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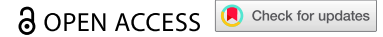


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RESEARCH PAPER



Effect of a native bacterial consortium on growth, yield, and grain quality of durum wheat (*Triticum turgidum* L. subsp. *durum*) under different nitrogen rates in the Yaqui Valley, Mexico

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ABSTRACT

A field experiment was carried out to quantify the effect of a native bacterial inoculant on the growth, yield, and quality of the wheat crop, under different nitrogen (N) fertilizer rates in two agricultural seasons. Wheat was sown under field conditions at the Experimental Technology Transfer Center (CETT-910), as a representative wheat crop area from the Yaqui Valley, Sonora México. The experiment was conducted using different doses of nitrogen (0, 130, and 250 kg N ha⁻¹) and a bacterial consortium (BC) (*Bacillus subtilis* TSO9, *B. cabrialesii* subsp. *tritici* TSO2^T, *B. subtilis* TSO22, *B. paralicheniformis* TRQ65, and *Priestia megaterium* TRQ8). Results showed that the agricultural season affected chlorophyll content, spike size, grains per spike, protein content, and whole meal yellowness. The highest chlorophyll and Normalized Difference Vegetation Index (NDVI) values, as well as lower canopy temperature values, were observed in treatments under the application of 130 and 250 kg N ha⁻¹ (the conventional Nitrogen dose). Wheat quality parameters such as yellow berry, protein content, Sodium dodecyl sulfate (SDS)-Sedimentation, and whole meal yellowness were affected by the N dose. Moreover, the application of the native bacterial consortium, under 130 kg N ha⁻¹, resulted in a higher spike length and grain number per spike, which led to a higher yield (+1.0 ton ha⁻¹ vs. un-inoculated treatment), without compromising the quality of grains. In conclusion, the use of this bacterial consortium has the potential to significantly enhance wheat growth, yield, and quality while reducing the nitrogen fertilizer application, thereby offering a promising agro-biotechnological alternative for improving wheat production.

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1 Introduction

Durum wheat is a fundamental source of carbohydrates, protein, vitamins, minerals, fiber, and other phytochemicals for global human nutrition¹. Globally, in 2021, around 770 million tons of this crop were produced on 220 million hectares². However, projections of rapid growth in the global human population indicate that by 2050, food demand will double compared to its current status³. This global food demand has led to the evolution of crop production systems that require the use of more productive crop varieties under intensive agricultural practices to maintain or increase their yield capacity and nutrition quality^{4,5}.

Mexico contributes to the global wheat production with 3.3 million tons, and the Yaqui Valley, located in the Sonora State, contributes ~50% to the national wheat production⁶. The Yaqui Valley, known as the birthplace of the Green Revolution, has conducted successful research programs to develop more productive genotypes for global wheat

production⁷. However, the intensive agricultural practices employed by farmers, including over-plowing, and high synthetic fertilization doses (300 kg N ha⁻¹, and 100 kg mono-ammonium phosphate ha⁻¹), combined with the semi-arid climatic conditions of the region, have negatively impacted the soil fertility. As a result, the current nitrogen use efficiency by wheat is about 31%^{8,9}. This nutritional imbalance generates i) high economic costs for farmers due to synthetic fertilizers representing 32.7% of the total cost for wheat production, and ii) environmental hazards by nitrogen leaching and volatilization, such as pollution of groundwater and surface waters, and biological and nutrient stresses^{10,11}. In addition, the increased demand for synthetic nitrogen fertilizers generates high energy costs, primarily derived from fossil fuels, producing greenhouse gasses such as carbon dioxide, nitrous oxide, and methane^{12,13}.

To address the challenges associated with the high rates of nitrogen fertilizers and enhance crop yield, the application of microbial inoculants has gained significant attention as

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a promising and sustainable alternative¹⁴. Microbial inoculants are bio-products containing mainly Plant Growth Promoting Rhizobacteria (PGPR), with the ability to colonize the whole or specific parts of plants to improve their nutrient uptake, yield, and control diseases. These benefits in plants are achieved through several mechanisms, such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production, biological fixation of nutrients, antibiotic production, and synthesis of phytohormones^{15,16}. Furthermore, PGPR sustain essential ecosystemic services, including social and ecological sustainability, adaptation and mitigation of climate change, biotechnological resources for humanity, water, and nutrients cycling, and increased food production^{17,18}.

However, many PGPR that show promotion properties *in vitro* do not always yield the expected effects when applied in field conditions. Therefore, a strategy for developing highly efficient bacterial inoculants is to isolate native microorganisms from the specific site where they will be applied¹⁹. By doing so, microorganisms are already adapted to the local edaphoclimatic conditions, particularly in the case of extreme conditions such as salinity or high content of heavy metals²⁰. In regions such as the study area, the Yaqui Valley, where extreme climatic conditions, low organic matter content, and large amounts of agrochemical residues are present, this approach becomes particularly relevant and unexplored.

Another strategy to increase the success of the microbial inoculants is to employ consortia, which involve the combination of two or more microbial strains that possess multiple functional traits and compatibility between them. This synergism within a consortium can enhance the beneficial effects on plants²¹. However, despite the economic and agricultural importance of the Yaqui Valley, there are no published studies on the use of native microorganisms from this region in field trials.

Thus, this study aims to fill this knowledge gap by evaluating the effects of a bacterial consortium, containing native strains (*Bacillus subtilis* TSO9, *B. cabrialesii* subsp. *tritici* TSO2^T, *B. subtilis* TSO22, *B. paralicheniformis* TRQ65, and *Priestia megaterium* TRQ8), under different nitrogen rates and across two agricultural seasons, on the growth, yield, and grain quality of durum wheat in the Yaqui Valley, Mexico. In detail, we hypothesized that the application of the native bacterial consortium can help reduce the amounts of chemical fertilizers used, maintaining or increasing the yield and quality of the wheat grain. By utilizing locally adapted microorganisms and assessing their performance in the field, this research intends to provide valuable insights into the potential of microbial-based strategies for sustainable and efficient wheat production in this specific agroecosystem.

2 Materials and methods

2.1. Bacterial strains and inoculant preparation

The bacterial consortium was developed using five native wheat-associated bacterial strains preserved in the Collection of Endophytic and Native Edaphic Microorganisms (www.itson.edu.mx/COLMENA). The strains *B. subtilis* TSO9, *B. cabrialesii* subsp. *tritici* TSO2^T,

B. subtilis TSO22, *B. paralicheniformis* TRQ65, and *Priestia megaterium* TRQ8 were isolated from the soil and rhizosphere of the CIRNO2008 variety wheat crop, grown in commercial plots located in the Yaqui Valley, Sonora, Mexico (27° 35' 53.14" N and 110° 2' 53.26" W), and were selected for this study based on their ability to colonize the rhizosphere of the wheat crop²², their antagonistic ability against phytopathogens, such as *Bipolaris sorokiniana*, the causal agent of the blur spot in this cereal²³ and previous knowledge of their ability to produce indoles, biosynthesize siderophores, solubilize phosphates, tolerate abiotic stress (saline, thermal, hydric, and chlorothalonil), and positively regulate wheat biometric traits^{24–31}.

For the preparation of the bacterial inoculant, the pre-inoculum of each strain was grown individually in an Erlenmeyer flask containing 250 mL of sterile mineral medium [MM, g L⁻¹, glucose, 10; (NH₄)₂SO₄, 4; K₂HPO₄, 5.32; KH₂PO₄, 6.4; MgSO₄·7 H₂O, 0.4; MnSO₄·H₂O, 0.044; CaCl₂, 0.021; FeSO₄·7 H₂O, 0.03], and then incubated for 48 h at 28 °C and 120 rpm. After the incubation period, each bacterial suspension was centrifuged at 4,000 rpm for 10 min, the pellet was washed twice and re-suspended in sterile distilled water, and the optical density (630 nm) of each strain was adjusted to 0.5 [10⁷ Colony Forming Units (CFU) mL⁻¹].

Finally, 1.5 L of the same sterile mineral medium was co-inoculated with each previously prepared pre-inoculum and incubated under the culture conditions previously described. The obtained biomass was centrifuged (4,000 rpm for 10 min) and adjusted to 10⁹ CFU mL⁻¹. Appropriate dilutions were made to obtain the inoculum concentration for field application (10⁷ CFU plant⁻¹), using sterile distilled water to ensure that the inoculum carrier was similar to the irrigation conditions and to minimize any potential effects of salts on crop germination. The relative abundance of each studied strain in the bacterial inoculum was TSO9 (20.5%), TSO2^T (20.8%), TSO22 (19.8%), TRQ8 (19.5%), and TRQ65 (19.4%), based on morphological (size, color, elevation, and shape), and metabolic (indole production, phosphate solubilization, siderophores production, and tolerance to saline, thermal, hydric, and chlorothalonil stress) traits, as well as the 16S rRNA gene sequencing by a Sanger platform^{22,23,30}.

2.2. Experimental set-up

The two field experiments were carried out during the winter season 2016–2017, and 2017–2018 at the Centro Experimental de Transferencia y Tecnología (CETT-910) (27° 21' 57.3" N, 109° 54' 55.3" W) of Instituto Tecnológico de Sonora, located in Ciudad Obregon, Sonora, Mexico (Figure 1).

Climate conditions during the studied seasons were: total season rainfall of 28.5 mm (2016–2017) and 13 mm (2017–2018), relative humidity of 66% (2016–2017) and 62% (2017–2018), and the average temperature was 19° C, in both seasons. The soil type (30 cm depth) in the experimental field was a Clay loam, with pH = 8.0 in H₂O, 0.86% organic matter content, 32 kg ha⁻¹ nitrogen, 50 kg ha⁻¹ phosphorus, and 2304 kg ha⁻¹ potassium.

The CIRNO C2008 durum wheat variety was used for these assays, which is the most planted variety in the Yaqui Valley

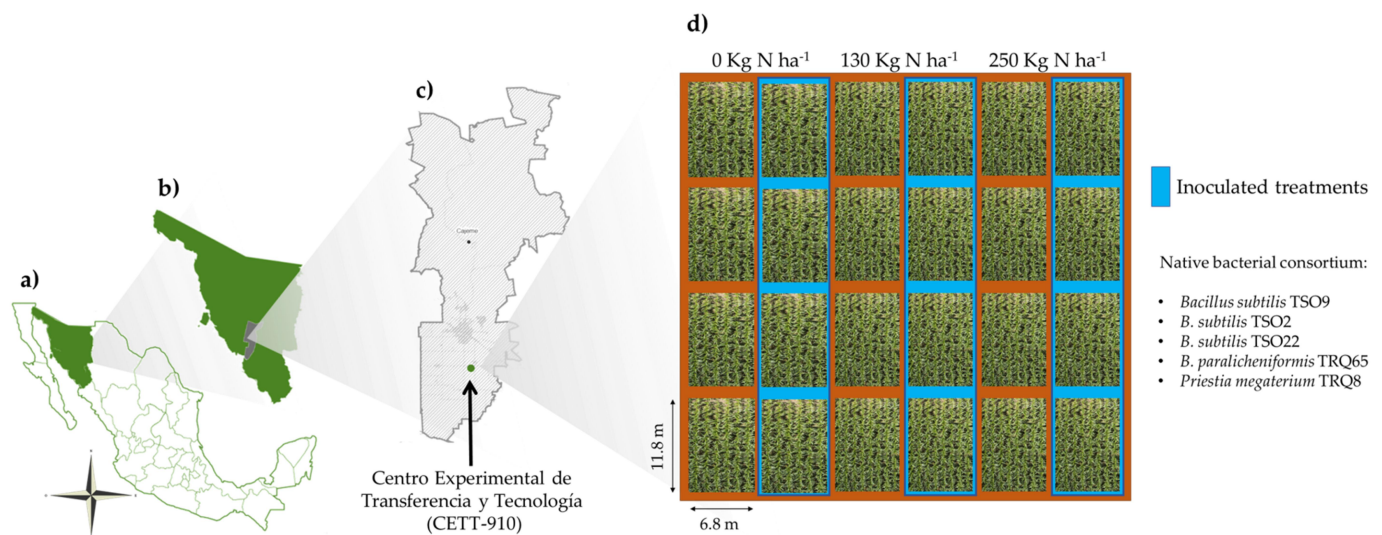


Figure 1. Centro Experimental de Transferencia y Tecnología (CETT-910) of Instituto Tecnológico de Sonora, located in Mexico (a), state of Sonora (b), and Municipality of Cajeme (c), in which the experimental design (d) consisting of nested plots of wheat under three doses of nitrogen (0, 50 and 100% N, respectively, added in the form of urea) and inoculation of the native bacterial consortium (CB) with four replicates each was established.

(80%)³². Field agricultural management was carried out according to the commercial wheat production in the Yaqui Valley. Seeding was carried out with a sowing machine (SUB-24) with three rows on the furrows and a seed density of 150 kg ha⁻¹.

2.3. Experimental design

A field experiment was carried out according to a three-factor (agricultural season, nitrogen percentage, and bacterial consortium application) with four replicates. In total, there were 24 plots (81.6 m², 6.8 m × 11.8 m) in each field experiment (Figure 1). Gravity irrigation was carried out, one pre-seeding irrigation (14 cm) two weeks before sowing, and three irrigations (12 cm) after sowing. There were three N rates dose [0%, 50%, and 100% of the recommended nitrogen fertilization (250 kg N ha⁻¹)]. Each of these rates was applied alone as well as in combination with the bacterial consortium. The bacterial consortium (10⁷ CFU plant⁻¹) was applied to the soil at 41, 68, and 89 days after sowing.

Urea (46:0:0; Tepeyac, Sonora, Mexico) was used as a nitrogen source, which was applied manually and fractioned as follows: pre-seeding (33%), first (33%), and second (33%) irrigation. Mono-ammonium phosphate (11:52:0; The Mosaic Company, Plymouth, MN) was used as a potassium source at the rate of 100 kg ha⁻¹ and was applied before sowing.

2.4. Data collection

Grain yield (GY, kg ha⁻¹) was quantified at the ripening stage and expressed at 14% moisture. The number of spikes m⁻² (NS m⁻²) was recorded before harvest. At harvest, plant height (PH, cm), spike length (SL, cm), number of grains per spike (NGS), straw yield (SY, kg ha⁻¹), and 1000-grain weight (TGW, g) were measured from 30 random spikes from each plot. Chlorophyll SPAD[®] units (model 2900P, Spectrum Technologies Inc., Plainfield, Illinois,

USA), Normalized Difference Vegetation Index (NDVI, Greenseeker, Trimble Inc., Westminster, Colorado, USA), and canopy temperature (infrared thermometer, Fluke 62 Max, Fluke Corp, Everett, WA) were also measured, at five phenological stages (S1: booting, S2: heading, S3: anthesis, S4: grain milk stage and S5: grain dough stage). The measures of the parameters previously mentioned were obtained in each experimental plot, using an area of 30.9 m². Finally, hectoliter weight, yellow berry, Nitrogen, protein, yellowness, SDS sedimentation volume, and whole-meal flour of wheat grains were determined following standard methods^{32–37}.

2.5. Statistical analysis

Data components were analyzed using analysis of variance (ANOVA) with STATGRAPHICS Plus 5.1. Differences between parameter means were assessed using the Tukey test. The significance level was set at $p \leq 0.05$ and ≤ 0.01 .

3 Results and discussion

In both agricultural seasons, wheat physiological traits such as chlorophyll content, NDVI, and canopy temperature were significantly more affected by the nitrogen fertilization doses than by the application of the studied bacterial consortium. The highest chlorophyll (56.23), NDVI (0.79), as well as lower canopy temperature (19.83°C) values, were observed in treatments under the application of 130 and 250 kg N ha⁻¹ (Table 1).

Regarding chlorophyll units observed in this study, similar findings have been reported by García-Mendivil *et al.*³³ who reported values from 52 to 58 units for the CIRNO C2008 variety. In addition, Mamrutha *et al.*³⁸ reported that six wheat genotypes, growing under 150 kg N ha⁻¹, showed chlorophyll content units from 35 to 49. These values are lower than those obtained in our study (52.13 to 55.35 at 130 kg N ha⁻¹),

Table 1. Effect of season, nitrogen fertilization level, and application of a bacterial consortium on wheat CIRNO C2008 physiological traits.

Factor	Chlorophyll, SPAD units										NDVI					Canopy temperature, °C																		
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5														
Season (A)	2016–2017	48.55 b	48.55 b	49.38 b	53.38 b	48.47 b	0.75 a	0.74 b	0.79 a	0.67	0.56 b	19.6 b	21.58	21.89 a	24.37 a	23.76 b	2017–2018	55.05 a	56.26 a	55.09 a	55.33 a	54.23 a	0.72 b	0.78 a	0.74 b	0.67	0.64 a	22.01 a	20.77	18.82 b	23.30 b	25.24 a		
N level, kg ha ⁻¹ (B)	0	50.75 b	51.55 b	51.05 b	51.48 b	47.14 b	0.71 c	0.70 b	0.72 b	0.62 b	0.52 b	21.35 a	21.17	21.09 a	25.13 a	25.89 a	130	52.13 ab	52.45 ab	53.04 a	55.35 a	53.34 a	0.76 a	0.79 a	0.78 a	0.70 a	0.63 a	20.26 c	19.83	20.00 b	22.90 c	23.94 b		
	250	52.52 a	53.22 a	52.62 a	56.23 a	53.56 a	0.73 b	0.79 a	0.78 a	0.70 a	0.64 a	20.80 b	22.52	19.96 b	23.48 b	23.68 b	Un-	52.59 a	52.79 a	52.38 a	54.68 a	51.37 a	0.73 a	0.75 b	0.76 a	0.67 a	0.60 a	20.87 a	22.44 a	20.46 a	24.16 a	24.79 a		
	Inoculated	51.02 b	52.79 a	52.10 a	54.03 a	51.33 a	0.73 a	0.77 a	0.76 a	0.67 a	0.60 a	20.74 a	19.91 a	20.24 a	23.51 b	24.22 b	Inoculated	51.02 b	52.79 a	52.10 a	54.03 a	51.33 a	0.73 a	0.77 a	0.76 a	0.67 a	0.60 a	20.74 a	19.91 a	20.24 a	23.51 b	24.22 b		
A x B	Inoculated	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	Inoculated	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
A x C	Statistical	**	**	**	*	**	**	**	*	**	**	**	**	-	**	**	Statistical	**	**	**	*	**	**	**	**	-	**	**	**	**	**	**	**	**
B x C	significance	-	-	-	**	**	**	**	**	**	**	**	**	**	**	**	significance	-	-	-	**	**	**	**	**	-	**	**	**	**	**	**	**	**
A x B x C		**	**	**	**	**	**	**	**	**	**	**	**	**	**	**		**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**

S1: booting, S2: heading, S3: anthesis, S4: grain milk stage, and S5: grain dough stage

Letters indicate differences between parameter means by factor using the Tukey test, $p \leq 0.05$. Asterisks indicate the significance level by the Tukey test [$p \leq 0.05$ (*) or ≤ 0.01 (**)]. Four replicates in each assay.

which may be related to the genetic background of wheat varieties, edaphic-climatic conditions, and agricultural practices in the agroecosystem. Furthermore, a study carried out in three agricultural seasons under 0% nitrogen fertilizer applied to wheat showed values from 32 to 48 chlorophyll content units³⁹, similar to the findings obtained in our treatments under the same nitrogen dose (Table 1).

A similar tendency was observed for NDVI values, with the lowest values (0.52) found in plants grown under 0 kg N ha⁻¹. However, these values increased according to the amount of N applied to wheat plants, reaching 0.79 at 130 and 250 kg N ha⁻¹ (Table 1). For CIRNO C2008, Argente *et al.*⁴⁰ reported NDVI values above 0.80 (at 250 kg N ha⁻¹) for healthy plants during the flowering stage. Similar values of 0.79 at stage S2 (heading) were observed in our study in plants grown under 130 and 250 kg N ha⁻¹ (Table 1). The decrease in NDVI values from S1 (0.76) to S5 (0.52) may be attributed to the translocation of nitrogenous compounds, which favors the filling of grains as an important signal of the plant senescence⁴¹ since chlorophyll content and NDVI in leaves help to estimate the status of N and the photosynthetic capacity of plants^{38,42}. Here, nitrogen doses significantly affected these parameters, with healthy plants observed under the application of 130 or 250 kg N ha⁻¹ (in soil naturally containing 32 kg N ha⁻¹).

The highest canopy temperature (25.8°C) was observed in plants grown under the application of 0 kg N ha⁻¹ (Table 1). This increase in temperature harms wheat production as it reduces the intake of water and nutrients from the soil by the root system^{43,44}. In addition, an elevated canopy temperature leads to a reduction in yield due to the acceleration of developmental processes, respiration rate, and assimilate partitioning, thus shortening the duration of the vegetative stage^{45,46}. Cultivars with lower canopy temperatures (12 to 25°C) are known to produce higher yields and reduce leaf senescence^{47,48}. In this study, although all studied treatments showed these optimal values of canopy temperature, the application of 130 or 250 kg ha⁻¹ of nitrogen fertilizer to wheat had the greatest impact on reducing canopy temperature (19.83–23.94°C) compared to the application of the native bacterial consortium and agricultural season (Table 1). These findings, along with the chlorophyll content and NDVI in leaves, suggest that healthy and productive wheat plants were obtained in soil with 32 kg N ha⁻¹, and fertilized with 130 or 250 kg N ha⁻¹.

On the other hand, plant height (ranging from 51.45 to 52.39 cm) did not show significant differences among the studied treatments in any of the evaluated factors (agricultural season, Nitrogen dose, and bacterial consortium) (Table 2), similar to the results reported in other studies⁴⁹. This is likely because plant height is mostly regulated by the genetic background^{50,51}. However, the number of spikes per square meter was significantly affected by the agricultural season, Nitrogen dose, and the bacterial consortium. The highest values were obtained in the 2016–2017 season (317.08), the Nitrogen dose of 130 and 250 kg N ha⁻¹ (306.81 and 309.81, respectively), and the un-inoculated treatment (307.56) (Table 2). Similar findings were found by Galindo *et al.*⁵² who reported a positive correlation between the amount of nitrogen applied to wheat and the number of spikes per square meter. On the other hand, Saleem *et al.*⁵³ reported a maximum number of winter wheat spikes at the jointing stage of 423.67 spikes m⁻² at a dose of 225 kg N ha⁻¹.

There was a significant interaction between the agricultural season, N rates, and inoculation with the bacterial consortium in terms of the number of spikes per meter (Table 2). At rates of 130 and 250 N kg ha⁻¹, the treatments showed a higher number of spikes per meter. In contrast, wheat plants inoculated with PGPR showed a lower number of spikes per square meter compared to the un-inoculated treatment, which is consistent with previous findings^{49,50}. These studies reported that the number of spikes per square meter was lower when single or combined bacterial strains were applied, compared to fertilized treatment with synthetic nitrogen, phosphorus, or in combination. However, despite the lower number of spikes per square meter in our inoculated treatment (290.95 spikes m⁻²), it has been reported that the optimal range for achieving maximum yields is between 200 to 300 spikes per square meter⁵⁴.

Spike length showed a significant negative difference in the high nitrogen treatment, with lengths of 7.20 cm and 6.98 cm at 130 and 250 kg N ha⁻¹, respectively. Conversely, a positive effect of the bacterial consortium on this parameter was observed, with lengths of 7.04 cm compared to 6.88 cm in the un-inoculated treatment (Table 2). In the case of durum wheat varieties, spike lengths from 7.0 to 8.5 cm have been normally reported⁵⁵. Besides, Gupta *et al.*⁴⁸ reported an average spike length of 6.8 cm for four wheat varieties. In contrast, Galindo

Table 2. Effect of season, Nitrogen fertilization level, and application of a bacterial consortium on wheat CIRNO C2008 quantitative traits.

Factor		Plant height, cm	Spike per m ²	Spike length, cm	Grains per spike	Straw, ton ha ⁻¹	Grain yield, ton ha ⁻¹
Season (A)	2016–2017	52.08 a	281.44 b	6.92 a	48.09 b	6.62 a	6.85 a
	2017–2018	51.54 a	317.08 a	7.00 a	53.62 a	7.04 a	7.04 a
N level, kg ha ⁻¹ (B)	0	51.59 a	281.16 b	6.64 c	49.91 a	6.36 a	5.54 b
	130	52.39 a	306.81 a	7.20 a	52.44 a	7.40 a	7.72 a
	250	51.45 a	309.81 a	6.98 b	50.10 a	6.74 a	7.57 a
Bacterial Consortium (C)	Inoculated	51.69 a	290.95 b	7.04 a	52.13 a	6.40 b	7.49 a
	Un-inoculated	51.93 a	307.56 a	6.88 b	49.40 b	7.26 a	6.40 b
A x B	Statistical	*	**	**	**	no	**
A x C	significance	-	**	-	**	-	-
B x C		-	**	**	-	**	**
A x B x C		*	**	**	**	**	**

Letters indicate differences between parameter means by factor using the Tukey test, $p \leq 0.05$. Asterisks indicate the significance level by the Tukey test [$p \leq 0.05$ (*) or ≤ 0.01 (**)]. Four replicates in each assay

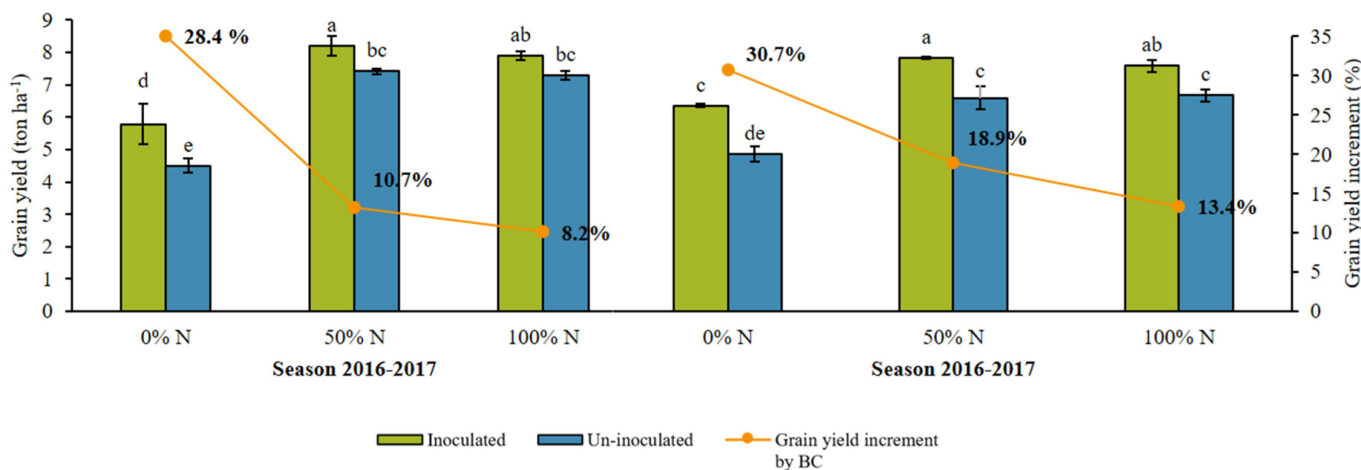


Figure 2. Effect of the application of a bacterial consortium on wheat CIRNO C2008 grain yield, under different seasons, and nitrogen fertilization levels. Letters indicate differences between parameter means using the Tukey test, $p \leq 0.05$. Four replicates in each assay.

*et al.*⁴⁹ quantified the impact of five nitrogen doses (0, 50, 100, 150, and 200 kg N ha⁻¹) and the inoculation with *Azospirillum brasilense* on winter wheat and found no significant differences in spike length (7.31 cm and 7.53 cm) between the control and bacterial application, respectively.

Regarding grains per spike, significant positive differences were observed in the 2017–2018 agricultural season (53.62) compared to the 2016–2017 season (48.09), as well as with the application of the bacterial consortium (52.13) compared to the un-inoculated treatment (49.40). However, the Nitrogen applied to wheat did not show a significant effect on this parameter (Table 2). Díaz-Zorita & Fernández⁵⁶ reported an increase in grains per spike of 2.62% in wheat inoculated with the bacterial genus *Azospirillum*; while García-Mendivil *et al.*³³ reported a greater accumulation of assimilates in the spike during the three weeks prior the anthesis stage, which was associated with a higher number of grains per spike.

Straw yields only showed a significant difference with the application of the bacterial consortium, resulting in a reduction from 7.26 to 6.40 tons ha⁻¹ (Table 2). However, the interaction between the N rates and inoculation with the bacterial consortium was significant for wheat grain yield. The consortium, compared to the un-inoculated treatments, showed significant positive differences in grain yield, increasing from 6.40 to 7.49 ton ha⁻¹ (equivalent to an increase in yield of 17%). Moreover, the increased Nitrogen doses (130 and 250 kg N ha⁻¹) also contributed to higher grain yield compared to 0 kg N ha⁻¹. (Table 2). Nitrogen is a macro-nutrient that increased the leaf area and enhances photosynthesis efficiency, thereby increasing yield (Tables 1 and 2)⁵⁷.

Concerning grain yield, several authors reported a positive response to N fertilization and PGPR inoculation in wheat. Hussain *et al.*³⁴ reported a 17.1% improvement in wheat grain yield when inoculated with *Bacillus* strain MWT-14 in combination with different Nitrogen-Phosphorous doses (0–0, 105–75, 150–100 kg ha N-P fertilizer). Singh & Kapoor⁵⁸ observed a significant increase (45%) in wheat grain yield, due to inoculation of *Bacillus circulans*, *Cladosporium herbarum*, and *Glomus* sp., which enhanced nutrient absorption. In both seasons, our data consistently demonstrated that the

grain yield in the inoculated plots with the studied consortium exhibited a notable increase across all doses of applied nitrogen (0, 130, and 250 N kg ha⁻¹). Specifically, the treatment inoculated with the consortium under 130 N kg ha⁻¹ increased the wheat grain yield to more than 1.0 tons ha⁻¹ compared to treatments utilizing the recommended nitrogen dose (Figure 2). Thus, since bacterial inoculants are bio-products containing bacteria that promote plant growth, health, and soil fertility restoration²², the studied bacterial consortium, combined with a reduced amount of Nitrogen fertilizer, represents a sustainable alternative to increase wheat yield under the specific soil, climatic, and crop conditions in the Yaqui Valley (Figure 2). This type of alternative has also been reported by Galindo *et al.*⁴⁹ where inoculation with *A. brasilense*, associated with the application of 100–150 N kg ha⁻¹, provided the highest grain yield of irrigated wheat cropped.

Regarding quality traits, the hectoliter weight reflects the density and milling quality of grains³² and did not show significant differences among the studied treatments, with values ranging from 80.40 to 80.92 kg l⁻¹ (Table 3). According to Chávez *et al.*⁵⁹, durum wheat should have a minimum hectoliter weight of 74 kg l⁻¹, with an average of 83 kg l⁻¹. Similarly, thousand kernel weight, another indicator of milling quality, did not show a statistical difference in any of the studied treatments, obtaining values from 49.49 to 53.43 g (Table 3), which are in the range (from 43.4 to 53 g) reported for durum wheat produced in Yaqui Valley⁵⁹.

The yellow berry percentage was only affected by the fertilization dose, with the highest yellow berry percentage observed in the lowest fertilizer doses (0 kg N ha⁻¹) (Table 3). Solís *et al.*⁶⁰ reported a yellow berry percentage of 70% in wheat crops growing with 0 kg N ha⁻¹, and 7% with 240 kg N ha⁻¹, demonstrating a direct relationship between the amount of Nitrogen applied and this parameter. Yellow berry is considered one of the most important industrial parameters affecting grain quality but not crop yield⁶⁰. The presence of yellow berry can reduce the percentage of protein in the grain by 2–4%⁶⁰. In addition, it was observed that the lower Nitrogen dose (0 kg N ha⁻¹) showed a lower protein content (9.55%), which was

Table 3. Effect of season, Nitrogen fertilization level, and application of a bacterial consortium on wheat CIRNO C2008 quality traits.

Factor	Season (A)	2016–2017	2017–2018	Hectolitre weight, kg l ⁻¹	1000 grains weight, g	Yellow berry, %	Protein, %	N, %	Whole meal SDS-Sedimentation, ml	SDS-Sedimentation index SDS/ PRO	Whole meal Yellowness Minolta
N level, kg ha ⁻¹ (B)	0	80.72 a	80.58 a	52.22 a	11.07 a	11.55 a	2.00 a	11.02 a	1.01 a	14.95 a	
	130	80.60 a	80.60 a	51.30 a	11.76 a	10.95 b	2.17 a	10.77 a	0.98 a	14.35 b	
	250	80.41 a	80.41 a	52.36 a	31.41 a	9.55 c	1.95 a	9.06 c	0.97 a	14.53 b	
Bacterial Consortium (C)	Inoculated	80.92 a	80.92 a	53.43 a	2.85 b	11.68 b	2.15 a	11.21 b	1.00 a	14.60 b	
	Un-	80.40 a	80.40 a	49.49 a	0.00 b	12.51 a	2.15 a	12.40 a	1.01 a	14.82 a	
	Inoculated	80.89 a	80.89 a	52.62 a	13.17 a	11.15 a	2.10 a	10.83 a	1.00 a	14.58 b	
	Statistical significance	-	-	50.89 a	9.66 a	11.34 a	2.07 a	10.95 a	0.99 a	14.72 a	
A x B	-	-	-	-	**	**	-	**	-	**	
A x C	-	-	-	-	-	-	-	-	-	**	
B x C	-	-	-	-	**	**	-	**	-	-	
A x B x C	-	-	-	-	**	**	-	**	-	**	

Letters indicate differences between parameter means by factor using the Tukey test, $p \leq 0.05$. Asterisks indicate the significance level by the Tukey test [$p \leq 0.05$ (*) or ≤ 0.01 (**)]. Four replicates in each assay

significantly increased by the Nitrogen dose applied to the crop (Table 3). Finally, the N concentration in plant tissue did not show significant differences in any of the studied treatments, obtaining values from 1.95 to 2.17%, which are similar to those reported for CIRNO C2008 (2.2%)⁶¹.

The whole meal yellowness, which contributes to the color of semolina and is a desirable trait in end products such as pasta and couscous, is mainly influenced by the genotype, with minor contributions from the environment and their interactions^{35,62}. In this study, whole meal yellowness was significantly lower at nitrogen doses of 0 and 130 kg N ha⁻¹ compared to the application of 250 kg N ha⁻¹. Also, significant differences in this parameter were observed concerning the agricultural season and bacterial consortium treatments (Table 3), however, these differences were very low between treatments, which in practical terms mean no difference. Furthermore, the yellowness values obtained in this study fall within the optimal range for high-quality grain³⁵.

Flour SDS sedimentation volume, which is used to characterize wheat flour and predict processing and end-product qualities, reflects the hydration and expansion capacity of gluten proteins^{63,64}. In our study, flour SDS sedimentation only showed a significant difference with different Nitrogen doses, observing that higher N doses increased the SDS Sedimentation volume (Table 3), which was expected as is well known that protein content influences SDS sedimentation volume^{35,64}.

Overall, the bacterial consortium application did not show any significant effects on the studied quality traits, indicating that gluten quality was not affected by its application, despite the increase in yield by 1.0 ton ha⁻¹.

4 Conclusions

The application of bacterial consortia that promotes plant growth is a promising alternative to increase crop production while reducing the environmental and economic costs associated with nitrogenous fertilizers. In this study, the application of a native bacterial consortium from the Yaqui Valley showed significant positive effects on wheat, even at a reduced Nitrogen fertilization rate of 130 kg N ha⁻¹, compared to the conventional dose of 250 kg N ha⁻¹. These effects were evidenced by the increase in spike length, the number of grains per spike, and overall grain yield, without compromising the quality parameters of the harvested grain.

Therefore, this native bacterial consortium (*B. subtilis* TSO9, *Bacillus cabrialesii* subsp. *tritici* TSO2^T, *B. subtilis* TSO22, *B. paralicheniformis* TRQ65, and *Priestia megaterium* TRQ8) is a promising sustainable alternative for wheat production. However, for the extensive use of this inoculant, it is crucial to assess its performance under various agro-edaphoclimatic conditions, since yield is determined by genetic and environmental factors. Thus, the development of new varieties and agronomic management, especially the appropriate doses and timing of nitrogen fertilizers, in conjunction with the use of PGPR are necessary to increase the efficient use of this nutrient by the crops. Besides, further exploration using omics approaches is necessary to identify the mechanisms of

action and understand its ecological role in agroecosystems.

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