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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 desarrollo 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

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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, 2014; 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; Kopittke *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}, K_2HPO_4: 0.5 g L^{-1}, $FeSO_4$: 0.05 g L^{-1}, $MnSO_4$: 0.01 g L^{-1}, $MgSO_4$: 0.01 g L^{-1}, NaCl: 0.02 g L^{-1}, $CaCl_2$: 0.01 g L^{-1}, Na_2MoO_4: 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 spectroscopically 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 10^7-10^8 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

Table 1. Formulation of microbial consortia for seedbed testing

Treatment ID	Description	Microbial consortia
T1	KS fungi	*Penicillium* consortia (H1, H10, H12, H13)
T2	PS fungi	*Penicillium* consortia (H2, H14), *Penicillium oxalicum* (H15), *Aspergillus* sp. (H16)
T3	NF fungi	*Penicillium* consortia (H5, H8), *Fusarium* sp. (H9)
T4	KS bacteria	*Pseudomonas* consortia (B1, B2, B4, B8, B10, B15)
T5	PS bacteria	*Pseudomonas* consortia (B6, B13, B14, B16)
T6	NF bacteria	*Pseudomonas* consortia (B3, B11, B12), *Serratia liquefaciens* (B5), *Bacillus* sp. (B7)
T7	All bacteria mixed	*Pseudomonas* consortia (B1-B4, B6, B8, B10- B16), *Serratia liquefaciens* (B5), *Bacillus* sp. (B7)
T8	PS fungi + NF bacteria	*Penicillium* consortia (H2, H14), *Penicillium oxalicum* (H15), *Aspergillus* sp. (H16), *Pseudomonas* consortia (B3, B11, B12), *Serratia liquefaciens* (B5), *Bacillus* sp. (B7)
T9	KS fungi + PS bacteria	*Penicillium* consortia (H1, H10, H12, H13), *Pseudomonas* consortia (B6, B13, B14, B16)
T10	NF fungi + KS bacteria	*Penicillium* consortia (H5, H8), *Fusarium* sp. (H9), *Pseudomonas* consortia (B1, B2, B4, B8, B10, B15)
Control	-	Uninoculated

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 N_2 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

Isolate	Sample number	MALDI-TOF Result	MALDI-TOF score	Reliability
Fungi	H1	*Penicillium camemberti*	1.871	Probable genus
	H2	*Penicillium camemberti*	1.795	Probable genus
	H3	Unidentified	-	
	H4	Unidentified	-	
	H5	*Penicillium camemberti*	1.899	Probable genus
	H6	Unidentified	-	
	H7	Unidentified	-	
	H8	Unidentified	-	
	H9	*Fusarium oxysporum*	1.816	Probable genus
	H10	*Penicillium expansum*	1.817	Probable genus
	H11	Unidentified	-	
	H12	*Penicillium commune*	1.873	Probable genus
	H13	*Penicillium aurantiogriseum*	1.825	Probable genus
	H14	*Penicillium expansum*	2.036	Genus-level, probable species
	H15	*Penicillium oxalicum*	2.371	Species-level
	H16	Possibly *Aspergillus niger*	1.645	-
Bacteria	B1	*Pseudomonas fragi*	2.01	Genus-level, probable species
	B2	*Pseudomonas libanensis*	2.108	Genus-level, probable species
	B3	*Pseudomonas brassicacearum*	1.862	Probable genus
	B4	*Pseudomonas libanensis*	1.948	Probable genus
	B5	*Serratia liquefaciens*	2.416	Species-level
	B6	*Pseudomonas libanensis*	1.976	Probable genus
	B7	*Bacillus altitudinis*	1.736	Probable genus
	B8	*Pseudomonas taetrolens*	1.89	Probable genus
	B9	Unidentified	-	
	B10	*Pseudomonas rhodesiae*	2.195	Genus-level, probable species
	B11	*Pseudomonas rhodesiae*	2.256	Genus-level, probable species
	B12	*Pseudomonas chlororaphis*	2.1	Genus-level, probable species
	B13	*Pseudomonas protegens*	2.002	Genus-level, probable species
	B14	*Pseudomonas fragi*	2.072	Genus-level, probable species
	B15	*Pseudomonas fragi*	2.01	Genus-level, probable species
	B16	*Pseudomonas fragi*	2.094	Genus-level, probable species

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.

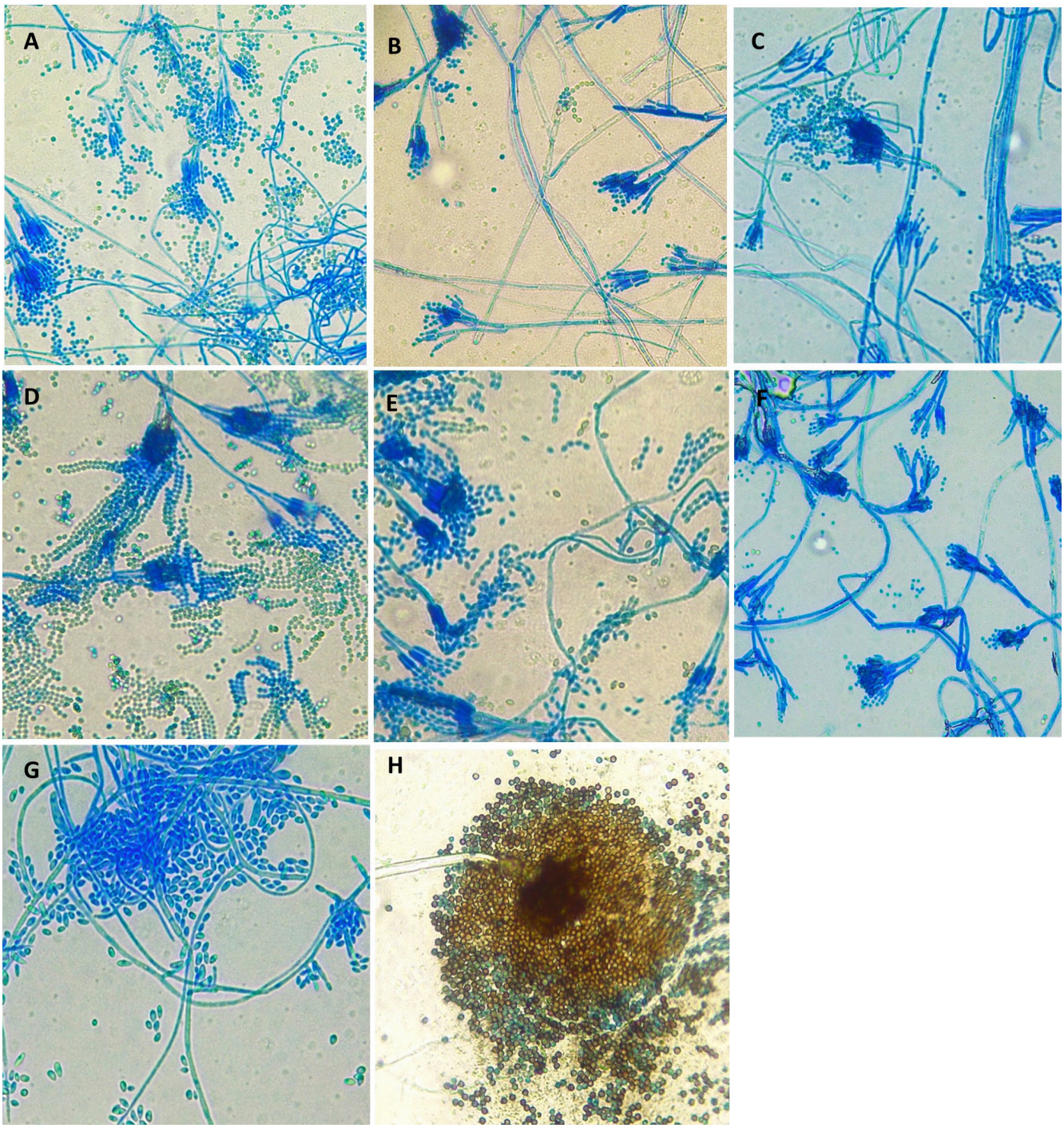

Figure 1. Microscopic images of lactophenol cotton blue-stained fungal isolates. A: *Penicillium* sp. (H5). B: *Penicillium* sp. (H14). C: *Penicillium* sp. (H12). D: *Penicillium* sp. (H13). E: *Penicillium oxalicum* (H15). F: *Penicillium* sp. (H3). G: *Fusarium* sp. (H9). H: *Aspergillus* sp. (H16). Magnification 1000x.

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-

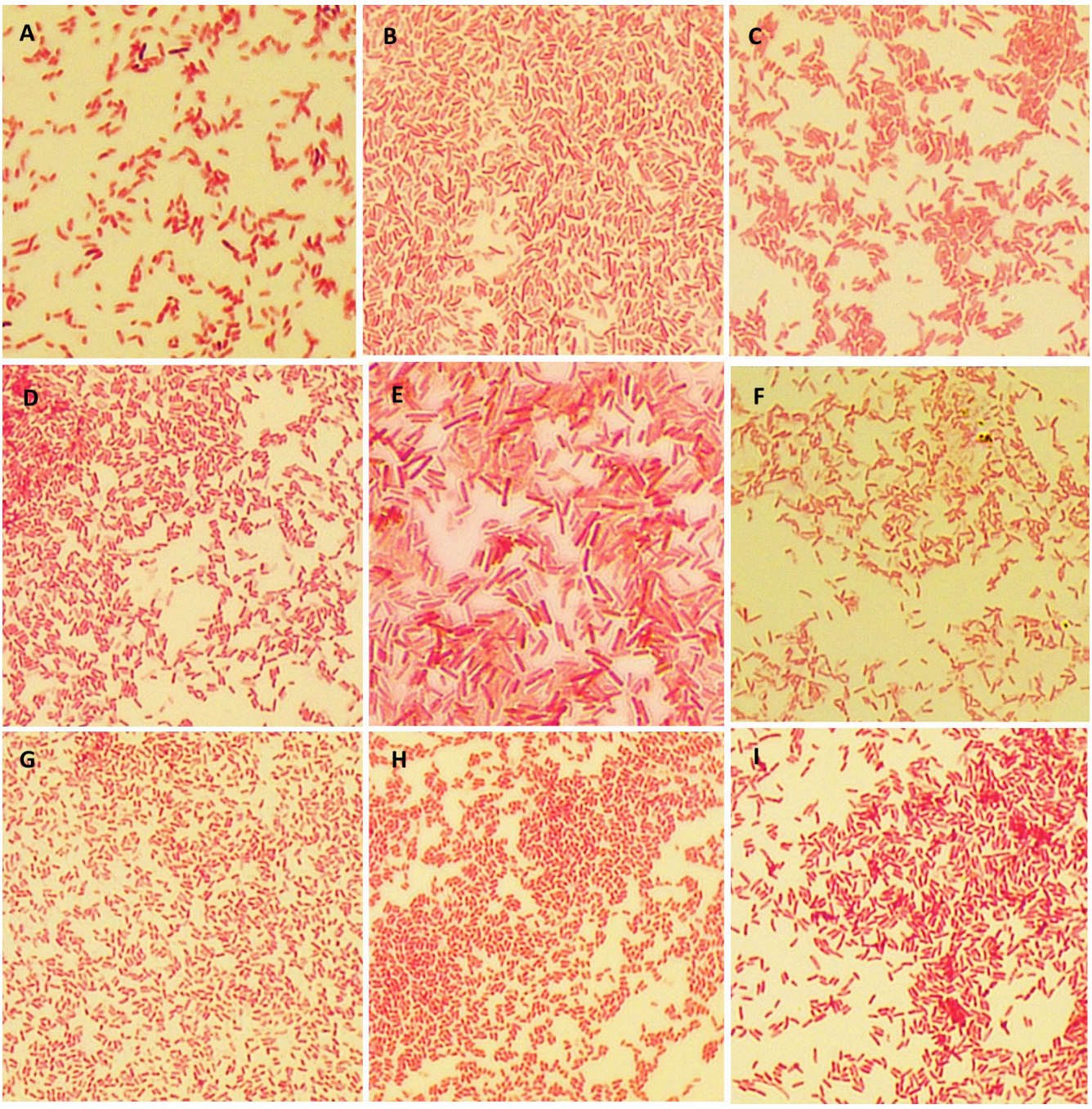

Figure 2. Microscopic images of Gram-stained bacterial isolates. A: *Pseudomonas* sp. (B1). B: *Pseudomonas* sp. (B4). C: *Pseudomonas* sp. (B3). D: *Serratia liquefaciens* (B5). E: *Bacillus* sp. (B7). F: *Pseudomonas* sp. (B8). G: *Pseudomonas* sp. (B11). H: *Pseudomonas* sp. (B12). I: *Pseudomonas* sp. (B13). Magnification 1000x.

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

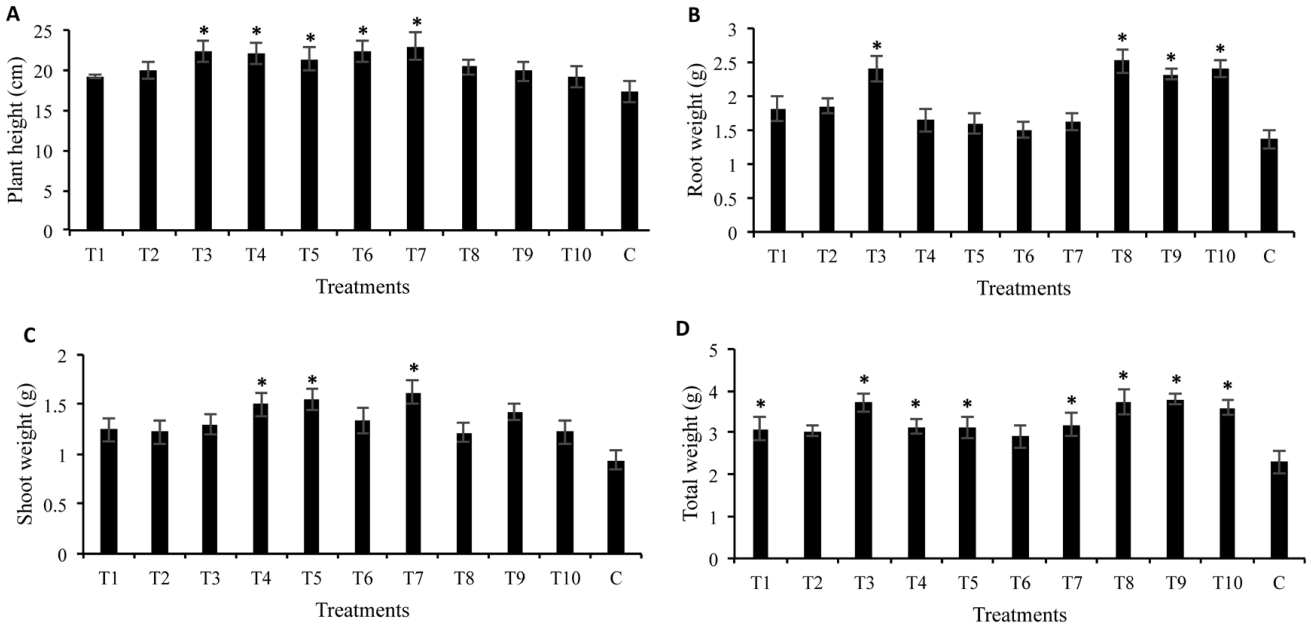

Figure 3. Microbial consortia effects on outdoor grown maize. A: Maximum height. B: Fresh root weight. C: Fresh shoot weight. D: Total fresh weight. * Significant difference from control ($P < 0.05$) using one-way analysis of variance and Tukey test (n=100), data represent average of each parameter ± 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.

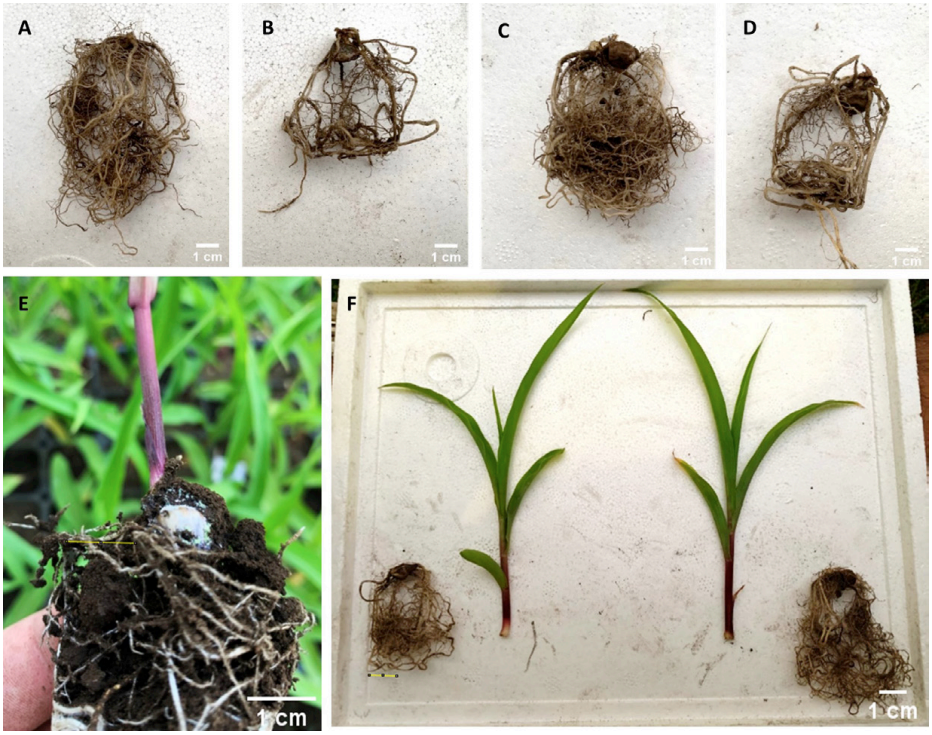

Figure 4. Maize growth measurements. A: Treatment 3, plant 2: maximum height 20.5 cm, root weight 2.5 g, shoot weight 1.2 g. B: Treatment 5, plant 2: maximum height 22 cm, root weight 1.3 g, shoot weight 1.7 g (not significantly different from the control). C: Treatment 8, plant 2: maximum height 20.5 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 3 D). 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 amyloliquefaciens* improved plant growth and drought stress tolerance in chickpea (Vurukonda *et al.*, 2016; Kumar *et al.*, 2016, Glick, 2014). *Azotobacter chrocoocum* 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 Kloepper, 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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