), under salinity conditions (Table I). When salinity was higher (0.12 M NaCl), inhibition of germination was observed in all varieties non inoculated (< 80%), compared with those inoculated, sticking out the 526 variety with *B. amyloliquefaciens.* However, when salinity conditions were reduced at 0 M NaCl), germination percentage was increasing until 97% in 526 Var. + with both beneficial microorganisms. The contrary was observed in varieties non biotreated, obtaining significant germination results in 76% at 0 M, and obtaining high values the 526 var. Similar behavior was showed in 0.06 M of NaCl, where 526 var., showed the highest values in germination with and even without Plant Growth promoting Bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF). According to the germination rate, results (data no-showed) of all varieties with bio-inoculants studied, showed a total germination (after inoculation and sowed – AIS-), among 8 to 17 days =d= (at 0 M NaCl = 9d with a 99.50%; at 0.06 M = 11d with 55%, and 0.12 M NaCl = 17 d with no more than 43%), while those non inoculated showed germination total rate till 11dAIS to 20d; at 0M with a 82.35%; at 0.06 with 43% in 16d AIS, and no more than 42% at 0.12 M NaCl in 20d, respectively. PGPB and AMF influenced plant growth of all varieties, during early seedling with significant differences among treatments for plant height, root length, fresh and dry weight (Table I). These results evidenced the potential on 526 var. to be selected and used in the next step. All high values were obtained at 0 M of NaCl with *B. amyloliquefaciens* and *Glomus intraradices*, and highlighting the same bionoculants at 0.06 and 0.12 M vs treatments without beneficial microorganisms.

Evaluation of *B. amyloliquefaciens* and *Glomus intraradices* inoculants, NaCl levels, and temperature regimes on germination and seedling growth of 526 variety

After inoculation with PGPB and AM during the germination stage under NaCl levels and temperature regimes, results showed significant differences among treatments (Table II). Optimal germination of 526 var. in 12/12 hr light/dark conditions occurred under all salinity conditions, were obtained at 0 M NaCl (P>0.05), and in these all treatments whit bio inoculants (P>0.05), but no significant between temperatures 5/15, 10/20, 15/25, and 25/35 °C (Table II).

Table I Effects of *Bacillus amyloliquefaciens* and *Glomus intraradices* on average values of germination (%), root length, plant height, fresh and dry weights of seedlings of varieties of *Sorghum* spp (Silo Miel 370 VCB (370), Sorgo Silo Miel 315 VC (315), Sorgo Silo Miel II (II) and Camino 526 CROPLAM WIN FIELD (526)) under three concentrations of NaCl after 25 days sowed.

Variety	Inoculant*	NaCl concentration (M)	Germination (%)	Plant height (cm)	Root length (cm)	Fresh weight (g)	dry weight (g)
370	Control	0	61.38d	10.20bc	11.60b	39.929a	7.591a
315	Control	0	79.64bc	11.40bc	11.15b	36.014ab	6.794a
II	Control	0	78.53bc	10.90bc	9.05bc	35.927ab	7.597a
526	Control	0	87.21b	11.65bc	12.10b	40.911a	8.581a
370	*B.amyloliquefaciens*	0	83.44b	12.25bc	19.25a	40.914a	10.584a
315	*B.amyloliquefaciens*	0	86.65b	15.85b	15.70ab	38.927a	8.597a
II	*B.amyloliquefaciens*	0	87.43b	17.95b	15.60ab	42.021a	10.801a
526	*B.amyloliquefaciens*	0	97.99a	24.80a	20.05a	43.924a	10.594a
370	*G.intraradices*	0	83.12b	13.20bc	15.65ab	39.917a	9.587a
315	*G.intraradices*	0	85.52b	12.55bc	16.60a	37.811a	7.481a
II	*G.intraradices*	0	84.31b	15.35b	14.55ab	41.014a	10.684a
526	*G.intraradices*	0	96.90a	24.25a	20.25a	43.817a	10.487a
370	Control	0.06	34.22g	5.30d	4.25d	11.047c	4.387b
315	Control	0.06	32.56g	4.65d	5.55cd	10.063c	4.519b
II	Control	0.06	37.78g	4.85d	5.30cd	11.066c	4.406b
526	Control	0.06	41.71f	5.40d	5.45cd	11.057c	4.397b
370	*B.amyloliquefaciens*	0.06	49.12e	9.05cd	7.70cd	12.743c	4.979b
315	*B.amyloliquefaciens*	0.06	48.21e	8.90cd	7.85cd	12.066c	4.269b
II	*B.amyloliquefaciens*	0.06	42.12f	8.70cd	7.70cd	12.147c	4.777b
526	*B.amyloliquefaciens*	0.06	51.44e	13.90b	8.60cd	13.743c	5.979b
370	*G.intraradices*	0.06	48.56e	6.00d	6.85cd	11.066c	5.569b
315	*G.intraradices*	0.06	48.22e	8.45cd	7.05cd	11.947c	4.777b
II	*G.intraradices*	0.06	44.32ef	7.30d	7.20cd	10.853c	4.199b
526	*G.intraradices*	0.06	50.70e	12.80bc	7.80cd	12.846c	4.076b
370	Control	0.12	15.21j	1.35ef	1.10e	7.740cd	0.904bc
315	Control	0.12	13.56j	1.65ef	1.50e	7.033cd	1.082bc
II	Control	0.12	13.12j	1.95ef	1.50e	7.049cd	1.518bc
526	Control	0.12	17.32j	1.55ef	1.25e	7.510cd	0.904bc
370	*B.amyloliquefaciens*	0.12	26.11h	3.10de	6.15cd	7.036cd	1.062bc
315	*B.amyloliquefaciens*	0.12	21.98i	3.90de	6.90cd	8.719c	1.018bc

Variety	Inoculant*	NaCl concentration (M)	Germination (%)	Plant height (cm)	Root length (cm)	Fresh weight (g)	dry weight (g)
II	*B.amyloliquefaciens*	0.12	29.11gh	2.55de	6.25cd	8.730c	0.944bc
526	*B.amyloliquefaciens*	0.12	29.23gh	3.95de	8.80bc	9.366c	1.062bc
370	*G.intraradices*	0.12	27.21h	3.85de	5.65cd	9.269c	1.018bc
315	*G.intraradices*	0.12	23.14hi	3.00de	5.55cd	8.730c	0.944bc
II	*G.intraradices*	0.12	28.22gh	3.15de	6.60cd	8.476c	1.082bc
526	*G.intraradices*	0.12	28.57gh	3.95de	8.70bc	9.709c	1.018bc

Means followed by the same letter are not significantly different at $P = 0.05$. Comparisons were made within columns using Duncan's Multiple Range Test. Values are means of five replicates. *Bacteria ($1x10^8$ CFU mL^{-1}); AMF (120 propagules·cm^3). control=C without inoculants.

Table II. Effects of *Bacillus amyloliquefaciens* and *Glomus intraradices* on average values of germination (%), root length, plant height, fresh and dry weights of seedlings in 526 Var. of *Sorghum* spp., under three concentrations of NaCl at temperatures of 5-15, 10-20, 15-25, 20.30 and 25-35°C.

Variety	Inoculant*	Temperature regimens °C	NaCl concentration (M)	Germination (%)	Plant Height (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
526	Control	5/15	0	77.05b	11.42b	16.82c	62.241b	6.882a
526	*B.amyloliquefaciens*	5/15	0	82.35ab	14.57b	18.77c	80.244a	8.901a
526	*G.intraradices*	5/15	0	82.00ab	14.02b	16.97c	79.247a	8.811a
526	Control	5/15	0.06	39.05d	5.17d	7.17e	14.267cd	2.703b
526	*B.amyloliquefaciens*	5/15	0.06	52.25c	8.67d	8.32e	20.283c	3.094b
526	*G.intraradices*	5/15	0.06	51.85c	8.57d	8.52e	22.276c	3.115b
526	Control	5/15	0.12	18.65e	2.32de	2.97ef	14.280cd	0.604c
526	*B.amyloliquefaciens*	5/15	0.12	42.05d	8.72d	5.52e	19.246c	0.768c
526	*G.intraradices*	5/15	0.12	41.65d	6.72d	5.42e	18.259c	0.751c
526	Control	10/20	0	78.00b	9.42d	15.51c	63.141b	7.012a
526	*B.amyloliquefaciens*	10/20	0	83.30ab	14.42b	19.82c	81.132a	8.911a
526	*G.intraradices*	10/20	0	82.95ab	13.22b	18.22c	80.238a	8.281a
526	Control	10/20	0.06	40.00d	6.72d	6.29e	15.137cd	2.453b
526	*B.amyloliquefaciens*	10/20	0.06	53.20c	8.87d	9.79e	21.273c	3.124b
526	*G.intraradices*	10/20	0.06	52.80c	7.37d	10e	23.276c	3.215b
526	Control	10/20	0.12	19.60e	3.97de	3.09e	15.260cd	0.658c
526	*B.amyloliquefaciens*	10/20	0.12	43.00d	7.82d	7.55e	20.226c	0.759c
526	*G.intraradices*	10/20	0.12	42.60d	7.49d	7.57e	19.239c	0.793c

Variety	Inoculant*	Temperature regimens °C	NaCl concentration (M)	Germination (%)	Plant Height (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
526	Control	15/25	0	85.20a	15.82bc	15.51c	65.361b	7.342a
526	*B.amyloliquefaciens*	15/25	0	99.50a	26.97a	30.05a	83.244a	8.341a
526	*G.intraradices*	15/25	0	99.15a	26.42a	28.47a	83.115a	8.341a
526	Control	15/25	0.06	40.20d	8.57d	6.18e	16.237cd	2.673b
526	*B.amyloliquefaciens*	15/25	0.06	53.40c	15.07b	14.85c	22.263c	3.784b
526	*G.intraradices*	15/25	0.06	53.00c	14.97b	15.05c	22.146c	3.215b
526	Control	15/25	0.12	19.80e	1.72e	1.96ef	16.260cd	0.638c
526	*B.amyloliquefaciens*	15/25	0.12	43.20d	5.12d	12.05cd	21.776c	0.732c
526	*G.intraradices*	15/25	0.12	42.80d	3.12de	11.96cd	20.479c	0.716c
526	Control	20/30	0	78.20bc	19.82ab	15.83c	64.131b	6.982a
526	*B.amyloliquefaciens*	20/30	0	83.50ab	25.97a	25.29ab	82.142a	8.841a
526	*G.intraradices*	20/30	0	83.15ab	25.42a	23.5b	82.338a	8.921a
526	Control	20/30	0.06	43.20d	9.57d	5.85e	17.137c	2.433b
526	*B.amyloliquefaciens*	20/30	0.06	56.40c	18.07b	19.72c	24.033c	3.334b
526	*G.intraradices*	20/30	0.06	56.00c	17.97b	19.59c	24.036c	3.235b
526	Control	20/30	0.12	22.80e	2.72de	1.76ef	17.270c	0.615c
526	*B.amyloliquefaciens*	20/30	0.12	46.20d	7.12d	16.82c	22.686c	0.769c
526	*G.intraradices*	20/30	0.12	45.80d	5.12d	16.59c	21.699	0.693c
526	Control	25/35	0	79.20b	14.32bc	15.5c	64.831b	6.222a
526	*B.amyloliquefaciens*	25/35	0	84.50ab	25.47a	33.29a	82.742a	8.991a
526	*G.intraradices*	25/35	0	84.15ab	24.92a	31.49a	82.128a	8.111a
526	Control	25/35	0.06	41.20d	8.07d	6.19e	16.327cd	2.213b
526	*B.amyloliquefaciens*	25/35	0.06	54.40c	16.57b	22.5b	22.363c	3.334b
526	*G.intraradices*	25/35	0.06	54.00c	16.47b	22.82b	22.956c	3.785b
526	Control	25/35	0.12	20.80e	1.22de	4.54e	16.380cd	0.670c
526	*B.amyloliquefaciens*	25/35	0.12	44.20d	5.62d	19.82c	21.126c	0.774c
526	*G.intraradices*	25/35	0.12	43.80d	3.62de	19.83c	20.139c	0.774c

Means followed by the same letter are not significantly different at $P = 0.05$. Comparisons were made within columns using Duncan's Multiple Range Test. Values are means of five replicates. *Bacteria ($1x10^8$ CFU mL^{-1}); AMF (120 propagules·cm^3). control=C without inoculants.

Table III. Results of two way ANOVA carried out on factors of salinity, thermoperiod, microorganisms and their interactions.

Independent variable	Salinity	Thermoperiod	microorganisms	Salinity*Thermoperiod
Percent germination	0.000000	0.000000	0.000000	0.6419056
High plant	0.000000	0.000000	0.016424	0.224755
Root length	0.153204	0.000000	0.000000	1.000000
Fresch weigh	0.000000	0.839958	0.000000	0.977109
Dry weight	0.000000	1.000000	0.000001	1.000000

Note: Numbers are F-values, $P = 0.05$

Continuation….

Table III. Results of two way ANOVA carried out on factors of salinity, thermoperiod, microorganisms and their interactions.

Independent variable	microorganisms* Salinity	microorganisms* Thermoperiod	microorganisms*Thermoperiod* salinity
Percent germination	0.059789	0.138434	1.000000
High plant	0.035483	0.923315	0.833949
Root length	0.003487	0.000000	1.000000
Fresch weigh	0.000000	0.998041	0.969960
Dry weight	0.000000	1.000000	1.000000

Note: Numbers are F-values, $P = 0.05$

However, was detected that numerically among treatments inoculated with the bacterium PGP and AMF, stood out *B. amyloliquefaciens* at 20/30 °C, with a minimum difference compared with *Glomus intraradices* (99.50 vs 99.15, respectively), while non-inoculated treatment, it showed 85.30%. According to with plant height variable, highest values were obtained with 0 M NaCl, with both beneficial inoculants compared with control non-bioinoculated and at all temperatures excepted 5/15 °C. Similar behavior was displayed in root length, fresh and dry weight variables. Different inoculants, temperatures and several concentrations of salinity individually, and their interaction, significantly affected the rate of germination of 526 variety seeds (Table III).

DISCUSSION

Sorghum acts as a dietary staple for millions of people living in about 30 countries in the subtropical and semi-arid regions of Africa, Asia, and Mexico (Rakshit *et al*. 2014; Hariprasanna and Rakshit 2016). It is a source of food and fodder, mostly in the traditional, smallholder farming sector. Maunder (Maunder 1999), and Juarez and Harrison (20), indicate that in dry arid zones, more than 80 % of the global *Sorghum* area is characterized by low yield levels due to the low content of organic matter, and in this sense, farmers should apply many chemical fertilizers to supply N deficiencies. To overcome the challenge of increasing food production with a significant reduction of agrochemical use and environmental pollution, and an increase of natural resource productivity, the use of soil microorganisms in basic crops like *Sorghum* is essential. Two groups of microorganisms consist of plant growth-promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF), which have been studied from the beginning of the twentieth century and their mode of action at the physiological level is currently well understood (Bashan 1998; Vessey 2003; Ruzzi and Aroca 1980).

Sorghum spp. includes different varieties which are sowed along of northwest of México in two seasons each year. *Sorghum* seeds are exposed to two different environments at planting seasons; from May to June, the temperature exceeds 47 °C, and the relative humidity does not exceed 5%; so the water potentials inside of halotolerant plants (sap) and in soil show values between 7 and 8 –Mpa, respectively; this phenomenon decreases until September and October below 5 and 4 -Mpa in gross sap and soil respectively. Therefore, this abiotic stress present in desert areas must be reduced and strengthened by the interaction of beneficial microorganisms as our study showed and with the variety "526" (Table I, II). Studies related to interaction plant and beneficial microorganisms agree with our findings (Baldani and Dobereiner 1980; Bashan *et al*. 2014; Bashan *et al*. 2012), who indicated that results depend on strain-host plant specificity. Specifically with *Sorghum* studies,

our results are strengthened with those found by Sarig *et al*. (Sarig *et al*. 1992), Issopi *et al*. (1995), James *et al*. (1997), Stein *et al*. (1997), they obtained favourable results with the inoculation of beneficial microorganisms like a vesicular-arbuscular mycorrhizas and *Acetobacter diazotrophicus*; *Herbaspirillum*, as a endophytic diazotroph colonizing vascular tissue in leaves; *Azospirillum brasilense* inoculation on growth dynamics and hydraulic conductivity; and the contribution of nitrogen fixation by Azoarcus sp. BH72 in *Sorghum*, respectively. However, disagree with Arsac *et al*. (1990), Okon and Labandera (1994), who stated that the effect is not strain-dependent among different plant species.

Our study shows that low temperatures reduce germination; the contrary was observed in germination percentage with an increase in temperature (Table 2). For its part, while the salinity was reduced, the germination was increased. At 15 °C and under 0.12 M NaCl, our 526 var. germinated and showed tolerance to salinity; however the germination of this seed was more effective when we inoculated PGPB; results agreeing with others results in others crops (Khan 1991); Khan and Weber (1986), reported maximum salinity tolerance ranges from 200 to 1720 mM NaCl in halophytes such as *S. bigelovii*, *S. europaea*, *S. stricta*, *Cressa cretica*, and *Suaeda moquinii*. These reports indicate that 526 variety could be classified into the salt-tolerant one during germination.

In this 2nd phase, the temperature increasing germination percentage in 2 and 13% at 20/30 and 15/25, respectively, compared with the 1st phase using 27 °C in a constant form (at 0 M NaCl and both bio-inoculants). Moreover, the same variety in the other variables (plant height, root length, fresh and dry weight variables), its values were significant vs control non inoculated when seeds were inoculated with PGPB and AMF. Considering a relationship between all the variables with the different temperatures and salinity studied, some plants showed variability in their length and weight in their roots and whole plant. This is attributed to the capacity of the plant to tolerate in its interior ions and the production of glycophytes and osmolytes, which are have a function of maintaining an ionic homeostasis between several tissues and intracellular compartmentalization; effects visualized in desert species of the Great Basin, subtropical maritime deserts of Pakistan, Haloxylon recurvum, Zygophyllum simplex and Arthrocnemum macrostachyum (Khan and Ungar 1997; Khan 1991; Gul, *B.*; Weber 1999), respectively.

All varieties studied in this study and especially "526" obtained, was benefit using beneficial microorganisms by *B. amyloliquefaciens* and *G. intraradices* abilities. Other studies indicate positive effects using plant growth-promoting microorganisms (Afek *et al*. 1990), results that coincide with those obtained in the present study, appreciating that the PGPB of this study and experiencing it with var. 526 *Sorghum*, positively was affected. These effects are possible to the participation of responsible substances in the growth of plants (Afek *et al*. 1990; Puente Bashan 1993; Goodfriend *et al*. 2000; Díaz *et al*. 2001). Similarly, some studies support *G. intraradices* in the promotion of plant development (Melgares *et al*. 2004), results that are consistent with the findings in this evaluation. Similar results were obtained for other plants and beneficial microorganisms (90). However, other assays although they were carried out with other plants and other beneficial microorganisms, also some inhibitive effects on germination were observed (Díaz *et al*. 2001).

Plants exposed to saline stress change their environment, and one of the first vegetative phases is germination. In this first stage, osmotic stress begins to manifest itself, mainly by stopping the emergence of the radicle and its subsequent roots. The recoil is associated with an accumulation of ionic stress that generates phytotoxicity, and showing an imbalance in nutrient intake and absorption (Asish and Anath 2005), oxidative stress at the subcellular level, mediated by reactive oxygen species (ROS) (Acosta-Motos *et al*. 2017), mainly. These responses to salinity in plants tolerant to NaCl, implement adaptations so as not to be affected by salinity and carry out their survival. There are mechanisms inside the cells that promote an adaptation that is reflected in their morphology, physiology, biochemistry and molecular (Acosta-Motos *et al*. 2017). In the germination stage, among the first existing metabolic actions to tolerate salt, it is determined by multiple routes that influence in water retention and / or acquisition, protecting organelles and maintaining homeostasis. Given this situation, that the seeds or new plants are subject, the beneficial microorganisms as a PGPB or AMF, release bio substances that generate hormonal changes within the plants, the production of volatile organic, benefiting in the taking of nutrients and a tolerance to abiotic stress such as salinity (Ruzzi and Aroca 2015). Same authors indicate that oxidative stress in different phases in the plant is linked to the electron transport chain in membranes of mitochondria and chloroplast during ATP production. Therefore,

numerous energetically demanding physiological processes like germination and seedling development are associated with oxidative stress (Bailly 2004). It is well known that mitochondria are the principal subcellular compartment of oxygen consumption and the principal source of reactive oxygen species (ROS) (Puntarulo *et al*. 1998). Most of the ATP required for early stages of development comes from mitochondria; therefore, generation of ROS during this process is mainly associated to cellular respiration. At later stages of development, photophosphorylation in chloroplast becomes the main source of ATP to cover the energy requirement for proper development; then, chloroplast also becomes a primary source of ROS (Asada 2006), and it is possible that the release of hormones by beneficial microorganisms inside and attached to plants, the production of volatile organic compounds, help to improve nutrient intake and improve tolerance to abiotic stress (García *et al*. 2018).

CONCLUSIONS

This study is the first step to obtain an ideal variety of *Sorghum*, which is a principal crop in dry arid and desert zones of México. This kind of study promotes beneficial microorganisms as a resource to increase the development and productivity of plants. Moreover, studies with the interaction of *B. amyloliquefaciens* and *G. intraradices* with 526 var. of *Sorghum* are necessary to decide the magnitude to which these observations can be observed and reproduced under one production system.

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Data availability. Data are available with the corresponding author (erueda04@santana.uson.mx) upon reasonable request

Compliance with ethical standards. There was strict adherence to the Global code of conduct for research in resource-poor settings following the Convention on Biological Diversity and Declaration of Helsinki.

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