Native microbial consortia improve maize shoot and root systems at early developmental stages in a seedbed assay

Consorcios de microorganismos nativos mejoran los sistemas caulinar y radicular del maíz en etapas tempranas de desarroll en un ensayo de semillero

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RESUMEN
Antecedentes: La agricultura es uno de los principales contribuyentes a la degradación ambiental. Los microorganismos del suelo son esenciales para mejorar el crecimiento de las plantas, el rendimiento de los cultivos y la tolerancia al estrés.

Objetivo: Caracterizar la respuesta temprana del maíz en condiciones de semillero a consorcios nativos de microorganismos aislados de zonas áridas.

Métodos: Dieciséis hongos y 16 bacterias de suelos áridos fueron identificados por MALDI-TOF MS y verificados por características morfológicas. Se evaluaron diez biofertilizantes con réplicas (n=100) en maíz bajo condiciones de semillero. Los consorcios se formularon basándose en caracteres promotores del crecimiento, incluyendo principalmente especies de Penicillium y Pseudomonas. Después de 45 días, los biofertilizantes se evaluaron midiendo altura de la planta y peso fresco de los brotes y raíces.

Resultados y Conclusiones: Penicillium y Pseudomonas fueron los géneros predominantes. Los consorcios bacterianos promovieron principalmente el desarrollo caulinar, mientras que la combinación de hongos y bacterias aumentó notablemente el desarrollo de las raíces. Ocho consorcios microbianos de zonas áridas tuvieron efectos positivos en la etapa temprana del desarrollo del maíz en condiciones de semillero en comparación con plantas no inoculadas.

Palabras clave: Agricultura sostenible, biofertilizante, microorganismos promotores del crecimiento de plantas, consorcios microbianos, microorganismos del suelo

ABSTRACT
Background: Agriculture is a major contributor to environmental and soil degradation. Soil microorganisms are essential to improve plant growth, crop yields and stress-tolerance.

Objective: To characterize maize early plant-response in a seedbed setting to native consortia of isolated microorganisms from arid zones.

Methods: Sixteen fungal and 16 bacterial isolates from arid soils were identified by MALDI-TOF MS and confirmed using morphological characteristics. Ten biofertilizers were tested in replicates (n=100) in maize under seedbed conditions. Consortia were formulated based on growth promoting traits, including mainly Penicillium and Pseudomonas species. After 45 days, biofertilizers were evaluated according to plant height, and shoot and root fresh weight.

Results and Conclusions: Penicillium and Pseudomonas were the predominant genera identified. Most strains are potential candidates for biofertilizer formulation based on their growth promoting traits. Bacterial consortia mainly promoted plant caulinar development, while the combination of fungal and bacterial species markedly increased root development. Eight biofertilizer consortia from arid zones had positive effects at early developmental stage of maize under seedbed conditions compared to uninoculated plants.

Keywords: Sustainable agriculture, biofertilizer, plant growth promoting microorganisms, microbial consortia, soil microorganisms
INTRODUCTION

The global rise in human population is driving a steady increase in the demand for food as it will be necessary to increment its production by 70 % in 2050, estimating that 90 % of the growth of agricultural production will be possible by obtaining higher yields, meaning crop intensification (FAO, 2009; Mahanty et al., 2017; Carrington, 2018; Adisa et al., 2019). The conventional agricultural system is designed for massive food production, increasing yields and decreasing production costs at the expense of high energy consumption and excessive use of fertilizers, pesticides and water; consequently, degrading the environment through air and water pollution, soil depletion, and loss of soil ecosystems and biodiversity (Horrigan et al., 2002; Glick, 2016; DeLonge et al., 2016; Mahanty et al., 2017; El-Ghamry et al., 2018; Kumari and Singh, 2019). Particularly, chemical fertilizers are extensively applied to sustain the growing demand for food. According to the FAO, worldwide consumption of chemical fertilizers was 191.98 million tons in 2019 (FAO, 2019), considering that plants use between 20 % and 50 % of the applied fertilizer, there is a high rate of fertilizer release into the environment (Drechsel et al., 2015; Tomer et al., 2016; Mahanty et al., 2017; El-Ghamry et al., 2018; Kumari and Singh, 2019).

The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) details that intensified cropping and rapid expansion of croplands have placed agriculture as the main driver of soil degradation (IPBES, 2018; Leahy, 2018). Soil degradation is one of the greatest environmental problems faced by humanity (FAO, 2015; IPBES, 2018; Watts, 2018; Kopitke et al., 2019); it is reported that 33 % of the world’s land and 52 % of agricultural land are moderately or severely degraded (ELD, 2015; FAO, 2015), projecting that in the next 25 years soil degradation will reduce global food production by 12 % to 50 %, increasing food prices by 30 % (United Nations, 2010; ELD, 2015). Agriculture faces major challenges to improve food security and implement more sustainable and less harmful production strategies (FAO, 2009). Implementation of sustainable agriculture is a viable alternative to meet these challenges as it involves the development of cost effective, eco-friendly and high efficiency procedures (Malusá et al., 2012; DeLonge et al., 2016; Busby et al., 2017; Prasad et al., 2017; El-Ghamry et al., 2018; Adisa et al., 2019; Elemike et al., 2019; Kumari and Singh, 2019). The use of biofertilizers is a very promising sustainable practice since they improve water use efficiency, increase crop yields from 10 to up to 40 % (Bhardwaj et al., 2014), reduce chemical fertilization (35 to 50 %) without compromising crops yield (Kumar et al., 2009; Isfahani and Besharati, 2012; Aggani, 2013; Saeed et al., 2015a, b; Nurbaity et al., 2016; Guardiola-Márquez et al., 2019), and improve plant resistance to adverse environmental conditions (Jochum et al., 2019; Ojuederie et al., 2019). Beneficial microorganisms from biofertilizers colonize the rhizosphere and root system of the plant and promote growth through different mechanisms such as siderophores production, atmospheric nitrogen fixation, solubilization of minerals (phosphorus, potassium), and production of phytohormones (auxins, cytokinins, gibberellins) and enzymes (phosphatases, catalases) (Vessey, 2003; Bardi and Malusa, 2012; Malusa and Vassilev, 2014; Alori et al., 2017; Mahanty et al., 2017; El-Ghamry et al., 2018; Gouda et al., 2018).

Even though biofertilizers represent a promising alternative, most of them are produced from commercial microbial strains that may not be adapted to adverse climatic conditions, hindering their colonization and survival (Horrigan et al., 2002; Aggani, 2013; Gupta et al., 2015; Gouda et al., 2018). In this sense, the use of native communities of soil microorganisms is essential for the development of biofertilizers for arid environments. The efficiency of microbial inocula increases when native species of plant growth promoting (PGP) microorganisms are used as they show higher ability to increase crop yields and plant stress-resistance, greater resistance against pathogens and higher colonization rates since they have greater adaptability to local environmental conditions (Berruti et al., 2016; Emam, 2016; Sood et al., 2018). Also, several publications have shown that the use of microbial consortia as inocula is more effective to increase crop yields and growth promotion properties in plants, in comparison with individual strains (El-Afry et al., 2012; Wang et al., 2012; Naseem and Bano 2014; Kumar et al., 2016; Vurukonda et al., 2016; Ojuederie et al., 2019). A final consideration is that plants have different nutritional requirements at each developmental stage and microbial inocula represent a different metabolic expense for plants. Indeed, plant age can affect and change microbial communities (Roesti et al., 2006). Therefore, it
is important to evaluate the effects of biofertilizers at different stages of plant development, being early stages important for plant establishment. Hence, the objective of this study was to evaluate the early plant-response of maize (Zea mays) to isolated native fungal and bacterial consortia from arid zones in a seedbed setting.

**MATERIALS AND METHODS**

**Microbial selection and identification**

Microbial species were previously isolated from root and soil samples of six economically relevant crops: watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai), onion (Allium cepa L.), walnut (Juglans regia L.), pepper (Capsicum annuum L.), alfalfa (Medicago sativa L.) and maize (Zea mays L.) from arid soils in northern Mexico. Crops were sampled at three different fields each, giving a total of 18 fields distributed in four towns located in the south center (Meoqui, Delicias and Saucillo) and one in south east (Camargo) of the State of Chihuahua. These towns exhibit extreme semiarid climate, average daytime temperature is 32 °C and rainfall is 328 mm per year (INEGI, 2018).

Isolates were screened for N-fixation, and phosphate and potassium solubilization. N-fixation was determined on N-free solid malate medium (Nfb) (malate: 5 g L−1, KOH: 4 g L−1, K2HPO4: 0.5 g L−1, FeSO4·7H2O: 0.05 g L−1, MnSO4·H2O: 0.01 g L−1, MgSO4·7H2O: 0.01 g L−1, NaCl: 0.02 g L−1, CaCl2·2H2O: 0.01 g L−1, Na2MoO4·2H2O: 0.002 g L−1, pH 6.8, 1 % Bromothymol blue solution). Nitrogen fixing microorganisms were identified by a color change from pale green to blue (Syed-Ab-Rahman et al., 2018; Kuan et al., 2016; Goswami et al., 2015). Phosphate solubilization and potassium solubilization were evaluated with commercial Pikovskaya agar medium and Aleksandrov agar medium, respectively. Media were added with 1 % Bromothymol blue (BTB) solution to improve visualization of the clearing zones. P and K solubilizing microorganisms were identified by a color change from pale green to yellow and by the presence of clear halo zones around the colonies (Syed-Ab-Rahman et al., 2018; Kuan et al., 2016; Lima-Rivera et al., 2016; Rajawat et al., 2016; Sharon et al., 2016; Goswami et al., 2015; Zhang and Kong, 2014; Gupta et al., 2012). Plates were incubated at 30 °C for 5 days for bacteria, and 20 °C in darkness for 10 days for fungi.

Positive strains to each growth-promoting trait, with visual morphological differences, were sub-cultured by transferring them into the same agar medium to obtain pure colonies. Isolates were classified by the size of the clear halo zone measured from the edge of the colony. For P and K solubilization the halo zone was classified as level 1 (1 mm), level 2 (2-4 mm) and level 3 (≥5 mm). In the case of N-fixation, 5 mm, 10-14 mm and ≥15 mm, for level 1, 2 and 3, respectively. Selected isolates (level 3) were transferred back to enriched media for storage at 4 °C and subsequent characterization.

Isolates were identified by MALDI-TOF mass spectrometry (MALDI-TOF MS). For this, selected bacterial isolates (level 3 of PGP trait) were sub-cultured to obtain clearly separated colonies in standard solid medium without nutrient limitation [tryptic soy agar (TSA)]. Spore-free fungal cultures were prepared in 25 mL PDB medium incubated in the dark for 7 days at 20 °C and 130 rpm, cultures were washed three times with sterile distilled water. Bacterial samples were taken directly from TSA plates (48 h, 30 °C); plates were stored for 2 days at 4 °C until analysis. For fungal samples, a protein extract (ethanol/formic acid extraction) described by Bruker Daltonik (2011) from spore-free fungal cultures: PDB, 7 days, 20 °C, 130 rpm, was prepared for identification. One μL of the microbial material was transferred to the MSP 96 polished steel BC target. The sample spot was air dried at room temperature and covered with 1 μl of saturated α-cyano-4-hydroxy-cinnamic acid (CHCA) matrix solution in 50 % acetonitrile, 47.5 % water, 2.5 % trifluoroacetic acid. Each sample was analyzed by triplicate using a Microflex LT (Bruker Daltonics) MALDI-TOF mass spectrometer. Mass spectra were compared with reference mass spectra using the MALDI BIOTYPER 3.1 software with FILAMENTOUS FUNGI and BDAL databases. The software estimates a score value between 0 and 3 to determine the similarity between the sample and reference spectrum. Scores between 2.300 and 3.000 represented a high identification reliability at species level, whereas scores between 2.000 and 2.299 provided a high reliability of identification at the genus level (probable species identification), scores between 1.700 and 1.999 represented a probable genera identification and scores of 1.699 and below represented an unreliable identification (Schulthess et al., 2014; Bruker Daltonik, 2011).

From the identified microbial species, six fungal and nine bacterial species were used in this study. To verify
that the isolates were axenic and that their microscopic characteristics corresponded to the indicated species, cultures were examined microscopically using Gram and lactophenol cotton blue stain for bacteria and fungi, respectively. Cell morphology was observed using a light microscope using a Leica EC4 Camara (Leica DM750, Germany). Plant growth promoting activity of each identified species was determined by reports in scientific literature.

**Fungal and bacterial inocula propagation**

Selected bacterial strains were individually grown in glass bottle flasks containing 250 mL of tryptic soy broth (TSB) and incubated on a rotary shaker at 180 rpm and 30 °C for 72 h. Bacterial growth was monitored spectrophotometrically until an optical density (600 nm) between 0.8 and 1 was reached, which corresponded to plate counts of $10^7-10^8$ CFU mL$^{-1}$. Bacterial growth was confirmed by plate count in TSA plates (24 h, 30 °C), dilutions in which bacteria formed between 30 and 300 colonies were considered to perform plate count.

For fungal inocula, potato dextrose agar (PDA) plates were inoculated and spores of 10-day old cultures were collected. For this, 5 mL of sterile distilled water were added to each plate and colonies were scraped to create a spore suspension. Spores were then transferred to a sterile 50 mL Falcon tube and adjusted to maximum volume with sterile distilled water. A Neubauer chamber was used to perform spore count.

Bacterial and fungal inocula were stored at 4 °C for a maximum of one week until biofertilizer formulation and application.

**Microbial consortia formulation**

To prepare the microbial consortia, a final concentration of $10^7-10^9$ CFU mL$^{-1}$ and $10^6$ spores mL$^{-1}$ were used for bacteria and fungi, respectively. Inocula were mixed according to their growth promoting traits (nitrogen fixation (NF), phosphorus solubilization (PS), potassium solubilization (KS)), resulting in ten treatments (Table 1). It is also important to evaluate the effect on plants resulting from the type of interaction between fungi and bacteria. Controls were uninoculated plants. Equal volumes of each cell suspension were mixed according to the microbial consortia formulation.

**Experimental design and culture conditions**

Experiments were carried out as a completely randomized design in plastic seedbeds of 72 cavities (5 cm in diameter, 5 cm depth), filled to ¾ of their capacity with commercial disinfected non-sterile black soil Nutrigarden®. Treatments were evaluated in maize (Zea mays) hybrid H-70, each cavity was sown with two seeds at 0.5 – 1 cm deep, considering ten replicates per treatment. Seedlings were thinned down to one plant per plot after emergence. Inoculation was done at day 4, 14 and 24 by applying 0.2 mL of the microbial consortia formulation to each cavity of each microbial consortium.

<table>
<thead>
<tr>
<th>Table 1. Formulation of microbial consortia for seedbed testing</th>
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<tbody>
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<td><strong>Treatment ID</strong></td>
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<tr>
<td>T1</td>
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<td>T3</td>
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<td>T4</td>
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<td>T7</td>
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<td>T8</td>
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<td>T9</td>
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<tr>
<td>T10</td>
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<tr>
<td>Control</td>
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</table>

NF, PS and KS refers to nitrogen fixation, phosphorus solubilization and potassium solubilization, respectively.
treatment in the seedbed. The assay was done from March to May of 2020 in outdoors conditions with a natural light cycle and an average maximum and minimum temperature of 33 °C and 12 °C, respectively, during the experimental period. Plants were watered twice a day (10 mL) with tap water during the first three weeks of the experiment; later irrigation was reduced 50 % for the next three weeks.

**Plant growth measurements**

Forty-five days after planting, plants were harvested. The effect of the inoculants was determined according to plant height (cm), plant fresh weight (g), root fresh weight (g) and total fresh weight (g). Plant height was evaluated two times (at the middle and end of the experimental period), from the soil surface to the highest point of the plant. Weight was recorded using a compact balance (AandD Weighing EK-600i), averaging three measurements to set the final value. Root weight was recorded after shaking the plant to remove all soil particles.

**Statistical analysis**

Data was analyzed using one-way analysis of variance (ANOVA) and the Tukey test to determine statistical significance of the treatments on plant development. Significance level was P <0.05. Normality was confirmed with Shapiro-Wilk Test. The Real Statistics Resource Pack for Microsoft Excel 365 was used to perform the analysis.

### RESULTS

**Microbial selection and identification**

Bacterial and fungal species were tested for N₂ fixation, and solubilization of phosphate and potassium using N-free solid malate medium (Nfb), Pikovskaya agar medium and Aleksandrov agar medium, respectively. All media were added with 1 % bromothymol blue solution to improve halo and colony visualization and resolution. Positive microorganisms for each PGP trait were sub-cultured in the same medium, where they were isolated and classified depending on the activity level of the trait. Level 1 to 3 corresponded to 1 mm, 2-4 mm and ≥ 5 mm, respectively. For bacteria from level 3, 16 isolates with the largest halos were selected which contemplated six nitrogen fixers, four phosphate solubilizers and six potassium solubilizers. For fungi from level 3, 16 strains were selected, including five nitrogen fixers, six phosphate solubilizers and six potassium solubilizers.

Sixteen bacterial and 16 spore-free fungal cultures were identified by MALDI-TOF MS obtaining 81 % identification efficiency. Fungal isolates generated lower identification scores than bacteria, most fungi scores were between 1.7 and 1.8 (probable genera), while bacteria were from 1.8 to 2.1 (genus-level, probable species). Score differences can be associated with database variation, bacterial databases have greater species diversity and are more studied and updated than fungal databases. Nine out of 16 (56.3 %) fungi samples were identified, including four genera and eight species. The *Penicillium* genus was the most predominant (Table 2). For bacteria, 15 out of 16 (93.75 %) samples were identified, including three genera and nine species. In this case, *Pseudomonas* was the most abundant genus. Because some identified species had scores below two, microscopic and macroscopic morphological characteristics were used to confirm their identification.

**Morphological studies of microbial isolates**

Microscopic characteristics of the isolates were studied using light microscopy and specific stains for bacteria and fungi (i.e., Gram stain and lactophenol cotton blue stain, respectively). Consequently, MALDI-TOF microbial identification was verified with these observations. Figure 1 shows microscopy images of the fungal strains. Fungal strains classified as *Penicillium* species coincided with typical morphological characteristics of this genus, a filamentous fungus with simple or branched conidiophores ending in phialides organized in a brush-like bunch (Figure 1 A-F) (Smith et al., 1990). Morphology of the other fungal strains was in accordance with the species identification, *Fusarium* sp. (Figure 1 G) presented oval microconidia (Rahjoo et al., 2008) and *Aspergillus* sp. (Figure 1 H) presented conidiophores that terminate in a characteristic conidial head with conidia in one-celled circular structures (Diba et al., 2007).

Figure 2 shows the different bacterial isolates under Gram stain. As observed, all strains were Gram negative (i.e., they stained with safranin acquiring a pink-red color) and showed a rod-shaped bacilli morphology. These characteristics are in agreement with the characteristics of *Pseudomonas*, *Serratia* and *Bacillus*; *Bacillus* appeared as an elongated straight rod-shaped bacilli, while *Pseudomonas* and *Serratia* seem much smaller and slightly curved (Lindsay and Von Holy, 1999; Joung and Côté, 2002).
Table 2. MALDI-TOF mass spectrometric identification of fungal and bacterial isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sample number</th>
<th>MALDI-TOF Result</th>
<th>MALDI-TOF score</th>
<th>Reliability</th>
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</tr>
<tr>
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<td>Pseudomonas fragi</td>
<td>2.094</td>
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<td>Genus-level, probable species</td>
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</table>

Reliability score: 2.300 to 3.000 correspond to high reliability at the species level, 2.000 to 2.299 high reliability at the genus level and probable species identification, 1.700 to 1.999 probable identification at the genus level and < 1.699 unreliable identification.
Early plant response to microbial consortia in a seedbed assay

Ten microbial consortia were formulated and tested for initial plant response in maize. Forty-five days after planting, seedling growth parameters were assessed. Biofertilizer effect was measured according to maximum height, root and shoot fresh weight, and total fresh weight. Microbial consortia showed significant effects on maize growth compared to uninoculated plants, except for T2 whose effect was similar to the control in all parameters (Figure 3); also, plants grew healthy with no disease symptoms. Results showed that maximum height and shoot weight were signifi-
cantly influenced mainly by bacterial consortia (T4 to T7). These treatments had an increase in plant height of 24 to 33 % compared to the control, while only one fungal formulation (T3) had an effect on height, 30 % higher than the control (Figure 3 A). Shoot biomass was significantly improved by treatments T4, T5 and T7 by 60-70 % (Figure 3 C).

Regarding root weight, it was mainly influenced by the fungal formulation T3 and the combination of bacterial and fungal consortia (T8 to T10), achieving an increase in root weight between 71 and 85 % (Figure 3 B). Figure 4 shows a comparison of plants under treatments T3 (A), T5 (B), T8 (C) and T7 (D). They all showed similar shoot characteristics but evident differences in the root
Figure 3. Microbial consortia effects on outdoor grown maize. A: Maximum height. B: Fresh root weight. C: Fresh shoot weight. D: Total fresh weight. * Significant difference from control ($P < 0.05$) using one-way analysis of variance and Tukey test ($n=100$), data represent average of each parameter ± standard error. T1: Penicillium consortia (H1, H10, H12, H13). T2: Penicillium consortia (H2, H14), Penicillium oxalicum (H15), Aspergillus sp. (H16). T3: Penicillium consortia (H5, H8), Fusarium sp. (H9). T4: Pseudomonas consortia (B1, B2, B4, B8, B10, B15). T5: Pseudomonas consortia (B6, B13, B14, B16). T6: Pseudomonas consortia (B3, B11, B12), Serratia liquefaciens (B5), Bacillus sp. (B7). T7: Pseudomonas consortia (B1-B4, B6, B8, B10-B16), Serratia liquefaciens (B5), Bacillus sp. (B7). T8: Penicillium consortia (H2, H14), Penicillium oxalicum (H15), Aspergillus sp. (H16). Pseudomonas consortia (B3, B11, B12), Serratia liquefaciens (B5), Bacillus sp. (B7). T9: Penicillium consortia (H1, H10, H12, H13), Pseudomonas consortia (B6, B13, B14, B16). T10: Penicillium consortia (H5, H8), Fusarium sp. (H9), Pseudomonas consortia (B1, B2, B4, B8, B10, B15). C: Uninoculated plants.

Figure 4. Maize growth measurements. A: Treatment 3, plant 2: maximum height 20.5 cm, root weight 2.5 g, shoot weight 1.2 g. B: Treatment 5, plant 2: maximum height 22 cm, root weight 1.3 g, shoot weight 1.7 g (not significantly different from the control). C: Treatment 8, plant 2: maximum height 20.5 cm, root weight 2.4 g, shoot weight 1.1 g. D: Treatment 7, plant 2: maximum height 23.5 cm, root weight 1.5 g, shoot weight 1.7 g (not significantly different from the control). E: Fungal growth on roots. F: Comparison of plants from T5 (left) and T3 (right) with similar shoot growth, but significantly different root development.
system, T3 and T8 had a significant \( P < 0.05 \) increment in root biomass. Also, root branching and lateral root development were more predominant based on visual characteristics. In general, the total plant weight was significantly improved \( (P < 0.05) \) by all treatments except T2 and T6, which obtained similar values to the control (Figure 3D). The total weight of the significant treatments was between 35 % and 65 % higher than the control (uninoculated plants).

**DISCUSSION**

MALDI-TOF mass spectrometry has been used successfully to characterize bacteria and fungi from soil samples (Avanzi et al., 2017; Borowik et al., 2017; Al-Kaabi et al., 2018; Pandey et al., 2019; Nazir et al., 2020). In this research, comparable efficiencies and score values were obtained. MALDI-TOF MS technique is described as a rapid and reliable tool to identify and differentiate microorganisms at genera level (Avanzi et al., 2017; Al-Kaabi et al., 2018). This experiment was focused on two main genera, *Penicillium* and *Pseudomonas*. *Penicillium* genus was the most abundant fungal genus, it is universally distributed in most environments, distinguished by its activity as a decomposer of organic compounds (Park et al., 2019). This genus is composed of around 200 known species, some of them with important industrial applications (Altaf et al., 2018). Moreover, *Pseudomonas* was the most predominant bacterial genus. Globally distributed, *Pseudomonas* is one of the most studied genera for its multiple plant growth promoting traits (Preston, 2004; Santoyo et al., 2012; Radhapriya et al., 2015; Widnyana and Javandira, 2016; Dorjey et al., 2017).

In this study, formulations of different microbial consortia as potential biofertilizers showed that treatments that were most effective in promoting growth and shoot biomass (T4 to T7), consisted of bacteria mainly of the genus *Pseudomonas*. Bacterial strains from these consortia have been reported by several authors as having multifunctional plant growth-promoting attributes with significant beneficial effects on plant growth including enhanced nutrient uptake, production of indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, siderophores and ammonia (Selvakumar et al., 2009; Adediran et al., 2015; Chen et al., 2015; Singh et al., 2015; Yadav et al., 2015; Fox et al., 2016; Jain and Pandey, 2016; Ogata-Gutiérrez et al., 2016; Romero et al., 2016; Kamran et al., 2017; Kong et al., 2017; Etminani and Harighi, 2018; Andreolli et al., 2019; Kour et al., 2019). Plant height and shoot growth promotion are controlled by various factors, especially nutrition. In this sense, the experimental plants were grown on the same substrate without fertilization, differences in nutrient absorption may be attributed to the effect of microorganisms as they increase nutrient bioavailability from the substrate, mainly by phosphorus and potassium solubilization, and nitrogen fixation. The amount of available nutrients has a positive correlation with the increase in plant height, especially nitrogen since it is the most important nutrient for plant growth, essential for synthesis of amino acids that constitute proteins and chlorophyll for the process of photosynthesis (Richardson et al., 2009; Pirasteh and Li, 2017).

Also, plant growth regulators produced by microorganisms influence many physiological plant processes. *Pseudomonas*, *Serratia* and *Bacillus* strains used in this study are reported to produce the auxin IAA (Kang et al., 2006; Selvakumar et al., 2009; Yadav et al., 2015; Ogata-Gutiérrez et al., 2016; Romero et al., 2016; Kong et al., 2017; Kour et al., 2019), which is essential for plant growth, controlling leaf formation, vascular tissue differentiation, embryo development, cell elongation, microbial-plant interactions, branching, apical dominance, and also root initiation and development, including improvements in root length, root branching, root hairs and lateral root formation (Mohite, 2013; Dutta et al., 2015; Fahad et al., 2015; Chandra et al., 2018; Ojuederie et al., 2019). However, IAA levels may not be sufficient since bacterial consortia (T4 to T7) did not influence root weight, whereas shoot development could be influenced by the production of other phytohormones. Gibberellic acid (GA) is a terpenoid hormone that is directly involved in cell elongation of stems and germination (Sharma et al., 2018). Several authors reported GA production by different *Pseudomonas* species (Prabavathy et al., 2011; Ponmurugan et al., 2011; Qessaoui et al., 2019). Sharma et al. (2018) evaluated GA production in thirty *Pseudomonas* isolates, reaching production levels ranging from 116.1 - 485.8 mg L\(^{-1}\). Pandya et al. (2011) described that *Pseudomonas* species produce more GA than other PGPBs (*Bacillus* sp., *Azotobacter* sp. and *Rhizobium* sp.), achieving a maximum level of 290 mg L\(^{-1}\).
Other authors have also shown positive effects of using *Pseudomonas* species in consortium with other bacterial species, describing synergistic activities to enhance plant growth. *Pseudomonas putida* and *Bacillus amylolyticus* improved plant growth and drought stress tolerance in chickpea (Vurukonda et al., 2016; Kumar et al., 2016, Glick, 2014). Azotobacter *chroococcum* and *Pseudomonas* sp. induced anatomical changes in the dermal and vascular tissue of wheat plants, improving growth and stress resistance (El-Afry et al., 2012). *Pseudomonas fluorescens*, *P. jessenii*, *P. synxantha*, *B. cereus*, and *Arthrobacter nitroguajacolicus* prevented oxidative stress in rice exposed to stress conditions (Gusain et al., 2015).

Regarding root development, the mixture of fungal and bacterial consortia (T8 to T10) significantly influenced root weight. In addition to the plant growth-promoting properties of bacterial species, fungal strains, mainly *Penicillium* and *Aspergillus* species, are recognized as potential plant growth promoters (Xiao et al., 2009; Gong et al., 2014; Ruangsanka, 2014; Panchala et al., 2015; Yin et al., 2015; Anand et al., 2016; Li et al., 2016; Malusá et al., 2016; Sahoo and Gupta, 2017; Banerjee and Dutta, 2019; Wang et al., 2020). On the other hand, some strains of *Fusarium* are recognized as plant pathogens, causing fusarium wilt and rot disease (Shanmugam and Kanoujia, 2011; Shanmugam et al., 2011, Shen et al., 2015; Xiong et al., 2017). However, it is also described that some non-pathogenic strains of *Fusarium* are able to promote plant growth, reduce nematode infections and increase arbuscular mycorrhizal fungi (AMF) colonization rates (Diedhiou et al., 2003). Therefore, it is suspected that the *Fusarium* sp. strain used in this study is a non-pathogenic strain since plants did not show disease symptoms and this microorganism was a component of one of the treatments with the best effects on shoot and root development (T3).

Mixing fungi and bacteria has been reported as an effective bioformulation to increase plant growth (Thakkar and Saraf, 2015; Schoebitz et al., 2016; Bilal et al., 2018). It has been hypothesized that the PGP traits of the microorganisms that make up a consortium exert an additive and complementary effect to enhance plant growth, thus the consortium effectiveness depends on the synergistic interaction of its components (Rashid et al., 2016). Fungi have larger and more resistant structures that bacteria and have the advantage of spreading and expanding more easily through the soil and the rhizosphere, increasing their effective area (Ortíz-Castro et al., 2009; Chandra et al., 2018). It is reported that fungi reach higher levels of phosphorus solubilization than bacteria; the solubilization levels of *A. niger* are around 468 mg L\(^{-1}\) soluble phosphate (Bhattacharya et al., 2015), while *P. libanensis* solubilizes only 199.10 mg L\(^{-1}\) (Kour et al., 2019). P-solubilizing activities are complemented by higher levels of IAA production in bacteria since the architecture of the root system is regulated by the auxin levels and nutrient availability, both trigger the initiation and development of lateral roots (Ortíz-Castro et al., 2009).

In arid environments, primary roots are not affected by drought conditions but growth of lateral roots is significantly limited, mostly by repression of the lateral root meristem. It is known that small lateral roots are important to increase the absorptive surface to resist environmental stress (Basu et al., 2016; Ngumbi and Klopper, 2016; Fahad et al., 2017). Indirect and direct improvements to the architecture of the root system is an important factor to increase plant tolerance to drought. Although surface water evaporates easily, in deeper layers there is enough moisture to supply water to the plant. A strong and extensive root system is essential to increase water use efficiency and capacity of the plant to reach and absorb nutrients, which increases crop yields (Wasaya et al., 2018). Therefore, application of native soil microorganisms as consortia may deliver multifunctional growth-promoting traits to plants, which are essential to enhance crop growth, and could increase plant drought stress tolerance by improving the root system. Nevertheless, further research is required to describe the effect of these species when plants are exposed to drought stress or other harsh environmental conditions.

**CONCLUSIONS**

Fungal and bacterial strains were isolated from arid zones, characterized for plant growth promoting traits, identified by MALDI-TOF and propagated to generate ten microbial consortia that were evaluated in maize under seedbed conditions. Significant growth promotion effects were found at an early plant developmental stage in maize growth; consortia had significant improvements on shoot growth and root development. Bacterial consortia mainly promoted development of plant
aerial biomass, while combination of fungal and bacterial species notably increased root biomass by the development of lateral roots and root hairs. Next steps of optimization of the evaluated microbial consortia will contemplate selection of species with the best performance and generate a highly effective biofertilizer composed of a microbial consortium that can be tested under greenhouse and field conditions.

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