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Short-term effects of a photosynthetic microbial consortium and nitrogen fertilization on soil chemical properties, growth, and yield of wheat under greenhouse conditions

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Abstract

This study evaluated the effects of the application of a photosynthetic and N-fixing microbial consortium (biofertilizer) on some chemical properties of a vertisol soil and on the growth and yield of *Triticum aestivum* L. var. Barcenas S2002. The experimental design consisted on the chemical nitrogen fertilization (labeled as Q) based on 120 kg N ha^{-1} by using $(NH_4)_2SO_4$ as the N source, and its combination with the application of the microbial consortium (labeled as B), resulting in the following five treatments: Q (control), B, QB1 (75%Q + 25%B), QB2 (50%Q + 50%B), and QB3 (25%Q + 75%B). The QB1 and Q treatments favored the accumulation of NO_3^- in soil. Regarding the effects on plants, the application of B resulted in significantly enhanced 1000-grain weight and grain yield as compared to the application of Q; the grain nitrogen content was similar between Q, QB1, QB2, and QB3 treatments. In addition, the QB2 and QB3 treatments allowed high values of grain yield (50–83 g m^{-2}) and grain nitrogen content (3.1–3.5%) without showing significant differences when compared to Q treatment (100% of nitrogen chemical fertilization). These results allow a reduction of 75% of chemical fertilization for wheat production, due to complementary effects of the photosynthetic microbial consortium, which had beneficial effects on plant growth and yield as well as on soil parameters.

Keywords Cyanobacteria · Microbial consortium · Biofertilizer · Ammonium sulfate · *Triticum aestivum*

Introduction

Nitrogen (N) availability is one of the main nutrimental factors that limit agricultural production. Moreover, studies have shown that crops only take up between 30 and 50% of chemical fertilizers, which represents economical losses and creates environmental issues such as soil salinization, water eutrophication, and nitrogen oxide emissions to the atmosphere (Wang et al. 2018). Thus, worldwide researchers look for alternate non-harmful fertilizer sources like biofertilizers that are directed to enhance the fertility and biological properties of soils (Ghazal et al. 2018), and to improve plant growth and crop productivity (Karthikeyan et al. 2007; Prasanna et al. 2015; Bidyarani et al. 2016; Mondal et al. 2017).

Cyanobacteria play a very important role in both aquatic and terrestrial environments. Cyanobacteria were the first photosynthetic microorganisms that colonized the planet and allowed the subsequent establishment of other living organisms (Ramanan et al. 2016). In addition to being primary producers, some cyanobacteria genera are capable of fixing atmospheric nitrogen, by which they have gained great importance in agriculture by providing the required nitrogen for agricultural crops such as rice (Prasanna et al. 2008; Pereira et al. 2009; Padhy et al. 2016; Chittapun et al. 2018). Other agricultural crops in which cyanobacteria have been applied as biofertilizers are corn, beans, peas, sugarcane (Svircev et al. 1997; Osman et al. 2010), wheat (Hussain and Hasnain 2011; Swarnalakshmi et al. 2013; Babu et al. 2015; El-Beltagy et al. 2016; Ghazal et al. 2018), cotton (Prasanna et al. 2015), and chickpea (Bidyarani et al. 2016). Some research efforts have

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considered the application of a mixture of cyanobacteria on wheat under greenhouse conditions (Karthikeyan et al. 2007; Nain et al. 2010), and the inoculation of the *Nostoc* cyanobacterium in wheat seedlings in hydroponic systems (Gantar et al. 1993; Sood et al. 2011). Such studies demonstrated that cyanobacteria besides colonizing the roots also enhanced the seedling growth by stimulating a higher number of leaves in comparison to non-inoculated seedlings (Gantar et al. 1993; Sood et al. 2011).

The utilization of cyanobacteria as biofertilizers, termed algalization, on agricultural crops allows several benefits in soils like increased content of organic matter, improved particle bonding and water retention (Swarnalakshmi et al. 2013; Prasanna et al. 2015; Ghazal et al. 2018), releasing plant growth promoting substances (auxins, cytokinins, gibberellins, etc.) (Hussain and Hasnain 2011; Swarnalakshmi et al. 2013), enhancing P bioavailability, and preventing the growth and proliferation of weeds (Karthikeyan et al. 2007; Prasanna et al. 2008; Osman et al. 2010; Singh et al. 2011; Sood et al. 2011; Bidyarani et al. 2016). Inoculation of cyanobacteria can also increase the availability of Zn, Cu, Fe, and K in soil and plant uptake (Renuka et al. 2018). Furthermore, cyanobacteria are considered as potential biocontrol agents because they produce hydrolytic enzymes and biocidal compounds such as benzoic acid and majusculonic acid, which exhibit antagonistic effects towards many phytopathogens (bacteria, fungi, and nematodes) (Swain et al. 2017; Renuka et al. 2018). All these benefits support the relevance of cyanobacteria for sustainable agricultural crop production.

In the case of wheat production, the International Fertilizer Association (IFA) recommends doses of nitrogen fertilization ranging from 60 to 120 kg ha^{-1} (Wichmann 1992); however, the doses of nitrogen fertilization in Mexico varies from 240 to 303 kg ha^{-1} and more importantly, only 69% of this applied nitrogen is used by wheat plants (Solís-Moya and Rodríguez-Guillén 2000). In this regard, the evaluation of biological alternatives for accomplishing the nitrogen requirements in important agricultural crops like wheat is still needed. Thus, the present study evaluated the effects of the application of a photosynthetic and N-fixing microbial consortium on some soil chemical properties, and on the growth and yield of wheat plants under greenhouse conditions, when compared to the nitrogen chemical fertilization.

Materials and methods

Experimental settings

The experiment consisted on the utilization of a photosynthetic and N-fixing microbial consortium (biofertilizer), and the application of $(NH_4)_2SO_4$ as a nitrogen chemical fertilizer, in a vertisol soil sown with *Triticum aestivum* L. var. Barcenas S2002. Plastic pots with a diameter of 16.5 cm were used.

The experimental design consisted on the chemical nitrogen fertilization based on 120 kg N ha^{-1} by using $(NH_4)_2SO_4$ as the N source (labeled as Q), and its combination with the application of the microbial consortium (labeled as B), resulting in the following five treatments: Q (control), B, QB1 (75%Q + 25%B), QB2 (50%Q + 50%B), and QB3 (25%Q + 75%B). There were four replicates per treatment.

The chemical fertilizer (120–60–40 kg NPK ha^{-1}) was applied in granular form and was split in three applications (0, 40, and 69 days after sowing) as recommended for field conditions (Wichmann 1992). The P fertilization was based on the application of calcium diacid phosphate, and 50% (0.242 g $plot^{-1}$) of it was applied at the beginning of the experiment, and the remaining percentage at day 40. The source of K was KCl, and the corresponding dose (0.164 g $plot^{-1}$) was completely applied at the beginning of the experiment.

The volume of the biofertilizer (microbial biomass and supernatant) for each treatment (Table 1) was proposed based on the nitrogen fixation recorded from 1 L of biofertilizer (2.795×10^{-3} g N_2 day^{-1}). Additionally, considering that not all microorganisms applied to the soil could survive, it was proposed that 30% of the microorganisms could die once the inoculum was applied to the soil, which would reduce the nitrogenase activity to 70%, and this activity would be maintained during the following days until the second application. According to Table 1, some biofertilizer applications for QB2, QB3, and B treatments overpassed the 0.38 L, the maximum liquid volume before plant flooding in the pot. In order to avoid plant flooding, the biofertilizer applications were split and equally applied in following days.

Wheat seeds were submerged in distilled water for 1 h, then washed with 2% commercial powder detergent (Fábrica de Jabón La Corona) for 10 min and rinsing several times with distilled water. Then, seeds were surface disinfested by immersion in 70% ethanol for 40 s, followed by 4% NaClO for 10 min twice, and finally were rinsed several times with distilled water, and air dried at room temperature. Five seeds of wheat were sown in each plastic pot (16.5 cm diameter and 3 L of capacity) filled with the soil and maintained at 60% of field capacity. The emerged seedlings were kept for 8 days, and 1 day after, only two seedlings per pot were maintained for 123 days.

Both temperature and relative humidity (RH) in greenhouse were recorded by means of a HOBO 1996 ONSET. Average minimum and maximum temperatures were −4.3 and 68.2 °C, respectively; average minimum and maximum RH was 23.4 and 90%, respectively.

Table 1 Doses of nitrogen fertilization (chemical or biological sources) applied to the corresponding treatment

Treatments	1st application (0 days)		2nd application (40 days)		3rd application (69 days)	
	Q 30% (g pot^{-1})	B 20.3% (L pot^{-1})	Q 40% (g pot^{-1})	B 37.3% (L pot^{-1})	Q 30% (g pot^{-1})	B 42.4% (L pot^{-1})
Q (100%)	0.56	0	0.75	0	0.56	0
QB1 (75%Q + 25%B)	0.42	0.17	0.56	0.31	0.42	0.35
QB2 (50%Q + 50%B)	0.28	0.33	0.38	0.61	0.28	0.70
QB3 (25%Q + 75%B)	0.14	0.50	0.19	0.92	0.14	1.05
B (100%)	0	0.67	0	1.23	0	1.40

Q =100% nitrogen chemical fertilization [$(NH_4)_2SO_4$], *B*= 100% inoculation of the microbial consortium, *QB1*= 75%Q + 25%B, *QB2*= 50%Q + 50%B, *QB3*= 25%Q + 75%B

Preparation of the photosynthetic and N-fixing microbial consortium (biofertilizer)

The biofertilizer was obtained from a rice field at Alpuyeca, Morelos (Mexico), located at 18° 44′ north latitude and 90° 13′ west longitude at 1300 m.a.s.l. (Reyna-Velarde et al. 2012). Hernández-Melchor et al. (2016) performed the phylogenetic and morphological identification of the photosynthetic microbial consortium which is conformed by 21 identified microorganisms belonging to the phyla Proteobacteria (*Rhodobacter* sp. (KC979092), *Devosia insulae* (KC979075), *Pedomicrobium americanum* (KC979083), *Alphaproteobacteria* (KC979071), *Aquaspirillum delicatum* (KC979079), *Methylibium petroleiphilum* (KC979100), and *Nannocystis* sp. (KC979087)), Bacteroidetes (*Flavobacterium* sp. (KC979070) and *Flavobacterium aquatile* (KC979099)), Cyanobacteria (*Aphanizomenon aphanizomenoides* (KC979066), *Leptolyngbya* sp. (KC979068), and *Anabaena oscillarioides* (KC979098)), Chlorophyta (*Monoraphidium* sp., and *Chlorella* sp.), and Heterokontophyta (*Cyclotella meneghiniana*, *Melosira varians*, *Cocconeis placentula*, *Achnanthidium chlidanos*, *Navicula radiosa*, *Fragilaria ulna*, and *Nitzschia* sp.). This microbial consortium has high ability for fixing atmospheric nitrogen (until 10294 nmol C_2H_2 g^{-1} h^{-1} = 3431.3 nmol N_2 g^{-1} h^{-1}), which is determined by the nitrogenase activity through the acetylene reduction assay (Hernández-Melchor et al. 2016).

The biofertilizer was cultivated in $BG\text{-}11_0$ medium (Rippka et al. 1979) in a 20-L bubbled column with 18 L operational volume, in semicontinuous mode for satisfying nutritional requirements. Culture conditions for the biofertilizer propagation were as follows: aeration of 1.0 vvm (18 L min^{-1}), light intensity of 80 μmol photons m^{-2} s^{-1} with 12/12-h L/D cycle, and 21 ± 1 °C. The inoculation of the biofertilizer on soil and wheat plants was carried out when the exponential growth was achieved (10–12 days), as indicated in Table 1.

Soil parameters

The vertisol soil used in the present study was collected from Salamanca, Guanajuato (Mexico), without previous record for agricultural purposes. Physical, chemical, and biological properties of soil were determined: clay soil, pH 6.5, 13 μg g^{-1} NH_4^+ content, 46.3 μg g^{-1} NO_3^- content, and 8.7 μg g^{-1} P content (Bray-Kurtz); 57.5 μS cm^{-1} electrical conductivity (1:20); and 0.094 nmol C_2H_2 g^{-1} h^{-1} nitrogenase activity.

After 123 days of experimentation, soil samples were collected from all treatments. Each soil sample was air dried at room temperature, sieved, and homogenized before determining pH, NH_4^+, NO_3^-, and P contents (Bray-Kurtz), and electrical conductivity (EC) (Pansu and Gautheyrou 2006).

The nitrogenase activity was performed by means of the acetylene reduction assay adapted for soil (modified from Nain et al. 2010). Samples of fresh soil cores (0–5 cm) were collected at 123 days of experimentation and placed in 28-mL serological bottles hermetically sealed. Thereafter, 3 mL of air was eliminated from the head space, replacing it with 3 mL of acetylene. Then, bottles were incubated during 8 days under greenhouse conditions. There were four replicates per treatment. The presence of ethene or ethylene produced (nmol C_2H_4 g^{-1} h^{-1}) was evaluated with a Varian CP3380 FID gas chromatograph. The column temperature was maintained at 60 °C, the injector temperature was 200 °C, and the detector temperature was 250 °C. A Carbowax 1540/Porapak Q 1/8″ × 6 ft. column was used, and the carrier gas was N_2 at 15 psi.

Growth and yield evaluations in wheat plants

After 123 days of growing, plant height, biological yield measured as the biomass produced per unit of area, grain yield, 1000-grain weight, and the content of nitrogen and protein in grains were analyzed. The N content was determined by the micro-Kjeldahl method, and the protein content was estimated by multiplying the N content by 5.71 which corresponds to a constant factor for cereals (Hussain et al. 2006).

Statistical analysis

A completely randomized design was set with five treatments and four replicates each for either soil or plant analysis. Data were subjected to the analysis of variance and the mean comparison test (Tukey, $\alpha = 0.05$); in addition, a Pearson's correlation coefficient was performed among soil or plant parameters by using the statistical program SAS 5.1 (SAS Institute 2000).

Results

Effects on soil parameters

Soil pH showed significant differences ($P < 0.05$) between Q and QB1 treatments; At the beginning of the experiment, soil pH was 6.5 and at the end soil pH was 6.4 for Q treatment, but the soil from QB1 treatment showed the highest pH value (Table 2). In contrast, soils from B and QB3 treatments with the highest proportion of biofertilizer had an increase in pH values towards neutrality (Table 2).

The NH_4^+ content in soil did not show significant differences among treatments from the beginning to the end of the experiment (Table 2). The content of NO_3^- significantly increased ($P < 0.001$) in soil samples from Q, QB1, and QB2 treatments with respect to the value from the beginning of the experiment. Soil from Q treatment recorded the highest content of NO_3^-, followed by soil from QB1 treatment (Table 2); both values were 9.7 and 6.9 higher than that value obtained in the soil at the beginning of the experiment. On the contrary, QB3 treatment had no significant increases in the NO_3^- content respect to the beginning of the experiment, but in B treatment a slight decrease in NO_3^- was observed.

By comparing the initial value of P in the soil, all treatments resulted in non-significant effects on this parameter (Table 2). Moreover, soil from Q treatment registered the highest value of EC, which was 3.2 times higher than that obtained at the beginning of the experiment. The EC values tend to diminish as the percentage of biofertilizer increased in treatments (Table 2). Furthermore, a significant positive correlation ($r = 0.901$; $P < 0.0001$) between EC and the NO_3^- content was also accounted in soil under the experimental conditions.

At the beginning of the experiment, vertisol soil showed nitrogenase activity indicating the potential presence of diazotrophic microorganisms such as *Azotobacter* (data not shown). At the end of the experiment, the soil from B treatment had the highest nitrogenase activity (Table 2) equivalent to 0.071 nmol N_2 g^{-1} h^{-1}. Moreover, this activity was 2.3 times higher than that recorded at the beginning of the experiment. A positive significant correlation ($r = 0.778$; $P < 0.01$) was found between the nitrogenase activity and the P content in soil. In contrast, the lowest value of nitrogenase activity was achieved at QB1 treatment (Table 2). Additionally, a negative correlation ($r = -0.503$; $P = 0.023$) between nitrogenase activity and the NO_3^- content in soil was achieved.

Effects on growth and yield of wheat plants

After 123 days, plants with the single inoculation of the microbial consortium (B) showed significant differences ($P < 0.001$) when compared to the remaining treatments (Fig. 1a). Particularly, the plant height in B treatment was 14% higher than that achieved for plants in Q treatment.

Plants from Q treatment registered the highest biological yield; however, it was not significantly different in respect to QB3 treatment. In contrast, the lowest biological yield was obtained in plants from treatment QB1 (Table 3).

The 1000-grain weight of wheat only showed significant differences ($P < 0.05$) between B and Q treatments (Table 3). In the same manner, the highest grain yield (113.41 g m^{-2}) was recorded in plants from B treatment (Fig. 1b). The nitrogen content of grains was high in those

Table 2 Effects of chemical or biological fertilization and their combinations on pH, NH_4^+ content, NO_3^- content, phosphate content (P), electrical conductivity (EC), and nitrogenase activity of soil planted with wheat (*Triticum aestivum* L. var. Barcenas S2002), after 123 days of growth under greenhouse conditions

Treatments	pH	NH_4^+ (μg g^{-1})	NO_3^- (μg g^{-1})	P (μg g^{-1})	EC (μS cm^{-1})	Nitrogenase activity (nmol C_2H_4 g^{-1} h^{-1})
Q	6.4 ± 0.07 b	11.9 ± 7.4 a	448.0 ± 27.1 a	22.8 ± 0.4 a	183.5 ± 10.3 a	0.140 ± 0.024 ab
QB1	7.1 ± 0.06 a	2.4 ± 1.6 a	319.3 ± 10.7 b	27.1 ± 0.8 a	180.5 ± 4.4 a	0.109 ± 0.021 b
QB2	6.5 ± 0.11 ab	0.2 ± 0.07 a	187.9 ± 4.1 c	25.2 ± 1.3 a	143.8 ± 2.7 b	0.139 ± 0.009 ab
QB3	7.0 ± 0.29 ab	0.1 ± 0.1 a	63.7 ± 4.2 d	24.0 ± 1.8 a	138.5 ± 6.5 bc	0.147 ± 0.003 ab
B	6.8 ± 0.01 ab	0 ± 0 a	27.7 ± 3.6 d	24.8 ± 2.5 a	113.3 ± 1.1 c	0.213 ± 0.024 a
	$P < 0.05$	$P > 0.05$	$P < 0.001$	$P > 0.05$	$P < 0.001$	$P < 0.05$

Means ± standard error. Different lowercase letters in each column are statistically different (Tukey, $\alpha = 0.05$). $n = 4$

Q =100% nitrogen chemical fertilization [$(NH_4)_2SO_4$], *B*= 100% inoculation of the microbial consortium, *QB1*= 75%Q + 25%B, *QB2*= 50%Q + 50%B, *QB3*= 25%Q + 75%B

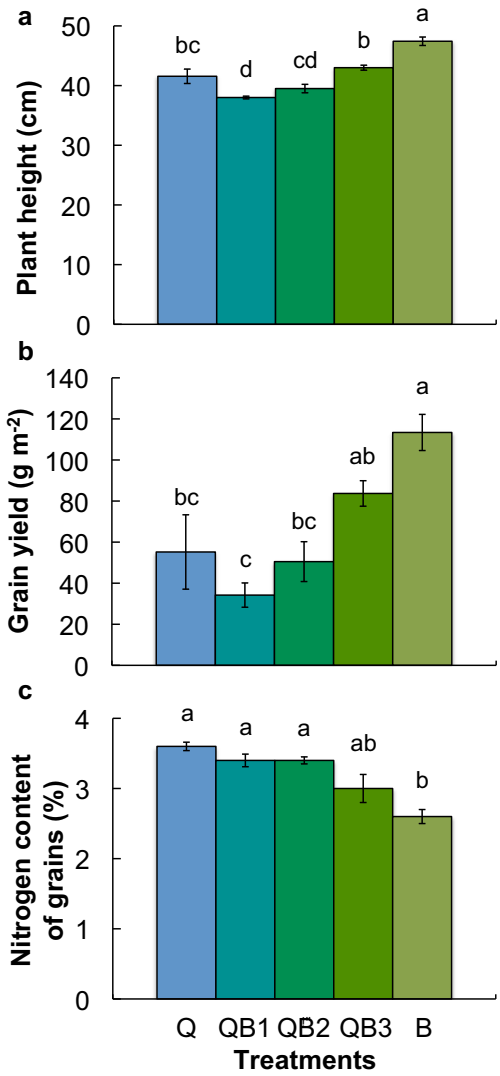

Fig. 1 Effect of chemical and biological fertilization and their combinations, **a)** on the height of wheat plants (*Triticum aestivum* L. var. Barcenas S2002), **b)** on the grain yield, and **c)** the nitrogen content of grains, after 123 days of growth under greenhouse conditions. Means ± standard error. Different letters on bars are statistically different (Tukey, $\alpha = 0.05$). $n = 4$. Abbreviations: Q = 100% nitrogen chemical fertilization [$(NH_4)_2SO_4$]; B = 100% inoculation of the microbial consortium; QB1 = 75%Q + 25%B; QB2 = 50%Q + 50%B; QB3 = 25%Q + 75%B

treatments in which chemical fertilization was applied (3.2% in average). Particularly, the nitrogen content of grains from plants grown in Q, QB1, and QB2 treatments showed significant differences ($P < 0.001$) in respect to B treatment (Fig. 1c). By considering such data and the constant factor of 5.7 utilized for estimating of protein content (Hussain et al. 2006), the results indicate that grains from plants grown at QB2 and QB3 treatments had a protein content of 19.8% and 17.6%, respectively, whereas those grains from Q treatment registered similar protein content (20.5%), but without showing significant differences among treatments.

Table 3 Effects of chemical or biological fertilization and their combinations, on the biological yield and weight of 1000-grains of wheat (*Triticum aestivum* L. var. Barcenas S2002), after 123 days of growth under greenhouse conditions

Treatments	Biological yield (g m^{-2})	1000-grain weight (g)
Q	368.22 ± 15.12 a	35.11 ± 1.13 b
QB1	288.33 ± 7.48 c	38. 40 ± 0.51 ab
QB2	327.03 ± 4.28 b	38.74 ± 0.78 ab
QB3	352.95 ± 3.13 ab	39.60 ± 0.94 ab
B	331.75 ± 5.56 b	42.21 ± 1.92 a
	$P < 0.001$	$P < 0.05$

Means ± standard error. Different lowercase letters in the same column are statistically different (Tukey, $\alpha = 0.05$). $n = 4$

Q= 100% nitrogen chemical fertilization [$(NH_4)_2SO_4$], *B*= 100% inoculation of the microbial consortium, *QB1*= 75%Q + 25%B, *QB2*= 50%Q + 50%B, *QB3*= 25%Q + 75%B

A significant negative correlation ($r = -0.673$; $P < 0.01$) was found between the nitrogen content of grains and grain yield, indicating that a higher yield of grain may result in low nitrogen content. In this way, the best results for both nitrogen content of grains and grain yield corresponded to the treatments with the combination of chemical fertilization and biofertilizer (QB2 and QB3), but without showing significant differences with Q treatment.

Discussion

Overall, cereals grow well in soils with pH values between 5.0 and 7.0 (Parsons 2004). In the present study, the pH values recorded in all treatments ranged from 6.8 to 7.3, which are similar to those pH values reported by Karthikeyan et al. (2007) and Nain et al. (2010) where the N-fixing cyanobacteria were applied as biofertilizer. Furthermore, the observed increase in soil pH from B and QB3 treatments is opposite to that described by Nisha et al. (2007) who detected a diminishing of pH values due to the inoculation of a mixture of cyanobacteria like *Anabaena dolium* HH-209, *Cylindrospermum sphaerica* HH-202, and *Nostoc calcicola* HH-201 as biofertilizer for wheat plants under greenhouse conditions.

NH_4^+ is the main component for the chemical fertilizer and the chemical by-product of the microbial consortium; however, no significant differences were recorded on the accumulation of this nitrogen form among treatments. Nitrogen is the element that plants take up in abundant quantity, mainly as NH_4^+ form. The latter may be explained in part because NH_4^+ cation is easily assimilated and is the most available form for being absorbed by plants and assimilated in the glutamine synthase/glutamate synthase cycle (GS/GOGAT) without energy cost (Barneix 2007; Kant et al. 2007).

On the other hand, NO_3^- is the second chemical form of inorganic N that plants assimilate. The accumulation of NO_3^- in the QB1, QB2, and QB3 treatments was identified at 55 days (data not shown). Treatments QB1, QB2, and Q recorded significant increases on the NO_3^- content at the end of the experiment, which was due to the nitrification process where bacteria such as *Nitrosomonas* and *Nitrobacter* could have participated (Haynes 1986). Moreover, soil from QB1 treatment was the only biological fertilization where the NO_3^- content was 1.4 times higher than that value reported by Nisha et al. (2007), who used a mixture of cyanobacteria (*A. dolium* HH-209, *C. sphaerica* HH-202, and *N. calcicola* HH-201) as biofertilizer for improving physico-chemical properties, structure, and microbial activities of a poor semiarid soil. A similar response was detected for the EC, whose values proportionally increased as the chemical fertilization increased, resulting in the accumulation of available salts in the soil.

As expected, the highest nitrogenase activity (0.213 nmol C_2H_4 g^{-1} h^{-1}) was achieved in the treatment with the single inoculation of the biofertilizer (B). This value was similar to that obtained in wheat plants inoculated with a dual culture of *Anabaena torulosa* and *Azotobacter chroococum* but was higher than that nitrogenase activity recorded with a dual culture of *A. torulosa* and *Mesorhizobium ciceri* (Swarnalakshmi et al. 2013). On the contrary, the reduction of the nitrogenase activity recorded in soils from Q, QB1, QB2, and QB3 treatments may be attributed to the inhibition of the nitrogenase enzyme due to the presence of available inorganic nitrogen forms (NH_4^+ and NO_3^-) as a consequence of the application of chemical fertilization. The latter concurs with experimental findings by Padhy et al. (2016) in rice crop, in which the nitrogen fixation was diminished or inhibited due to the accumulation of either inorganic (for instance NH_4^+ and NO_3^-) or organic of N-forms (urea and certain amino acids). Furthermore, the nitrogenase activity recorded in the B treatment represents approximately a third part of that value reported by Nain et al. (2010) in wheat plants fertilized with 40 kg N ha^{-1}, in combination with the inoculation of N-fixing bacteria and cyanobacteria. P fertilization stimulates the nitrogen fixation process and increases the amounts of inorganic nitrogen in the soil (Reed et al. 2007). The latter agrees with the present results since a positive significant correlation ($r = 0.778$; $P < 0.01$) was found between the nitrogenase activity and the P content in soil.

In regard to plant responses, the highest height (47.4 cm) was achieved in B treatment (Fig. 1a). Similar results were obtained by Reyna-Velarde et al. (2012) in rice seedlings inoculated with the same microbial consortium used in the present work. The above is opposite to findings by Karthikeyan et al. (2007) who achieved the maximum plant height with the application of chemical fertilization (120 kg N ha^{-1}).

The biological yield achieved in plants grown in QB3 treatment, with chemical fertilization of 30 kg N ha^{-1}, was similar to that obtained in Q treatment, with chemical fertilization of 120 kg N ha^{-1}. The maximum biological yield reported by Karthikeyan et al. (2007) for wheat was obtained with chemical nitrogen fertilization of 40 kg ha^{-1}, and the inoculation of the cyanobacteria *Hapalosiphon intricatus* and *Nostoc* sp. On the other hand, Swarnalakshmi et al. (2013) recorded the maximum biological yield with a dual inoculation of *A. torulosa* and *A. chroococuum* in wheat plants. In contrast, Nain et al. (2010) reported a biological yield of 1440.3 g m^{-2} by applying chemical nitrogen fertilization (40 kg ha^{-1}) and by inoculating a mixture of three bacteria and three cyanobacteria isolated from the rhizosphere soil of wheat plants.

The highest value of the 1000-grain weight was recorded from plants grown at B treatment (Table 3), which is similar to that reported by Hussain and Hasnain (2011), who inoculated wheat plants with *Anabaena* sp., a cytokinin and indole acetic acid–producing cyanobacteria. Thus, considering that *A. oscillarioides* is part of our microbial consortium, it is possible that this cyanobacterium may also release seed germination or plant growth promoting compounds. Preliminary experiments showed that the microbial consortium resulted in increased germination of wheat seeds up to 20% (Ramírez-López unpublished data), which concurs to the described beneficial effects of cyanobacteria. On the other hand, the 1000-grain weight values obtained with the three QB combinations (QB1, QB2, and QB3) and with B treatment were higher than that reported by Ghazal et al. (2018) for wheat plants inoculated with *Nostoc elepsosporum*, *Nostoc linckia*, and *Anabaena variabilis* grown in a sandy soil.

The grain yield obtained from plants grown in B treatment was 2 and 3 times higher than those values obtained in plants grown in Q and QB1 treatments, respectively (Fig. 1b). The maximum grain yield reported by Karthikeyan et al. (2007) was achieved with the combination of the cyanobacteria *Calothrix ghosei*, *Hapalosiphon intricatus*, and *Nostoc* sp. and chemical fertilization (40 kg N ha^{-1}). Additionally, Ghazal et al. (2018) obtained a grain yield of 250 g m^{-2} employing 75% of chemical nitrogen fertilization and nitrogen-fixing cyanobacteria. Regarding the nitrogen content of grains, at the beginning of the experiment the nitrogen content of grains before being sown was of 2.2%, which was significantly low ($P < 0.001$) when compared to that obtained for all treatments at the end of the experiment. Nevertheless, the best results for both nitrogen content of grains and grain yield corresponded to QB2 and QB3 treatments. Moreover, data from QB2, QB3, and B treatments were respectively 1.5, 1.4, and 1.2 times higher to that protein content achieved for the wheat seeds used for the experimental establishment.

Considering the present results obtained in both plants and grains, it is important to highlight that B, QB2, and QB3 treatments resulted in greater biological yield, 1000-grain weight, and grain yield than that achieved with other experiments where only a mixture of cyanobacteria was employed (Karthikeyan

et al. 2007; Swarnalakshmi et al. 2013; Ghazal et al. 2018). Additionally, the biofertilizer used in this work produces an extracellular matrix that surrounds the microorganisms and confers them protection against predators and environmental disturbances (Reyna-Velarde et al. 2012). The abovementioned could represent an advantage over those mixtures of cyanobacteria that do not produce biofilms. Multispecies biofilms have an essential role in maintaining the ecological balance in soil and confer benefits like increased resistance to antibacterial compounds, enhanced protection from desiccation and protozoan predation (Velmourougane et al. 2017).

The present data support a reduction about 50% of the nitrogen chemical fertilization when combined with the biofertilizer (QB2 treatment). This reduction in chemical fertilization was also described in other experiments in which cyanobacteria-based biofertilizers (*Anabaena iyengarii* var. *tenuis*, *Nostoc* sp., *Nostoc commune, N. linckia, Nostoc entophytum*, and *Oscillatoria angustissima*) were applied in rice (Pereira et al. 2009) and peas (Osman et al. 2010). Similarly, El-Beltagy et al. (2016) reported that a mix of *Nostoc muscorum*, *A. variabilis, Anabaena orientalis*, and *N. linckia* can support wheat growth, enhance soil fertility, and reduce the requirement of the chemical nitrogen fertilization for wheat cultivation by 25%. Moreover, Ghazal et al. (2018) found that *N. elepsosporum*, *N. linckia*, and *A. variabilis* might save almost 30% of the chemical nitrogen fertilization required for wheat production. In addition, such chemical fertilization reduction may contribute on significant environmental benefits by which salinization and acidification of soils, water eutrophication, NH_3 volatilization, and N_2O emissions may be eluded.

Conclusions

This study demonstrated that the combination of chemical nitrogen fertilization with the biofertilizer inoculation as QB1 treatment, as well as the single chemical fertilization (Q), resulted in enhanced accumulation of NO_3^- in soil. Regarding the plant effects, the single inoculation of the biofertilizer (B) resulted in improved 1000-grain weight and grain yield when compared to the unique chemical fertilization (Q). Besides, the biofertilizer enhanced the nitrogen content of grains with respect to that value recorded for wheat grains at the beginning of the experiment. The combination of chemical nitrogen fertilizer with the biofertilizer (QB2 and QB3 treatments) resulted in similar grain yield and nitrogen content of grains when compared to those values achieved with Q treatment. The latter allowed a significant reduction of chemical nitrogen fertilization (50 to 75%) for wheat production under our experimental conditions. Furthermore, the application of the biofertilizer represents an alternative and feasible strategy for plant fertilization.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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