

Genomic insights of a native bacterial consortium for wheat production sustainability

Marisol Ayala Zepeda^a, Valeria Valenzuela Ruiz^a, Fannie Isela Parra Cota^b, Cristina Chinchilla-Soto^c, Eulogio de la Cruz Torres^d, María Itria Ibba^e, María Isabel Estrada Alvarado^f, Sergio de los Santos Villalobos^{a,*}

^a Departamento de Ciencias Agronómicas y Veterinarias, Instituto Tecnológico de Sonora ITSON, Obregón, 85000, Mexico

^b Campo Experimental Norman E. Borlaug, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias INIFAP, Obregón, 85000, Mexico

^c Centro de Investigación en Contaminación Ambiental, Universidad de Costa Rica UCR, San José, 11501-2060, Costa Rica

^d Departamento de Biología, Instituto Nacional de Investigaciones Nucleares ININ, Ocoyoacac, 52750, Mexico

^e Centro Internacional de Mejoramiento de Maíz y de Trigo CIMMYT, Texcoco, 56237, Mexico

^f Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora ITSON, Obregón, 85000, Mexico

ARTICLE INFO

Keywords:

Nitrogen use efficiency
Genomic bioinformatic analysis
Wheat crop yield
Wheat quality
PGPB

ABSTRACT

The use of plant growth-promoting bacteria as bioinoculants is a powerful tool to increase crop yield and quality and to improve nitrogen use efficiency (NUE) from fertilizers in plants. This study aimed to bioprospecting a native bacterial consortium (*Bacillus cabrialesii* subsp. *cabrialesii* TE3^T, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65), through bioinformatic analysis, and to quantify the impact of its inoculation on NUE (measured through ¹⁵N-isotopic techniques), grain yield, and grain quality of durum wheat variety CIRNO C2008 grown under three doses of urea (0, 120, and 240 kg N ha⁻¹) during two consecutive agricultural cycles in the Yaqui Valley, Mexico. The inoculation of the bacterial consortium (BC) to the wheat crop, at a total N concentration of 123–225 kg N ha⁻¹ increased crop productivity and maintained grain quality, resulting in a yield increase of 1.1 ton ha⁻¹ (6.0 vs. 7.1 ton ha⁻¹, 0 kg N ha⁻¹ added, 123 kg N ha⁻¹ in the soil) and of 2.0 ton ha⁻¹ (5.9 vs. 7.9 ton ha⁻¹, 120 kg N ha⁻¹ added, 104 kg N ha⁻¹ in the soil) compared to the uninoculated controls at the same doses of N. The genomic bioinformatic analysis of the studied strains showed a great number of biofertilization-related genes regarding N and Fe acquisition, P assimilation, CO₂ fixation, Fe, P, and K solubilization, with important roles in agroecosystems, as well as genes related to the production of siderophores and stress response. A positive effect of the BC on NUE at the studied initial N content (123 and 104 kg N ha⁻¹) was not observed. Nevertheless, increases of 14 % and 12.5 % on NUE (whole plant) were observed when 120 kg N ha⁻¹ was applied compared to when wheat was fully fertilized (240 kg N ha⁻¹). This work represents a link between bioinformatic approaches of a native bacterial inoculant and the quantification of its impact on durum wheat.

Introduction

At present, wheat is the most extensively grown crop worldwide and it has become the most important source of food, as it provides about 20 % of all protein and calories consumed worldwide (Igrejas and Branlard, 2020; Reynolds and Braun, 2022). In Mexico, wheat is the second most-produced cereal, and this country is the third exporter of durum wheat in the world (SIAP, 2023a).

This region served as the origin of the Green Revolution with the

scientific works led by Dr. Norman E. Borlaug, where the wheat yield increased from 1.4 ton ha⁻¹ in 1950 to 5 ton ha⁻¹ in 1980 (Fischer et al., 2014). This increment (or yield change) was possible due to the development of high-yielding and pest-resistant varieties, but also to higher rates of chemical fertilizer applications, which have doubled since 1980 in the Yaqui Valley, and currently ~300 kg N ha⁻¹ are applied to the wheat production (Millar et al., 2018).

Currently, nitrogen use efficiency (NUE) by crops is less than 40 % and has been reported in 31 % for the wheat crop in the Yaqui Valley

* Corresponding author.

E-mail address: sergio.delossantos@itson.edu.mx (S. de los Santos Villalobos).

<https://doi.org/10.1016/j.crmicr.2024.100230>

Available online 5 March 2024

2666-5174/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

(Ortiz-Monasterio and Raun, 2007), which means that the rest is lost from the agroecosystems by surface runoff and leaching, as well as by volatilization and denitrification (IAEA, 2017). That situation leads to economic losses associated with agricultural production, adverse health, and environmental effects, such as nitrous oxide N_2O emissions, eutrophication of aquatic ecosystems, and groundwater nitrate contamination which is used for human consumption (Beman et al., 2005; Lares-Orozco et al., 2016; Millar et al., 2018; Wongsanit et al., 2015). Thus, to increase NUE, there are two main approaches: crop breeding and/or agronomic management (Ortiz-Monasterio, 1998).

In this sense, for tracing nitrogen in the agroecosystem the use of ^{15}N -enriched fertilizers is a feasible strategy, to precisely quantify the actual recovery of this nutrient by crops (Kennedy et al., 2008). This ^{15}N -isotopic technique is based on the fact that the sources of the N taken up by a non-nitrogen-fixing plant are only the soil and the fertilizers; therefore, nitrogen fertilizers can be artificially isotopically enriched in ^{15}N atoms above the natural abundance (0.3663 atom % ^{15}N) and then applied to the crop of interest. Any enrichment in ^{15}N atoms found in the plant tissues is then attributed to the applied synthetic fertilizer.

Besides, to increase the NUE in plants, the use of beneficial microorganisms is a suitable strategy. Plant growth-promoting bacteria (PGPB) have shown the ability to improve the acquisition of nutrients by plants, protecting crops from pests, diseases, and various types of stress (Montoya-Martínez et al., 2022).

Thus, studies combining PGPB and chemical fertilizers have demonstrated increases in nutrient absorption and efficiency by the crops, improving quality and yield, being a promising strategy to address actual and future agricultural challenges (Kumar et al., 2016; Goicoechea and Antolín, 2017, 2017; García-Montelongo et al., 2023). However, the bioprospection stage for identifying promising PGPB is a key step for developing bacterial inoculants, due to several known or unknown action modes found, and the expression of their metabolic potential under variable conditions in the field (Cordova-Albores et al., 2021). In this context, genomic approaches -based on mining- can be successfully used for designing promising inoculants, from their compatibility to ecological functions in the agroecosystem. For example, in the research carried out by Ortega-Urquieta et al. (2022), wheat plants showed significant ($p < 0.05$) increments in the leaf number (68.3 %) and the stem diameter (87.9 %) when plants were inoculated with *Priestia sp.* TSO9. The genome of this strain showed the presence of genes related to auxin biosynthesis, siderophore production, growth, adaptability, and colonization, which could be associated with wheat growth promotion.

For years, extensive work has been carried out by Laboratorio de Biotecnología del Recurso Microbiano (LBRM) in Mexico to bioprospect native PGPB to increase wheat yield and quality. Among the studied strains, *Bacillus cabrialesii* subsp. *cabrialesii* TE3^T, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65, have shown several plant-growth-promoting traits. For example, Rojas-Padilla et al. (2020) demonstrated that these strains can produce indoles (from 8.21 to 39.29 $\mu\text{g/mL}$), as well as possess the ability to solubilize phosphorus (solubilization index from 1.37 to 1.43). In addition, *P. megaterium* TRQ8 showed a siderophore production index of 8.17. Additionally, these strains can tolerate thermal (43.5 °C), hydric [polyethylene glycol (PEG), 10 %, -0.84 mPa], saline (NaCl, 5 %, 6.8 dS m^{-1}), and chemical stress (Chlorothalonil) (Robles-Monoya et al., 2020). Also, Rojas-Padilla et al. (2022) synthesized calcium alginate microbeads containing strain TRQ8, TRQ65, and TE3^T, where their co-inoculation to wheat plants (strain TRQ8 + TRQ65 and TRQ8 + TE3^T) under greenhouse conditions, increased several biometric parameters, such as root length (increase of 7.9 % to 19.2 %), stem length (from 8.4 % to 11.7 %), stem dry weight (up to 30.5 % more than the control), root dry weight (up to 12.8–77.3 % more than the control), and chlorophyll content (increasing ~7 % in SPAD units compared to the control).

On the other hand, Chaparro-Encinas et al. (2022) carried out a transcriptomic analysis of wheat seedlings inoculated with strain

TRQ65. This strain stimulated cell growth and plant biomass via reprogramming the gene expression patterns associated with systemic response in wheat (induced systemic resistance, ISR, and systemic acquired resistance, SAR). Also, a morphometrical assay confirmed wheat growth promotion. A significant ($p < 0.05$) increase in shoot length (93.48 %), root dry weight (48.33 %), and shoot dry weight (117.02 %) was observed in inoculated plants.

The biological control potential of strains TRQ65 and TE3^T through the production of antifungal metabolites has been studied and they showed inhibition against *Bipolaris sorokiniana* TPQ3, the causal agent of spot blotch in wheat (Villa-Rodríguez et al., 2019; Valenzuela-Ruiz et al., 2019). However, they did not show antagonistic action among them, when they were inoculated as a consortium (TRQ65 + TRQ8 + TE3^T) in wheat; on the contrary, they showed a synergistic effect with positive impacts in the studied morphometric variables in the early stages of development (Rojas-Padilla et al., 2020).

Despite the promising in vitro or greenhouse results of these strains on wheat plants, an insight into their genomic background associated with the above-mentioned traits, as well as their beneficial effects on wheat under commercial field conditions, at different doses of N fertilizer to quantify the NUE, have not been studied. Thus, this work further studies the inoculation of these strains in interaction with the biotic and abiotic factors of the agro-system in the field. Besides, understanding their genomic potential may help comprehend the complex plant, microorganism, and soil interactions, and how these may improve plant growth through different mechanisms. These of which the genomic profile will evidence and aid in generating strategies to optimize their function and efficacy in the field.

Thus, this study aimed to bioprospecting a native bacterial consortium (*B. cabrialesii* subsp. *cabrialesii* TE3^T, *P. megaterium* TRQ8, and *B. paralicheniformis* TRQ65), based on the first approach of the genomic analysis and evaluation under three doses of urea (0, 120, and 240 kg N ha^{-1}), for exploring its impact on NUE -through ^{15}N -isotopic techniques-, yield, and quality of durum wheat (CIRNO C2008), under two initial N concentrations in the soil (two consecutive agricultural cycles) in the Yaqui Valley, Mexico.

Materials and methods

Bacterial consortium characteristics and isolation

The native bacterial consortium (BC) consisted of three strains: *B. cabrialesii* subsp. *cabrialesii* TE3^T, *P. megaterium* TRQ8, and *B. paralicheniformis* TRQ65. The bacterial strains TRQ8 (Robles-Montoya et al., 2019) and TRQ65 (Valenzuela-Ruiz et al., 2019) were isolated from rhizospheric soil from commercial wheat fields located in the Yaqui, Valley, Mexico (27.3692°, 110.3886°), while TE3^T was isolated as an endophytic bacterial strain from wheat leaf tissue in the Yaqui Valley (de los Santos-Villalobos et al., 2019). These strains are cryopreserved at -80 °C in nutrient broth (NB) culture medium with glycerol (30 %), in the Colección de Microorganismos Edáficos y Endófitos Nativos (COLMENA, www.itson.mx/COLMENA) (de los Santos-Villalobos et al., 2021).

Genomic analysis of the studied bacterial consortium

The bacterial strains were characterized and taxonomically identified through a polyphasic approach (Robles-Montoya et al., 2020; Morales Sandoval et al., 2021). Thus, after obtaining raw reads from LANGE BIO (Institute of Genomic Services “LANGE BIO—CINVESTAV”, Irapuato Guanajuato, Mexico), who sequenced on the Illumina MiSeq platform (2 × 300 bp) (Illumina, San Diego, CA, USA) raw read quality was assessed using FastQC version 0.11.5 (Andrews, 2010). Then, adapter sequences and low-quality bases were eliminated using Trimmomatic version 0.32 (Bolger et al., 2014), using a sliding window of 4:24, headcrop of 12, and a minlen of 50. The previous parameters will

ensure that raw read quality is above 24 on a Phred scale. Following this, a *de novo* assembly was created utilizing SPAdes version 3.14.1 (Bankovich et al., 2012), implementing the "-careful" parameter to enhance error correction in reads. The resulting contigs from the assembly were then arranged by Mauve Contig Mover 2.4.0 (Darling et al., 2004), using reference genomes *Bacillus paralicheniformis* KJ-16^T (GCA_001042485.2) for strain TRQ65 (Valenzuela-Ruiz et al., 2019), *Bacillus megaterium* 15, 308 (GCA_001591525.1) for strain TRQ8 (Robles-Montoya et al., 2019), and *Bacillus inaquosorum* KCTC 13429^T (GCA_003148415.1) for strain TE3^T (de los Santos-Villalobos et al., 2019; Valenzuela-Ruiz et al., 2023). Furthermore, genome annotation was conducted using the Rapid Annotation Using Subsystem Technology (RAST) server version 2.0 (Overbeek et al., 2014) with the RASTtk pipeline, relying on The PathoSystems Resource Integration Center (PATRIC) under standard default parameters (Davis et al., 2020), where genes related to plant growth promotion were identified concerning interest subsystems (secondary metabolism). To further complement these findings, another annotation was carried out in the plant-associated bacteria web resource (PLaBase) v1.01 PGPT-Pred annotation of bacterial plant growth promoting traits (PGPT), using the blast+hmmer algorithm to depict PGPT ontology (Patz et al., 2021). Lastly, InteractiVenn (Heberle et al., 2015) was used to compare PGPT-related genes. The results generated were visualized with Krona (Ondov et al., 2011).

Inoculation of the studied native bacterial consortium to wheat

First, the frozen glycerol (-80°C) bacterial strains were pre-cultured on Petri dishes containing Nutrient Agar (MCD Lab, Cat. 7141) as a culture medium and incubated for 24 h at 28°C . Each PGPB strain was separately inoculated in 20 mL of a sterile minimal salt medium [composed of 10 g L^{-1} of glucose, 4 g L^{-1} of $(\text{NH}_4)_2\text{SO}_4$, 5.32 g L^{-1} of K_2HPO_4 , 6.4 g L^{-1} of KH_2PO_4 , 0.4 g L^{-1} of $\text{MgSO}_4\cdot\text{H}_2\text{O}$, 0.044 g L^{-1} of $\text{MnSO}_4\cdot\text{H}_2\text{O}$, 0.021 g L^{-1} of CaCl_2 , and 0.03 g L^{-1} of $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$], incubated at 28°C , and 180 rpm during 24–48 h, until obtaining an optical density (630 nm) of ~ 1.0 . Then, 20 mL PGPB strains culture medium were individually inoculated in 500 mL of the above-mentioned culture medium at the same conditions for 48 h. The number of viable bacterial cells was quantified as colony-forming units per milliliter (CFU mL^{-1}). Once a density of 1×10^7 CFU mL^{-1} was obtained for each strain, they were combined equitably to obtain the BC, which was applied manually directly to the soil (1×10^6 cells plant^{-1}), at sowing, at 45 days after sowing (tillering stage) with the first irrigation, and 80 days after sowing (wheat grain filling) with the second irrigation.

Study site description

The study was conducted during the winter crop season (December to May) 2018–2019 and 2019–2020 at the Experimental Technology Transfer Center (CETT-910) of the Instituto Tecnológico de Sonora (ITSON) in the Yaqui Valley, Mexico (Latitude: $27^{\circ}21'57.74''\text{N}$, Longitude: $109^{\circ}54'55.91''\text{W}$). Durum wheat (*Triticum turgidum* L. subsp. *durum*, CIRNO C2008 variety) was sown under field conditions (seed density 120 kg ha^{-1}), with conventional crop management (primary tillage, flood irrigation, integrated weed management) on a vertisol soil (Wilding et al., 1983).

Durum is a tetraploid species of wheat, and the main grain used for making pasta. It is adapted to more diverse environments than bread wheat, and it performs in semiarid regions, such as the Yaqui Valley, in Sonora, where 84 % of the national production of durum wheat is grown (SIAP, 2023b).

The soil in this site (CETT-910) has been monitored for its climatic and edaphic properties and only wheat has been sown there for the past 10 years (no crop rotation is practiced). Furthermore, this experimental center is located within the Yaqui Valley, where wheat has been conventionally cultivated in the surroundings for decades. Soil properties were determined according to specifications of the Official Mexican

Standard NOM-021-SEMARNAT-2000, and are shown in Table 1 along with climatic conditions.

Both sown dates were on the same day each year (December 20th, 2018, and December 20th, 2019). The source of phosphate fertilizer was mono-ammonium phosphate (100 kg ha^{-1}), and urea was used as nitrogen fertilizer (according to each treatment) which was fractionated equally at pre-planting (33 %), at the first irrigation event (33 %), and at the second (33 %) irrigation. The harvest date was 140 days after sowing.

Field experiment

The experiment consisted of a split-plot arrangement, 6 treatments, and 4 replicates: with and without the inoculation of the BC, under three nitrogen fertilization doses (0, 120, 240 $\text{kg N (urea) ha}^{-1}$; the last is the recommended N dose for wheat production in the region). Thus, the experimental design was: i) 0 kg N ha^{-1} ; ii) 0 kg N ha^{-1} + BC; iii) 120 kg N ha^{-1} ; iv) 120 kg N ha^{-1} + BC; v) 240 kg N ha^{-1} , and vi) 240 kg N ha^{-1} + BC. Each plot contained an internal microplot for the application of ^{15}N -isotopically enriched urea at 1 atom % ^{15}N (Shanghai Research Institute of Chemical Industry Co.; provided by the International Atomic Energy Agency), according to the dosage of 120 and 240 kg N ha^{-1} (Fig. 1). The original enrichment of the labeled urea was 5.09 atom % ^{15}N , and it was diluted with conventional urea (natural abundance of ^{15}N) to obtain an enrichment of 1 atom % ^{15}N following Zapata & Axman's (1990) equation and procedure.

Measurement of yield and quality parameters

Grain yield was obtained at the ripening stage (after harvest) and expressed at 14 % moisture. The number of spikes m^{-2} was recorded before harvest. At harvest, from the 6 central furrows, spike length, the number of grains per spike, and straw yield were measured. The harvest index was estimated by dividing grain yield by above-ground biomass.

Each sample was analyzed for hectoliter weight and 1000-grain weight using a SeedCount digital image system SC5000 (Next Instruments, Australia). The percentage of grain protein at 12.5 % humidity (%) was estimated by near-infrared spectroscopy (DA 7200 NIR, Perten Instruments, Sweden), whose calibration was based on official methods AACC 39-10.01 and 46-11.02 (AACC, 2021); whole meal yellowness (b^*) was measured using a colorimeter (Konica Minolta, Japan); sodium dodecyl sulfate (SDS) sedimentation volume test was performed according to Peña et al. (1990). Additionally, the SDS sedimentation index obtained by measuring the ratio between the SDS sedimentation volume and the sample protein content was calculated.

^{15}N analyzes

Soil, straw, and grain samples were collected at harvest, from the central furrow of the ^{15}N -microplots (an internal area of $0.8\text{ m} \times 1\text{ m}$)

Table 1
Edaphoclimatic characteristics of the study site.

Edaphoclimatic characteristics	Crop cycle 2018–2019	Crop cycle 2019–2020
Climatic conditions		
Relative humidity (%)	66.6	67.3
Average temperature ($^{\circ}\text{C}$)	18.1	19.0
Chill hours	460	375
Precipitation (mm)	26	46
Soil properties		
pH	7.9	8.1
Organic matter content (%)	0.8	0.5
Bulk density (g cm^{-3})	1.15	1.24
Texture	Sandy clay loam	Sandy clay loam
Soil nitrogen content (kg N ha^{-1})	123	104

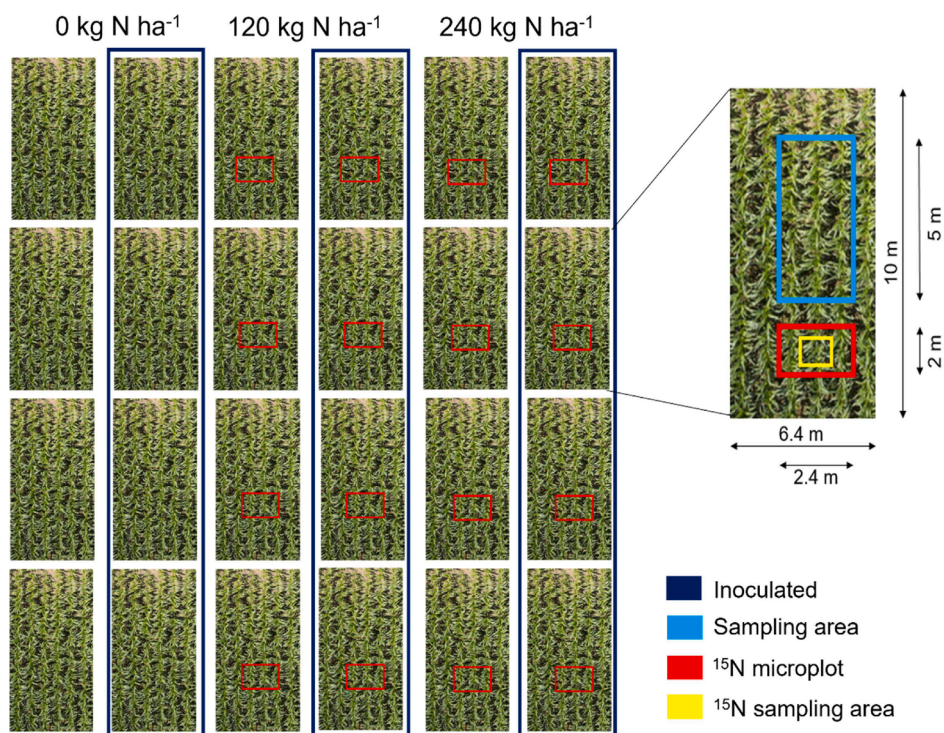


Fig. 1. Experimental design consisting of 6 treatments and 4 replicates with and without the inoculation of the BC on the wheat crop, under the three nitrogen doses (0, 120, 240 kg N ha⁻¹). An application of 1 % ¹⁵N-labeled urea was carried out in internal microplots.

(Zapata and Axmann, 1990). The soil was collected at 30 cm depth, dried at room temperature, and sifted with a No. 100 mesh sieve (a particle size of 0.149 mm). Straw and grain were milled and filtered with a strainer, to obtain smaller sample sizes (straw particle size: <2 mm, grain particle size: <0.3 mm).

All ¹⁵N-enriched samples were processed with a continuous flow Elemental Analyzer coupled with an Isotope Ratio Mass Spectrometer (EA-IRMS), at the Stable Isotope Facility (SIF) in the Department of Plant Sciences of the University of California. The equipment determined the percentage of ¹⁵N atoms on all samples (soil, straw, and grain; a ¹⁵N-enriched urea sample was a control). Then, the NUE in the samples was calculated according to Zapata & Axmann (1990). Nitrogen losses from the agroecosystem (% N losses) were calculated by subtracting from the total of N applied as urea (100 %) the sum of nitrogen use efficiency (% NUE_{plant}) of the whole plant plus the percentage of N applied as fertilizer that remained in the soil profile (0–30 cm) [% N losses = 100 % - % NUE_{plant} + % N_{remaining} in the soil].

Statistical analysis

Data components (quantitative traits for wheat yield: spikes m⁻², grains spike⁻¹, spike size, grain yield, straw yield, and harvest index; quality traits: hectolitre weight, 1000 grain weight, SDS sedimentation test, SDS/Protein index, and wholemeal yellowness; nitrogen use efficiency of ¹⁵N labeled urea in grain, straw and the whole plant, ¹⁵N losses, and ¹⁵N remnant in the soil) were analyzed in an analysis of variance (ANOVA) in Statgraphics Plus v 5.1. Statistical results were validated with a significance level of $p < 0.05$. Differences between the parameters' means were assessed using Duncan's Multiple Range Test at a 95 % confidence level.

Results and discussion

Genomic analysis of the studied bacterial consortium

Post-genome quality processing, assembly, and annotation of the

results may be observed in Table 2. Genome size ranged from 4125,766 to 5454,548 base pairs (bp), and %GC content ranged from 37.7 to 45.5 %. The number of coding DNA sequences (CDS) ranged from 4282 to 5882; these were classified into 334–340 subsystem features identified by RAST (Overbeek et al., 2014). The annotated genomes may be found under the GenBank accession numbers GCA_004124315, SAZD000000000, and RYZX000000000 for strain TE3^T, TRQ65, and TRQ8, respectively.

Furthermore, the genome annotation revealed genes related to PGPT (Table 3), including 4 CDS (in all three strains evaluated) related to auxin biosynthesis, specifically to the production of indole acetic acid. Auxins, particularly indole-3-acetic acid (IAA), play a pivotal role in the growth and development of wheat plants. Indole-3-acetic acid is a type of auxin that functions as a key plant hormone, influencing various aspects of wheat growth and development (Tan et al., 2021), including the promotion of root development and enhancement of the formation of lateral roots in wheat (Lv et al., 2021), reproductive growth, callus formation and regeneration (Kazan, 2013), the alleviation of biotic and abiotic stress (Egamberdieva, 2009), and improved efficiency in nutrient and water absorption. Indoles also stimulate the germination process,

Table 2
Genome characteristics.

Genome characteristic	<i>Priestia megaterium</i> TRQ8	<i>Bacillus paralicheniformis</i> TRQ65	<i>Bacillus cabrialesii</i> subsp. <i>cabrialesii</i> TE3 ^T
Size bp	5454,548	4475,481	4078,428
GC content (%)	37.7	45.5	44.1
N50	661,424	676,421	264,831
L50	3	3	6
Contigs	85	32	55
Number of coding sequences	5882	4809	4205
Number of RNAs	153	91	111
Number of subsystems	336	340	334

Table 3

Plant growth-promoting coding DNA sequences identified in the genome of the studied bacterial strains.

Characteristic	Plant growth promoting coding DNA sequences		
	<i>Priestia megaterium</i> TRQ8	<i>Bacillus paralicheniformis</i> TRQ65	<i>Bacillus cabrialesii</i> subsp. <i>cabrialesii</i> TE3 ^T
Auxin biosynthesis	4	4	4
Siderophore production	17	33	15
Phosphorus metabolism	20	11	14
Stress response	66	41	43

initiate, and increase xylem and root development, control vegetative development processes, and are involved in photosynthesis (Spaepen and Vanderleyden, 2011; Tsavkelova et al., 2006). As previously mentioned, Rojas-Padilla et al. (2020) demonstrated that these strains could produce indoles (from 8.21 to 39.29 µg/mL), confirming the function of these annotated genes. The putative proteins identified by RAST related to the production of Indole acetic acid are APRT, PRAI, IGS, TSa, TSb, TM, IAH, N3, TT, IPAC, IAD, AAD, AO, IAR, TO, BoundA, ATFS, N2, AUX1, and some enzymes were also identified, such as IAA acetyltransferase and Indole-3-glycerol phosphate synthase.

Also, Rojas-Padilla et al. (2020) reported that these strains can solubilize phosphorus (solubilization index from 1.37 to 1.43), where genomic annotation presented from 11 to 20 CDS relating to phosphorus metabolism in the genome of the studied bacterial strains (Table 3). Thus, these bacteria convert insoluble phosphorus in the soil into a soluble form, making it more accessible to wheat plants, consequently improving nutrient uptake and utilization critical for energy transfer, photosynthesis, and nutrient storage (El Mazlouzi et al., 2020). Genes related to the solubilization of phosphorus were found on all three strains (Supplementary Table 1), such as ackA, acyP, yccX, lpd, pdhd, maeA, sfcA, poxL, pta, actP, among others. However, strain-specific genes regarding phosphorus solubilization were also identified for each strain (Supplementary Table 1), such as for strain TE3^T: fdhA; for strain TRQ65: ybhC, pflD, dml, and kdpA-D; and for strain TRQ8: frc, nagK, dkgA, kgtP, Ira6, lldP, fumA, among others.

In addition, all three strains presented genes related to the production of siderophores ranging from 15 to 33 CDS (Table 3) (troA-D, bmr, fur, fmnA, feuD, narX, eutH, urtB among others) (Supplementary Table 1), these related to the production of anthrachelin and bacillibactin. Siderophores, as iron-chelating compounds, play a crucial role in enhancing wheat growth and production by facilitating iron uptake and utilization (Srivastava, 2023). Iron is an essential micronutrient for plants, including wheat, and is vital for various physiological processes, including photosynthesis, respiration, and DNA synthesis (Villarreal-Delgado et al., 2018). As we indicated above, *Priestia megaterium* TRQ8 has shown the ability to produce siderophores (production index of 8.17) (Rojas-Padilla et al., 2020). While TRQ65 and TE3^T do present putative genes related to this bioactivity, further research is necessary to quantify this ability.

Stress response mechanisms in PGPB are crucial adaptations that enable these microorganisms to thrive in challenging environments, encompassing both biotic and abiotic stress factors. The genome of the studied strains presents genes related to stress response (mainly osmotic and oxidative stress), ranging from 41 to 66 CDS. These characteristics are invaluable for bioprospection, as stress-tolerant PGPB have great potential for developing sustainable agricultural solutions that enhance plant growth, productivity, and resilience in challenging environmental conditions.

To complement these findings, the PLabAse v1.01 web source was used under the annotation of bacterial plant growth-promoting traits (proteins) (PGPTs) (Patz et al., 2021), where the results obtained

showed that for both strains TRQ8 and TRQ65, 12 % of their genes and 11 % for strain TE3^T are related to biofertilization traits (Table 4).

Among the mentioned PGPT predicted through Rast 2.0, PLabAse also elucidates nitrogen acquisition mechanisms ranging from 13 to 14 % of the total genes, from 64 to 102 CDS identified (Table 4). This includes atmospheric N fixation (8 % for all three strains) including genes nifS and nifU; which are involved in transforming atmospheric N to ammonia (a usable form of nitrogen for plants), contributing to wheat growth by providing a direct source of nitrogen for enhancing plant vigor, tillering, and overall biomass (Gul et al., 2023). Also, the number of genes identified in the studied strains involved in urea usage ranged from 7 to 16 %, for denitrification ranged from 13 to 25 %; these processes play an important role in the nitrogen cycle and soil fertility (Mahapatra et al., 2022).

Thus, more than 50 % of the biofertilization-related genes are shared among *Bacillus cabrialesii* subsp. *cabrialesii* TE3^T, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65, which are related to nitrogen acquisition, phosphorus assimilation, carbon dioxide fixation, iron acquisition, phosphate, and potassium solubilization (Fig. 2; and Supplementary Table 1). The analyzed strains also presented strain-specific genes associated with PGP traits. The results showed that TE3^T presented genes related to iron uptake (troA, troB, troC among others), bacillibactin transport (bmr), siderophore export (macA), urea acquisition (urtA-D) and phosphorus solubilization (fdhA); TRQ65 presented genes related to rhizobactin metabolism (rhbE) among others and TRQ8 presented the most strain-specific genes related to iron uptake, P solubilization S metabolism, and others (Supplementary Table 1).

The genomic bioinformatic analysis involves the systematic analysis of bacterial genomes to identify and apply their potential for various purposes, including enhancing plant growth and agricultural sustainability. Thus, the application of these as a consortium may optimize their PGP traits together. In the case of wheat, the studied PGPB have a great genomic diversity that can positively influence wheat growth by enhancing nutrient availability, disease resistance, and stress tolerance. Thus, to quantify the genomic background of the studied strains (in a consortium, BC) as wheat growth promoters, commercial field assays were carried out under two amounts of initial N in the soil and three N fertilizers doses, as follows.

Yield parameters

In 2018–2019, the effect of the inoculation of the BC at 0 kg N ha⁻¹ in the number of spikes per square meter resulted in an increment of 28 % compared to its uninoculated control (337 inoculated vs. 264 uninoculated) (Table 5), as well as an increase of 26 % compared to the treatment of the complete N fertilization (268). This parameter showed no

Table 4

Priestia megaterium TRQ8, *Bacillus paralicheniformis* TRQ65, and *Bacillus cabrialesii* subsp. *cabrialesii* TE3^T plant growth-promoting traits annotated using PGPT-pred.

Function	Coding DNA sequences		
	<i>Bacillus cabrialesii</i> subsp. <i>cabrialesii</i> TE3 ^T	<i>Bacillus paralicheniformis</i> TRQ65	<i>Priestia megaterium</i> TRQ8
Carbon Dioxide fixation	13	10	15
Iron acquisition	90	99	107
Nitrogen acquisition	64	74	102
Phosphate solubilization	166	179	245
Potassium solubilization	143	161	207
Sulfur assimilation and mineralization	27	24	45

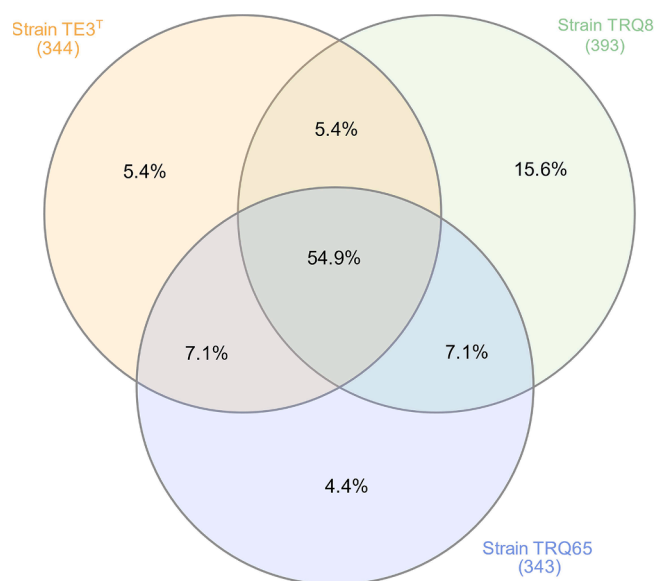


Fig. 2. Biofertilization-related genes (nitrogen acquisition, phosphorus assimilation, carbon dioxide fixation, iron acquisition, phosphate, and potassium solubilization) among *Bacillus cabrialesii* subsp. *cabrialesii* TE3^T, *Priestia megaterium* TRQ8, and *Bacillus paralicheniformis* TRQ65. This comparison was generated through InteractiVenn (Heberle et al., 2015), where a total of 1080 genes were input and analyzed.

significant difference between both treatments at 120 kg N ha⁻¹ (inoculated, 317 vs. uninoculated, 315). A similar impact occurred during the succeeding cycle (2019–2020), where the treatment of 0 kg N ha⁻¹ + BC had an increment of 37 % concerning its uninoculated control (207 vs. 151), and resulted in no significant difference among the rest of the treatments comparing the control vs. the inoculated treatment. The treatments that received the complete fertilizer dose (240 kg N ha⁻¹) showed no significant difference among them in the number of spikes per square meter (268, uninoculated vs. 288, inoculated).

It has been reported that N contributes to plant and spike generation by increasing vegetative growth (Sokurt and Çiğ, 2019); moreover, the number of spikes m⁻² is the result of earlier dynamics from the start of stem elongation to anthesis (Slafer et al., 2022), which are the stages of maximum nitrogen absorption. That would indicate that the treatments with higher values of spikes m⁻² have a balance in the optimal conditions, such as radiation, temperature, photoperiod, water, and nutrients (especially N) needed for the spikes to grow, which could have been facilitated by the studied BC considering that the beneficial effects were observed under treatments without N fertilization.

In the case of the number of grains per spike (Table 5), a tendency of

higher values with higher doses of N was found only in 2019–2020, but the inoculation did not have a significant effect compared to their controls, as well as in the parameter of spike size at both cycles.

On the other hand, the impact of the inoculation of the BC on grain yield in 2018–2019 (an initial N content of 123 kg N ha⁻¹ in the soil) was observed when no fertilization was added to the wheat crop (0 kg N ha⁻¹ + BC), obtaining an increment of 1 ton ha⁻¹ more than the uninoculated treatment (Table 5). The following year (an initial N content of 104 kg N ha⁻¹ in the soil), significant differences ($p < 0.05$) were obtained when 120 and 240 kg N ha⁻¹ were applied to the crop, resulting in 2.0 and 0.5 ton ha⁻¹ more grain yields, respectively, compared to the uninoculated controls at the same doses of N fertilization.

It has been reported in the literature that bacteria promoting plant growth do not represent benefits to the plant if large doses of chemical fertilizers are applied to the crops (Glick, 2012). That phenomenon could be attributed to nutrient imbalances in the soil (da Silva Freitas et al., 2023), modulating the positive effects of the studied BC.

Thereby, in this study, the positive effect of the inoculation is the result of the plant growth promotion mechanisms of the bacterial consortium. As previously mentioned, *B. cabrialesii* subsp. *cabrialesii* TE3^T, *P. megaterium* TRQ8, and *B. paralicheniformis* TRQ65 solubilize P (Robles-Montoya et al., 2019; Rojas Padilla et al., 2020; Valenzuela-Aragon et al., 2018; Valenzuela-Ruiz et al., 2019), where in their genomes 33–34 % of the total genes related to biofertilization traits were related to P solubilization (Table 4). This nutrient is indispensable for vegetative development, as it is a constituent of DNA and RNA nucleic acids, essential for protein reproduction and synthesis; as well as for the development at every phenological stage. Also, P stimulates germination, seed formation, root and stem development, yield, and quality, among other important processes (Malhotra et al., 2018). Alongside IAA production, N fixation, K and S assimilation, and iron acquisition mechanisms are involved in plant growth promotion.

IAA and P assimilation-related genes found in these strains have been associated with the plant's photosynthetic ability, where PGPB can stimulate photosynthesis in wheat plants by improving chlorophyll content, increasing the efficiency of photosystem II, and enhancing stomatal conductance (Spaepen and Vanderleyden, 2011; El Mazlouzi et al., 2020). This leads to higher rates of photosynthesis, resulting in increased production of glucose and fructose, the primary products of photosynthesis.

The above-mentioned can also be explained by the impact observed in the harvest index, which resulted in significant increments in 2018–2019 for the three different doses of N applied when the BC was inoculated, and in the medium dose in the following year (Table 5), compared to the uninoculated treatments. Harvest index is the ratio of grain to total shoot dry matter or a measure of the efficiency of the plant to produce grains, it depends on a combination of factors such as the genotype, the environment, and crop management (Porker et al., 2020);

Table 5

Quantitative traits for wheat yield under the studied experimental design.

Crop cycle	Treatment	Number of spikes m ⁻²	Number of grains spike ⁻¹	Spike size (cm)	Grain yield (ton ha ⁻¹)	Straw yield (ton ha ⁻¹)	Harvest index
2018–2019	0 kg N ha ⁻¹	264 ± 33 a	55 ± 12 a	7.1 ± 0.5 c	6.0 ± 0.6 a	6.3 ± 0.4 bc	0.49 ± 0.01 a
	0 kg N ha ⁻¹ + BC	337 ± 38 c	50 ± 7 a	6.7 ± 0.7 ab	7.1 ± 0.6 b	6.3 ± 0.4 bc	0.53 ± 0.01 cd
	120 kg N ha ⁻¹	315 ± 13 bc	53 ± 8 a	6.7 ± 0.5 ab	6.5 ± 0.5 ab	6.4 ± 0.4 c	0.50 ± 0.01 ab
	120 kg N ha ⁻¹ + BC	317 ± 30 bc	52 ± 6 a	6.6 ± 0.4 a	6.7 ± 0.4 ab	5.8 ± 0.4 ab	0.54 ± 0.02 d
	240 kg N ha ⁻¹	268 ± 38 a	53 ± 5 a	6.7 ± 0.5 ab	6.6 ± 0.7 ab	6.3 ± 0.5 bc	0.51 ± 0.01 bc
	240 kg N ha ⁻¹ + BC	288 ± 46 ab	51 ± 9 a	6.8 ± 0.3 bc	6.5 ± 0.1 ab	5.5 ± 0.2 a	0.54 ± 0.01 d
2019–2020	0 kg N ha ⁻¹	151 ± 16 a	45 ± 8 a	6.4 ± 0.8 a	3.5 ± 1.0 a	2.9 ± 1.1 a	0.57 ± 0.01 a
	0 kg N ha ⁻¹ + BC	207 ± 47 b	53 ± 10 ab	6.8 ± 0.7 bc	5.6 ± 0.8 b	5.3 ± 0.6 c	0.55 ± 0.03 a
	120 kg N ha ⁻¹	216 ± 44 b	51 ± 8 a	6.6 ± 0.7 ab	5.9 ± 1.3 b	4.0 ± 0.9 ab	0.60 ± 0.01 b
	120 kg N ha ⁻¹ + BC	215 ± 35 b	53 ± 10 ab	6.9 ± 0.6 bc	7.9 ± 0.7 c	4.7 ± 0.4 bc	0.63 ± 0.01 c
	240 kg N ha ⁻¹	230 ± 31 b	60 ± 9 b	7.1 ± 0.7 c	7.5 ± 0.5 c	4.9 ± 0.5 bc	0.61 ± 0.01 b
	240 kg N ha ⁻¹ + BC	234 ± 35 b	60 ± 8 b	7.1 ± 0.6 c	8.0 ± 1.3 c	5.2 ± 0.7 c	0.61 ± 0.01 bc

Values indicate mean ± standard deviation. Different letters in the same column indicate a significant difference, using the Duncan test ($p < 0.05$). BC corresponds to the treatments inoculated with the studied bacterial consortium. $n = 15$ per replicate, and four replicates per treatment.

thus, during the last decades, increasing the harvest index has been a tool for increasing crop productivity (Camargo-Alvarez et al., 2023).

Other studies have reported increases in yields due to the inoculation of microorganisms at similar N doses as the initial N contents (123 and 104 kg per hectare) reported in this study. Diosnel et al. (2019) and Galindo et al. (2019) reported an increase in wheat yields inoculating *Azospirillum brasilense* along with 130 and 140 kg N ha⁻¹, respectively. Kumar et al. (2017) applying 120 kg N ha⁻¹ obtained higher wheat grain yield (6–22 % more, according to the inoculated microorganism), and higher straw yield (6–28 % more) due to the inoculation of bacteria (single strain or in consortium) in comparison with the control treatment.

Besides, Ibarra-Villarreal et al. (2023) also obtained wheat grain yield increases of about ~1 ton ha⁻¹ (on average, under nitrogen fertilization rates of 0, 130, and 250 kg N ha⁻¹) compared to the uninoculated controls, for two consecutive agricultural cycles, when they inoculated a consortium composed of *B. subtilis* TSO9, *B. cabrialesii* subsp. *tritici* TSO2^T, *B. subtilis* TSO22, *B. paralicheniformis* TRQ65, and *Priestia megaterium* TRQ8, with an initial N content of 32 kg N ha⁻¹ in the soil. The inoculated treatments fertilized with half of the recommended dose of N, resulted in 1 ton ha⁻¹ more, compared to treatments that received the total recommended nitrogen dose of 250 kg N ha⁻¹. Consequently, the authors conclude that the application of the studied bacterial consortium, in combination with a diminished amount of N fertilizer represents a sustainable alternative to increase wheat yield.

Grain quality

In 2018–2019, the treatment with the highest yield (0 kg N ha⁻¹ + BC, 7.1 ton ha⁻¹) showed the lowest grain protein content (13.4 %); and the lowest value in the SDS sedimentation test (14.0 mL) (Table 6). However, all the values of protein obtained in the different treatments for that crop cycle surpassed the minimal protein content required for this variety (between 12.8 and 15.6 %) (Félix-Fuentes et al., 2010; Figueroa-López et al., 2010).

Neither the increase in the dose of fertilizer nor the application of BC had a significant effect on 1000-grain weight and hectolitre weight (Table 6), except in the treatments with 0 kg N ha⁻¹, where the BC increased both traits in the 2018–2019 crop cycle (38.1 g vs. 32.4 g). That year, the treatments of 0 kg N ha⁻¹ + BC, 120 kg N ha⁻¹, and 120 kg N ha⁻¹ + BC had the highest values and did not show a significant

difference between them (38.1 g, 36.2 g, and 35.8 g, respectively).

Nonetheless, all values of hectolitre weight in this study (74.4–76.8 kg/hL, in 2018–2019) turned out slightly lower than the values reported in the literature for this wheat variety, which are between 78 and 84 kg/hL (Félix-Fuentes et al., 2010; Guzman et al., 2016a). Besides, hectolitre weight and 1000-grain weight for the first cycle are negatively associated with the protein content and the SDS-sedimentation volume.

In 2019–2020, hectolitre weight and 1000-grain weight resulted in higher values than the previous cycle (81.7–82.2 kg/hL, and 53.8–59.7 g, respectively). On the contrary, in the 2019–2020 cycle, grain protein content, as well as wholemeal flour yellowness, SDS-sedimentation test, and the SDS/protein index remained lower than in the previous crop cycle. These results suggest that the composition of the grain could have been less protein content compared to other components (e.g. starch, lipids, or water). The SDS-sedimentation volume determines the hydration and expansion of gluten proteins in a slightly acidic medium, giving a general idea of the quality of gluten and its extensibility, thus making a suitable flour to make pasta (Guzman et al., 2016b).

Further, elevated protein content and a high value of wholemeal yellowness are required for high-quality durum wheat crops (Zarco-Hernandez et al., 2005). In the wholemeal yellowness analysis, no significant differences between the studied treatments in 2018–2019 (16.28–16.50, Table 6) were observed. The increased b values (wholemeal yellowness) recorded in the first cycle are likely determined by the reduced grain size of the samples and, therefore, a greater concentration of the pigments. In both cycles, however, neither the N treatment nor the addition of the BC is associated with this trait which is expected (mostly genetically determined). Tabbita et al. (2023) observed that this trait was the most stable (among yield and quality traits) across management conditions, as observed in this study; thereby, all obtained values met the requirements for this trait on CIRNO C2008 (Guzman et al., 2016a). Similarly, all percentages of protein content obtained (13.4–15.3 %, Table 6) exceeded the minimal requirement for this variety that year (between 12.8 and 15.6 %) (Félix-Fuentes et al., 2010; Figueroa-López et al., 2010).

Quality traits of protein content and SDS sedimentation test value obtained in 2018–2019 indicate that plants that did not receive nitrogen fertilization and were inoculated with the BC, improved residual soil N absorption, and produced the highest yield with desired quality traits. Some studies with the inoculation of PGPR have reported increases in protein content. Rana et al. (2012) reported an increment of 18.6 % of

Table 6
Wheat quality traits under the studied experimental design.

Crop cycle	Treatment	Hectolitre weight (kg hL ⁻¹)	1000-grain weight (g)	Protein (%)	SDS sedimentation test (mL)	SDS/Protein index	Wholemeal yellowness (Minolta, b)
2018–2019	0 kg N ha ⁻¹	74.4 ± 1.3 a	32.4 ± 1.2 a	15.1 ± 1.0 b	15.4 ± 0.5 b	1.02 ± 0.06 a	16.5 ± 0.2 a
	0 kg N ha ⁻¹ + BC	76.8 ± 0.6 c	38.1 ± 1.0 c	13.4 ± 0.7 a	14.0 ± 0.6 a	1.05 ± 0.1 a	16.4 ± 0.1 a
	120 kg N ha ⁻¹	76.4 ± 1.3 bc	36.2 ± 3.0 bc	14.5 ± 0.5 b	15.3 ± 1.0 b	1.06 ± 0.04 ab	16.3 ± 0.4 a
	120 kg N ha ⁻¹ + BC	76.6 ± 0.3 bc	35.8 ± 0.5 bc	14.6 ± 0.2 b	16.4 ± 0.5 c	1.12 ± 0.04 b	16.3 ± 0.3 a
	240 kg N ha ⁻¹	75.4 ± 0.7 ab	34.1 ± 1.8 ab	15.3 ± 0.4 b	16.0 ± 0.0 bc	1.05 ± 0.03 a	16.5 ± 0.1 a
	240 kg N ha ⁻¹ + BC	75.4 ± 0.9 ab	34.3 ± 1.9 ab	15.1 ± 1.0 b	15.5 ± 0.4 b	1.04 ± 0.06 a	16.4 ± 0.2 a
2019–2020	0 kg N ha ⁻¹	81.7 ± 0.4 a	58.9 ± 1.1 c	10.1 ± 0.3 a	9.0 ± 0.7 a	0.90 ± 0.08 a	14.1 ± 0.2 a
	0 kg N ha ⁻¹ + BC	81.9 ± 0.7 a	58.2 ± 1.1 bc	10.8 ± 0.5 bc	9.5 ± 0.4 ab	0.88 ± 0.02 a	14.4 ± 0.2 b
	120 kg N ha ⁻¹	82.2 ± 0.2 a	59.7 ± 2.2 c	10.7 ± 0.2 b	10.3 ± 0.7 bc	0.97 ± 0.08 ab	14.1 ± 0.1 a
	120 kg N ha ⁻¹ + BC	81.8 ± 0.4 a	54.9 ± 2.7 a	11.3 ± 0.4 cd	10.9 ± 0.6 cd	0.97 ± 0.03 ab	14.6 ± 0.1 b
	240 kg N ha ⁻¹	82.1 ± 0.5 a	53.8 ± 0.9 a	11.7 ± 0.3 d	11.6 ± 0.5 d	0.99 ± 0.05 b	14.7 ± 0.2 b
	240 kg N ha ⁻¹ + BC	82.0 ± 0.5 a	55.8 ± 1.3 ab	11.5 ± 0.3 d	11.1 ± 0.5 cd	0.97 ± 0.02 ab	14.5 ± 0.3 b

Values indicate mean ± standard deviation. Different letters in the same column indicate a significant difference, using the Duncan test ($p < 0.05$). BC corresponds to the treatments inoculated with the studied bacterial consortium. $n = 20$ per replicate, and four replicates per treatment.

protein, as well as an increase of 11.4 % in grain yield concerning the inoculated control (NPK 60:60:60), due to the inoculation of *Providencia* sp. PW5 at the same NPK doses.

In summary, the inoculation of the CB does not cause significant differences in the analyzed quality traits (the only exception is for the first cycle, at 0 kg N ha⁻¹ addition, where the inoculation causes an increase in both hectolitre weight and 1000-grain weight values), as quality traits tend to be more stable than yield parameters, and are more dependant on the wheat genotype; while yield shows fluctuations in different agricultural cycles, which depend to a large extent on the availability of nutrients and water (Figuerola-López et al., 2010). Thus, the improvement in yields due to the inoculation of the BC did not compromise the quality traits of the wheat production.

¹⁵N-Nitrogen use efficiency

In 2018–2019, NUE (Table 7) increased from 25.3 % (240 kg N ha⁻¹ applied) up to 39.3 %, when 120 kg N ha⁻¹ was applied, where 30.8 % of the applied N was detected in the grains; on the other hand, in 2019–2020, NUE increased from 34.4 % (240 kg N ha⁻¹ applied) up to 46.9 %, when half of the fertilization was added to wheat.

NUE for the wheat crop under the complete N dose conventionally applied in the Yaqui Valley (240 kg N ha⁻¹) in 2018–2019 (25.3 %) and 2019–2020 (34.4 %) turned out similar to what was reported by Ortiz-Monasterio and Raun (2007), which was 31 % for the same crop and region. While two decades ago, globally, the NUE of cereal crops was reported at 33 %; and in 2015 it was 35 % for the world, 41 % in the United States, 30 % in China, and 21 % for the cereals of India (Omara et al., 2019).

Furthermore, in 2018–2019, with the complete N fertilization dose, only 12.6 % remained in the superficial part of the soil (30 cm depth) after the harvest, which suggests that 62.1 % (Table 7) of the applied N is

Table 7
Nitrogen (¹⁵N) use efficiency in wheat under the inoculation of a native bacterial consortium.

Crop cycle	Treatment	Nitrogen use efficiency of ¹⁵ N labeled urea (%)			¹⁵ N remnant in the first 30 cm of soil (%)	Losses of ¹⁵ N (%)
		Grain	Straw	Whole plant		
2018–2019	120 kg N ha ⁻¹	30.8 ± 16.8 a	8.5 ± 3.7 a	39.3 ± 20.5 a	6.3 ± 2.1 ab	54.4 ± 18.4 a
	120 kg N ha ⁻¹ + BC	29.1 ± 7.7 a	7.2 ± 2.5 a	36.3 ± 10.2 a	2.5 ± 1.2 a	61.2 ± 11.3 a
	240 kg N ha ⁻¹	19.2 ± 1.1 a	6.1 ± 1.1 a	25.3 ± 2.2 a	12.6 ± 3.1 b	62.1 ± 0.8 a
	240 kg N ha ⁻¹ + BC	20.9 ± 10.8 a	5.4 ± 0.2 a	26.3 ± 11.0 a	12.6 ± 5.1 b	61.1 ± 5.9 a
2019–2020	120 kg N ha ⁻¹	44.2 ± 1.3 b	2.7 ± 0.5 a	46.9 ± 0.8 b	2.9 ± 1.5 a	50.2 ± 2.3 a
	120 kg N ha ⁻¹ + BC	39.1 ± 2.6 ab	3.7 ± 2.3 a	42.8 ± 4.9 ab	2.2 ± 2.1 a	55.0 ± 2.8 a
	240 kg N ha ⁻¹	30.4 ± 12.2 ab	4.0 ± 1.5 a	34.4 ± 13.7 ab	2.5 ± 2.3 a	63.2 ± 11.4 a
	240 kg N ha ⁻¹ + BC	17.8 ± 10.6 a	3.2 ± 0.8 a	21.0 ± 9.8 a	19.7 ± 2.9 b	59.3 ± 12.6 a

1 atom % ¹⁵N excess labeled urea was used in this experiment. BC corresponds to the inoculation of the bacterial consortium. Values indicate mean ± standard deviation. Different letters in the same column indicate a significant difference using the Duncan test ($p < 0.05$).

lost in the environment through leaching or volatilization, which causes adverse effects on the environment and represents economic losses to the farmers of the Yaqui Valley. The losses of N for that treatment in the following crop season were 63.2 %.

In both cycles, it was clear that the increase in NUE was attributable more to the reduction of the fertilization by half, than to the inoculation of the BC, even when there were notable and significant impacts on crop grain yield. Those findings showed that metabolic processes involved in N accumulation cannot be completely associated with wheat growth promotion mechanisms used by these PGPB strains. The above mentioned, suggests that these strains would impact and metabolize the N that is already uptaken. Also, the genomic annotation presented here highlights that 13–14 % of the reported genes related to biofertilization growth-promoting traits are involved in N acquisition, including N fixation and N transformation to ammonia which may be a direct source of N for the wheat plant; although, there is not experimental evidence of nitrogen fixation by these strains.

In this sense, it is important to coordinate wheat stages of high nitrogen demand with the applications of N (amount, ratio, and type of N fertilizer), as well as sustainable alternatives (e. g. bacterial inoculants) according to the requirements of N during the growth period. Also, adequate fractionation of N fertilization is necessary to improve N assimilation rates and grain quality (González-Figueroa et al., 2022). For example, at post-anthesis carbohydrates produced by photosynthesis contributes 60–90 % to the final single-spike yield; on the other hand, flag-leaf photosynthesis contributes 30–50 % of grain-filling assimilation in wheat (DING et al., 2022). On the other hand, N uptake efficiency is mostly determined by N accumulation at pre-anthesis, because strong root physiological activities promote N acquisition from the soil during the vegetative period, and these activities limit N uptake during the reproductive period (DING et al., 2022).

Additionally, it has been reported that higher doses of N cause higher loss and less use of N by the wheat crop, since it exists a limit in the nutritional demand of the plant, where it can absorb a certain amount of nutrients for a certain time, and the N that is not absorbed, get lost in the environment; which means that fertilization use efficiency diminishes at higher doses of applied N (Galindo et al., 2016, 2019). Besides, the beneficial effect of the inoculation of PGPB is lost with higher doses of N (Glick, 2012). For these reasons, the positive effect of the BC on NUE at the studied doses concerning their uninoculated controls was not observed, possibly due to the elevated residual soil N content (123 and 104 kg N ha⁻¹).

Grageda Cabrera et al. (2018), in their work of inoculation of mycorrhizae on wheat, obtained increases in grain yield of up to 1.29 ton ha⁻¹, increased the amount of N derived from the fertilizer to up to 15 kg, and enhanced nitrogen fertilizer utilization up to 11 %, compared to the uninoculated plants, using 120 kg of ammonium sulfate. On the other hand, Serret et al. (2008) found that NUE decreased by ~ half when nitrogen fertilizer increased from 50 to 100 kg ha⁻¹, and continued diminishing less abruptly with higher N doses because N utilization and absorption decreased when N doses increased from 50 to ≥100 kg ha⁻¹.

The remnant N in the first 30 cm of the soil with 100 % N (240 kg N ha⁻¹) was the same in both inoculated and uninoculated treatments (12.6 %, Table 7), which decreased until 6.3 % when the dose of N applied was reduced by half, and decreased until 2.5 % when that N dosage was combined with the inoculation of the BC (in 2018–2019). On the other hand, slightly fewer N losses were observed at the treatments of 120 kg N ha⁻¹ than at 240 kg N ha⁻¹ applied in both cycles (Table 7).

Thereby, based on yield results (Table 5), the higher value in 2018–2019 was obtained in the treatment where the BC was inoculated without the input of N fertilization (7.1 ± 0.6 ton ha⁻¹), and in 2019–2020 the positive effect was observed under 0 and 120 kg N ha⁻¹, it can be inferred that NUE could be even higher at lower doses of N applied on this study (120 kg N ha⁻¹). Hence, studying intermediate doses between 0 and 120 kg N is recommended to fully explain the BC

effect.

Conclusion

The inoculation of the studied native bacterial consortium (*B. cabrialesii* subsp. *cabrialesii* TE3^T, *P. megaterium* TRQ8, and *B. paralicheniformis* TRQ65) to the wheat crop, in the presence of a total N concentration (soil N content + fertilization) of 123–225 kg N ha⁻¹ increases crop yield and maintains an adequate grain quality for this cereal under the edaphoclimatic conditions of the Yaqui Valley. A positive effect of the inoculum on NUE at rates of 120 and 240 kg N ha⁻¹ under soils with 123 kg of N ha⁻¹ and 104 kg of N ha⁻¹, was not observed, but increases of 14 % and 12.5 % of NUE of the total plant were observed when the fertilization was diminished by half (120 kg N ha⁻¹).

In general, positive effects on wheat yield, quality, and nitrogen use efficiency were more dependent on nitrogen fertilization rates, rather than the inoculation of the BC: more NUE, with less N fertilization; better grain quality traits with more N fertilization; and the yield parameters were less homogeneous, with significant positive effects on wheat grain yield between 123 and 225 kg N ha⁻¹ in the soil.

Before their massive application in the field, it is important to keep working on the study and selection of potential strains with a genomic background that has a greater number of biofertilization-related genes to improve nitrogen acquisition, phosphorus assimilation, carbon dioxide fixation, iron acquisition, phosphate and potassium solubilization, as well as genes related to the production of siderophores (anthrachelin and bacillibactin), and stress response (mainly osmotic and oxidative stress), prioritizing native microorganisms, and their interaction with the plant, to design efficient strategies concerning an appropriate agricultural management.

The results obtained in this study highlight the importance of carrying out a physicochemical analysis of the soil to acknowledge the initial nutritional status of the soil (with a special focus on N content), to establish the nitrogen fertilization recommendation that the crop requires at which the inoculation of the BC would keep having the desired positive effect and increasing productivity.

CRedit authorship contribution statement

Marisol Ayala Zepeda: Formal analysis, Writing – original draft, Data curation, Methodology, Conceptualization, Investigation. **Valeria Valenzuela Ruiz:** Conceptualization, Investigation, Formal analysis, Writing – review & editing. **Fannie Isela Parra Cota:** Conceptualization, Supervision, Writing – review & editing, Project administration. **Cristina Chinchilla-Soto:** Conceptualization, Methodology, Writing – review & editing. **Eulogio de la Cruz Torres:** Conceptualization, Methodology, Writing – review & editing. **María Itria Ibba:** Formal analysis, Methodology, Writing – review & editing. **María Isabel Estrada Alvarado:** Conceptualization, Methodology, Writing – review & editing. **Sergio de los Santos Villalobos:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing, Data curation, Funding acquisition, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Authors greatly acknowledge the FAO/IAEA-RLA5077 project “Enhancing Livelihood through Improving Water Use Efficiency Associated with Adaptation Strategies and Climate Change Mitigation in Agriculture (ARCAL CLVIII)” for providing funding, equipment, and material. As well as the CONAcYT 253663 project “Fortalecimiento de la infraestructura del Laboratorio de Biotecnología del Recurso Microbiano del ITSON para la creación de COLMENA: Colección de Microorganismos Edáficos y Endófitos NATivos, para contribuir a la seguridad alimentaria regional y nacional” for its financial support.

The authors would like to thank Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHACYT) for master and doctorate Scholarship No. 605648 (Marisol Ayala Zepeda) and No. 712969 (Valeria Valenzuela Ruiz).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.crmicr.2024.100230](https://doi.org/10.1016/j.crmicr.2024.100230).

References

- American Association of Cereal Chemists (AACC), 2021. AACC Approved Methods of Analysis - 11th Edition. <http://methods.aacnet.org/toc.aspx>.
- Andrews, S., 2010. FastQC: a Quality Control Tool For High Throughput Sequence Data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.L., Pham, S., Pribelski, A.D., Pyshkin, A.V., Sirtkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19 (5), 455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Beman, J.M., Arrigo, K.R., Matson, P.A., 2005. Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean. *Nature* 434 (7030), 211–214. <https://doi.org/10.1038/nature03370>.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Camargo-Alvarez, H., Elliott, R.J.R., Olin, S., Wang, X., Wang, C., Ray, D.K., Pugh, T.A. M., 2023. Modeling crop yield and harvest index: the role of carbon assimilation and allocation parameters. *Model. Earth. Syst. Environ.* 9, 2617–2635. <https://doi.org/10.1007/s40808-022-01625-x>.
- Chaparro-Encinas, L.A., Parra-Cota, F.I., Cruz-Mendivil, A., Santoyo, G., Peña-Cabriales, J.J., Castro-Espinoza, L., de los Santos-Villalobos, S., 2022. Transcriptional regulation of cell growth and reprogramming of systemic response in wheat (*Triticum turgidum* subsp. *durum*) seedlings by *Bacillus paralicheniformis* TRQ65. *Planta* 255 (3), 56.
- Cordova-Albores, L.C., Zelaya-Molina, L.X., Ávila-Alistac, N., Valenzuela-Ruiz, V., Cortés-Martínez, N.E., Parra-Cota, F.I., Burgos-Canul, Y.Y., Chávez-Díaz, I.F., Fajardo-Franco, M.L., de los Santos-Villalobos, S., 2021. Omics sciences potential on bioprospecting of biological control microbial agents: the case of the Mexican agrobiotechnology. *Rev. Mex. Fitopatol.* 39 (1), 147–184. <https://doi.org/10.18781/r.mex.fit.2009-3>.
- Da Silva Freitas, G., Moreira, A., Prudencio, M.F., Heinrichs, R., Nogueira, M.A., Hungria, M., Soares Filho, C.V., 2023. Foliar spray inoculation with plant growth promoting bacteria associated with nitrogen doses in *Megathyrus maximus* cv. BRS Zuri. *Agronomy* 13 (4), 1040.
- Darling, A.C., Mau, B., Blattner, F.R., Perna, N.T., 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res.* 14 (7), 1394–1403. <https://doi.org/10.1101/gr.2289704>.
- Davis, J.J., Wattam, A.R., Aziz, R.K., Brettin, T., Butler, R., Butler, R.M., Chlenski, P., Conrad, N., Dickerman, A., Dietrich, E.M., Gabbard, J.L., Gerdes, S., Guard, A., Kenyon, R.W., Machi, D., Mao, C., Murphy-Olson, D., Nguyen, M., Nordberg, E.K., Olsen, G.J., Olson, R.D., Overbeek, J.C., Overbeek, R., Parrello, B., Pusch, G.D., Shukla, M., Thomas, C., VanOeffelen, M., Vonstein, V., Warren, A.S., Xia, F., Xie, D., Yoo, H., Stevens, R., 2020. The PATRIC bioinformatics resource center: expanding data and analysis capabilities. *Nucleic Acids Res.* 48 (D1), D606–D612. <https://doi.org/10.1093/nar/gkz943>.
- de los Santos-Villalobos, S., Díaz-Rodríguez, A.M., Ávila-Mascareño, M.F., Martínez-Vidales, A.D., Parra-Cota, F.I., 2021. COLMENA: a culture collection of native microorganisms for harnessing the agro-biotechnological potential in soils and contributing to food security. *Diversity (Basel)* 13 (8), 337.
- de los Santos-Villalobos, S., Robles, R.I., Parra-Cota, F.I., Larsen, J., Lozano, P., Tiedje, J. M., 2019. *Bacillus cabrialesii* sp. nov., an endophytic plant growth-promoting bacterium isolated from wheat (*Triticum turgidum* subsp. *durum*) in the Yaqui Valley, Mexico. *Int. J. Syst. Evol. Microbiol.* 69 (12), 3939–3945. <https://doi.org/10.1099/ijsem.0.003711>.
- Ding, Y., Zhang, X., Ma, Q., Li, F., Tao, R., Zhu, M., Li, C., Zhu, X., Guo, W., Ding, J., 2022. Tiller fertility is critical for improving grain yield, photosynthesis, and

- nitrogen efficiency in wheat. *J. Integr. Agric.* <https://doi.org/10.1016/j.jia.2022.10.005>. *Fao* 2019.
- Diosnel, A.M., Milciades, A.M.A., Walter, V., Miguel, B., Ever, M.C., Marcos, J.M., Rafael, F., Modesto, da S.H., Wilfrido, D.L., 2019. Effect of different doses of *Azospirillum brasilense* and nitrogen fertilizer in wheat crop. *Afr. J. Agric. Res.* 14 (7), 461–466. <https://doi.org/10.5897/ajar2018.13621>.
- Egamberdieva, D., 2009. Alleviation of salt stress by plant growth regulators and IAA-producing bacteria in wheat. *Acta Physiol. Plant.* 31, 861–864. <https://doi.org/10.1007/s11738-009-0297-0>.
- El Mazlouzi, M., Morel, C., Chesseron, C., Robert, T., Mollier, A., 2020. Contribution of external and internal phosphorus sources to grain P loading in durum wheat (*Triticum durum* L.) grown under contrasting P levels. *Front. Plant Sci.* 11, 870. <https://doi.org/10.3389/fpls.2020.00870>.
- Félix-Fuentes, J.L., Figueroa-López, P., Fuentes-Dávila, G., Valenzuela-Herrera, V., Chavez-Villalba, G., Mendoza-Lugo, J.A., 2010. Cirno C2008, Folleto 71. Variedad de trigo cristalino para el noroeste de México.
- Figueroa López, P., Félix Fuentes, J., Fuentes Dávila, G., Valenzuela Herrera, V., Chávez Villalba, G., Mendoza Lugo, J., 2010. Cirno C2008, nueva variedad de trigo cristalino con alto rendimiento potencial para el estado de Sonora. *Rev. Mexicana Cienc. Agric.* 1 (5), 745–749.
- Fischer, T., Byerlee, D., Edmeades, G., 2014. Crop Yields and Global Food Security: Will Yield Increase Continue to Feed the World? 634ACIAR Monograph.
- Galindo, F.S., Carvalho, M., Teixeira, M., 2016. Corn Yield and Foliar Diagnosis Affected by Nitrogen Fertilization and Inoculation with *Azospirillum brasilense*, pp. 1–18. <https://doi.org/10.1590/18069657rbcs20150364>.
- Galindo, F.S., Teixeira Filho, M.C.M., Buzetti, S., Rodrigues, W.L., Santini, J.M.K., Alves, C.J., 2019. Nitrogen fertilization efficiency and wheat grain yield affected by nitrogen doses and sources associated with *Azospirillum brasilense*. *Acta Agric. Scand. Section B: Soil Plant Sci.* 69 (7), 606–617. <https://doi.org/10.1080/09064710.2019.1628293>.
- García-Montelongo, A.M., Montoya-Martínez, A.C., Morales-Sandoval, P.H., Parra-Cota, F.I., de los Santos-Villalobos, S., 2023. Beneficial microorganisms as a sustainable alternative for mitigating biotic stresses in crops. *Stresses* 3 (1), 210–228.
- Glick, B.R., 2012. Plant Growth-Promoting Bacteria : Mechanisms and Applications, 2012.
- Goicoechea, N., Antolín, M.C., 2017. Increased nutritional value in food crops. *Microb. Biotechnol.* 10 (5), 1004–1007. <https://doi.org/10.1111/1751-7915.12764>.
- González-Figueroa, S.S., Vera-Núñez, J.A., Peña-Cabriales, J.J., Báez-Pérez, A., Grageda-Cabrera, O.A., 2022. Uso Eficiente De Nitrógeno En Aplicaciones Fraccionadas De Fertilizante Marcado Con 15N En Trigo. *Rev. Fitotec. Mex.* 45 (4), 437. <https://doi.org/10.35196/rfm.2022.4.437>.
- Grageda Cabrera, O.A., Vera Núñez, J.A., Peña Cabriales, J.J., 2018. Efecto de los biofertilizantes sobre la asimilación de nitrógeno por el cultivo de trigo. *Rev. Mexicana Cienc. Agric.* 9 (2), 281–289. <https://doi.org/10.29312/remexca.v9i2.1071>.
- Gul, S., Javed, S., Azeem, M., Aftab, A., Anwaar, N., Mehmood, T., Zeshan, B., 2023. Application of *Bacillus subtilis* for the alleviation of salinity stress in different cultivars of wheat (*Triticum aestivum* L.). *Agronomy* 13 (2), 437. <https://doi.org/10.3390/agronomy13020437>.
- Guzman, A. C., Autrique, J.E., Mondal, S., Singh, R.P., Govindan, V., Morales-Dorantes, A., Posadas-Romano, G., Crossa, J., Ammar, K., Peña, R.J., 2016a. Response to drought and heat stress on wheat quality, with special emphasis on bread-making quality, in durum wheat. *Field Crops Res.* 186, 157–165. <https://doi.org/10.1016/j.fcr.2015.12.002>.
- Guzman b, C., Mondal, S., Govindan, V., Autrique, J.E., Posadas-Romano, G., Cervantes, F., Crossa, J., Vargas, M., Singh, R.P., Peña, R.J., 2016b. Use of rapid tests to predict quality traits of CIMMYT bread wheat genotypes grown under different environments. *LWT - Food Sci. Technol.* 69, 327–333. <https://doi.org/10.1016/j.lwt.2016.01.068>.
- Heberle, H., Meirelles, G.V., da Silva, F.R., Telles, G.P., Minghim, R., 2015. InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics* 16, 169.
- IAEA, (International Atomic Energy Agency), 2017. Stable Nitrogen Isotope Helps Scientists Optimize Water and Fertilizer Use. <https://www.iaea.org/newscenter/news/stable-nitrogen-isotope-helps-scientists-optimize-water-fertilizer-use>.
- Ibarra-Villarreal, A.L., Villarreal-Delgado, M.F., Parra-Cota, F.I., Yepez, E.A., Guzmán, C., Gutierrez-Coronado, M.A., Valdez, L.C., Saint-Pierre, C., de Los Santos-Villalobos, S., 2023. 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. *Plant Signal. Behav.* <https://doi.org/10.1080/15592324.2023.2219837>.
- Igrejas, G., Branlard, G., 2020. The importance of wheat. In: Igrejas, G., Ikeda, T.M., Guzmán, C. (Eds.), *Wheat Quality For Improving Processing and Human Health*. Springer, Cham, Switzerland, pp. 1–7.
- Kazan, K., 2013. Auxin and the integration of environmental signals into plant root development. *Ann. Bot.* 112 (9), 1655–1665. <https://doi.org/10.1093/aob/mct229>.
- Kennedy, I.R., Choudhury, A.T.M.A., Kecskés, M.L., Rose, M.T., 2008. Efficient nutrient use in rice production in Vietnam achieved using inoculant biofertilisers. In: *Proceedings of a project (SMCN/2002/073) workshop held in Hanoi, Vietnam, 12–13 October 2007*. ACIAR Proceedings No. 130, 137pp.
- Kumar, A., Maurya, B.R., Raghuwanshi, R., Meena, V.S., Tofazzal Islam, M., 2017. Co-inoculation with enterobacter and rhizobacteria on yield and nutrient uptake by wheat (*Triticum aestivum* L.) in the alluvial soil under Indo-Gangetic Plain of India. *J. Plant Growth Regul.* 36 (3), 608–617. <https://doi.org/10.1007/s00344-016-9663-5>.
- Kumar, M., Singh, D.P., Prabha, R., Rai, A.K., & L., S. 2016. Role of microbial inoculants in nutrient use efficiency. In D.P. Singh, H. B. Singh, & R. Prabha (Eds.), *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 2: Functional Applications* (pp. 1–308). Springer, <https://doi.org/10.1007/978-81-322-2644-4>.
- Lares-Orozco, M.F., Robles-Morúa, A., Yepez, E.A., Handler, R.M., 2016. Global warming potential of intensive wheat production in the Yaqui Valley, Mexico: a resource for the design of localized mitigation strategies. *J. Clean. Prod.* 127, 522–532. <https://doi.org/10.1016/j.jclepro.2016.03.128>.
- Lv, X., Zhang, Y., Hu, L., Zhang, Y., Zhang, B., Xia, H., Du, W., Fan, S., Kong, L., 2021. Low-nitrogen stress stimulates lateral root initiation and nitrogen assimilation in wheat: roles of phytohormone signaling. *J. Plant Growth Regul.* 40, 436–450. <https://doi.org/10.1007/s00344-020-10112-5>.
- Mahapatra, S., Yadav, R., Ramakrishna, W., 2022. *Bacillus subtilis* impact on plant growth, soil health, and environment: Dr. Jekyll and Mr. Hyde. *J. Appl. Microbiol.* 132 (5), 3543–3562. <https://doi.org/10.1111/jam.15480>.
- Malhotra, H., Vandana, Sharma, S., Pandey, R., 2018. Phosphorus nutrition: plant growth in response to deficiency and excess. In: Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., Hawrylak-Nowak, B. (Eds.), *Plant Nutrients and Abiotic Stress Tolerance*. Springer, Singapore, pp. 171–190. <https://doi.org/10.1007/978-981-10-9044-8>.
- Millar, N., Urrea, A., Kahmark, K., Shcherbak, I., Robertson, G.P., Ortiz-Monasterio, I., 2018. Nitrous oxide (N₂O) flux responds exponentially to nitrogen fertilizer in irrigated wheat in the Yaqui Valley, Mexico. *Agric. Ecosyst. Environ.* 261 (February), 125–132. <https://doi.org/10.1016/j.agee.2018.04.003>.
- Morales Sandoval, P.H., Valenzuela Ruiz, V., Ortega Urquieta, M.E., Martínez Vidales, A. D., Félix Pablos, C.M., Chávez Luzania, R.A., Parra Cota F.I., de los Santos Villalobos, S., 2021. Taxonomía bacteriana basada en índices relacionados al genoma completo. *La sociedad Académica.* 58:1–11.
- Montoya-Martínez, A.C., Parra-Cota, F.I., de los Santos-Villalobos, S., 2022. Beneficial microorganisms in sustainable agriculture: harnessing microbes' potential to help feed the world. *Plants* 11 (3), 372.
- Omara, P., Aula, L., Oyebiyi, F., Raun, W.R., 2019. World cereal nitrogen use efficiency trends: review and current knowledge. *Agrosyst. Geosci. Environ.* 2 (1), 1–8. <https://doi.org/10.2134/age2018.10.0045>.
- Ondov, B.D., Bergman, N.H., Philliply, A.M., 2011. Interactive metagenomic visualization in a web browser. *BMC Bioinformatics* 12 (1), 385, 2011 Sep 30.
- Ortega-Urquieta, M.E., Valenzuela-Ruiz, V., Mitra, D., Hyder, S., Elsheery, N.I., Kumar Das Mohapatra, P., Parra-Cota, F.I., de los Santos-Villalobos, S., 2022. Draft genome sequence of *Priestia* sp. strain TSO9, a plant growth-promoting bacterium associated with wheat (*Triticum turgidum* subsp. *durum*) in the Yaqui Valley, Mexico. *Plants* 11 (17), 2231. <https://doi.org/10.3390/plants11172231>.
- Ortiz-Monasterio, J.I.R., 1998. Nitrogen management in irrigated spring wheat. In: *Bread Wheat: Improvement and Production, 1*. Stanford University, pp. 433–452.
- Ortiz-Monasterio, J.I., Raun, W., 2007. Paper presented at international workshop on increasing wheat yield potential, CIMMYT, Obregon, Mexico, 20-24 March 2006: reduced nitrogen and improved farm income for irrigated spring wheat in the Yaqui Valley, Mexico, using sensor-based nitrogen management. *J. Agric. Sci.* 145 (3), 215–222. <https://doi.org/10.1017/S0021859607006995>.
- Overbeck, R., Olson, R., Pusch, G., Olsen, G., Davis, J., Disz, T., Edwards, R., Gerdes, S., Parrello, B., Shukla, M., Vonstein, V., Wattam, A., Xia, F., Stevens, R., 2014. The SEED and the rapid annotation of microbial genomes using sub-systems technology (RAST). *Nucleic Acids Res.* 42 (D1), 206–214. <https://doi.org/10.1093/nar/gkt1226>.
- Patz, S., Gautam, A., Becker, M., Ruppel, S., Rodríguez-Palenzuela, P., Huson, D.H., 2021. PlaBase: a comprehensive web resource for analyzing the plant growth-promoting potential of plant-associated bacteria. *bioRxiv*. <https://doi.org/10.1101/2021.12.13.472471>.
- Peña, R.J., Amaya, A., Rajaram, S., Mujeeb-Kazi, A., 1990. Variation in quality characteristics associated with some spring 1B/1R translocation wheats. *J. Cereal Sci.* 12 (2), 105–112. [https://doi.org/10.1016/S0733-5210\(09\)80092-1](https://doi.org/10.1016/S0733-5210(09)80092-1).
- Porker, K., Straight, M., Hunt, J.R., 2020. Evaluation of G × E × M interactions to increase harvest index and yield of early sown wheat. *Front. Plant Sci.* 11, 994. <https://doi.org/10.3389/fpls.2020.00994>.
- Rana, A., Joshi, M., Prasanna, R., Shivay, Y.S., Nain, L., 2012. Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. *Eur. J. Soil Biol.* 50, 118–126. <https://doi.org/10.1016/j.ejsobi.2012.01.005>.
- Reynolds, M.P., Braun, H.J., 2022. Wheat improvement. In: Reynolds, M.P., Braun, H.J. (Eds.), *Wheat Improvement: Food Security in a Changing Climate*. Springer International Publishing, Cham, pp. 3–15.
- Robles-Montoya, R.I., Parra-Cota, F.I., de los Santos-Villalobos, S., 2019. Draft genome sequence of *Bacillus megaterium* TRQ8, a plant growth-promoting bacterium isolated from wheat (*Triticum turgidum* subsp. *durum*) rhizosphere in the Yaqui Valley, Mexico. *3 Biotech* 9 (5), 1–5. <https://doi.org/10.1007/s13205-019-1726-4>.
- Robles-Montoya, R.I., Valenzuela-Ruiz, V., Parra-Cota, F.I., Santoyo, G., de los Santos-Villalobos, S., 2020a. Description of a polyphasic taxonomic approach for plant growth promoting rhizobacteria (PGPR). In: Singh, J.S., Vimal, S.R. (Eds.), *Microbial Services in Restoration Ecology*. Elsevier Inc, pp. 259–269. <https://doi.org/10.1016/B978-0-12-819978-7.00017-8>.
- Robles-Montoya, R.I., Chaparro-Encinas, L.A., Parra-Cota, F.I., de los Santos-Villalobos, S., 2020b. Improving biometric traits of wheat seedlings with the inoculation of a consortium native of *Bacillus*. *Rev. Mexicana Cienc. Agric.* 11 (1), 229–235.
- Rojas-Padilla, J., Chaparro-Encinas, L.A., Robles-Montoya, R.I., De los Santos-Villalobos, S., 2020. Promoción de crecimiento en trigo (*Triticum turgidum* L. subsp. *durum*) por la co-inoculación de cepas nativas de *Bacillus* aisladas del Valle del Yaqui, México. *Nova Scientia* 12 (24), 1–27. <https://doi.org/10.21640/ns.v12i24.2136>.

- Rojas-Padilla, J., de-Bashan, L.E., Parra-Cota, F.I., Rocha-Estrada, J., de los Santos-Villalobos, S., 2022. Microencapsulation of *Bacillus* strains for improving wheat (*Triticum turgidum* subsp. *durum*) growth and development. *Plants* 11, 1–16. <https://doi.org/10.3390/plants11212920>.
- Serret, M.D., Pardo, A., Araus, J.L., 2008. The effects of urea fertilization and genotype on yield, nitrogen use efficiency, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in wheat. *Ann. Appl. Biol.* 153 (2), 243–257. <https://doi.org/10.1111/j.1744-7348.2008.00259.x>.
- aSIAP, 2023. SIAP (Servicio de Información Agroalimentaria y Pesquera). 2023 Avance De Siembras y Cosechas. Resumen por Estado. <https://nube.siap.gob.mx/cierre-agricola/>.
- ^bSIAP, 2023. SIAP (Servicio de Información Agroalimentaria y Pesquera). 2023. Escenario Mensual De Productos Agroalimentarios. Dirección de Análisis Estratégico. https://www.gob.mx/cms/uploads/attachment/file/870875/Trigo_cri_stalino_Octubre.pdf.
- Slafer, G.A., García, G.A., Serrago, R.A., Miralles, D.J., 2022. Physiological drivers of responses of grains per m² to environmental and genetic factors in wheat. *Field Crops Res.* 285, 108593.
- Sonkurt, M., ÇİĞ, F., 2019. The effect of plant growth-promoting bacteria on the development, yield, and yield components of bread (*Triticum aestivum* L.) and durum (*Triticum durum*) wheats. *Appl. Ecol. Environ. Res.* 17 (2).
- Spaepen, S., Vanderleyden, J., 2011. Auxin and plant-microbe interactions. *Cold Spring Harb. Perspect. Biol.* 1–14.
- Srivastava, N. 2023. Siderophore production in iron uptake and plant biofortification. In: Chhabra, S., Prasad, R., Maddela, N.R., Tuteja, N. (Eds.) *Plant Microbiome for Plant Productivity and Sustainable Agriculture. Microorganisms for Sustainability*, vol 37. Springer, Singapore. https://doi.org/10.1007/978-981-19-5029-2_13.
- Tabbitta, F., Ortiz-Monasterio, I., Piñera-Chavez, F.J., Ibba, M.I., Guzmán, C., 2023. On-farm assessment of yield and quality traits in durum wheat. *J. Sci. Food Agric. March*.
- Tan, S., Luschnig, C., Friml, J., 2021. Pho-view of auxin: reversible protein phosphorylation in auxin biosynthesis, transport and signaling. *Mol. Plant* 14 (1), 151–165. <https://doi.org/10.1016/j.molp.2020.11.004>.
- Tsavkelova, E.A., Klimova, S.Y., Cherdynseva, T.A., Netrusov, A.I., 2006. *Microbial Producers of Plant Growth Stimulators and Their Practical Use: A Review. September 2014*. <https://doi.org/10.1134/S0003683806020013>.
- Valenzuela-Ruiz, V., Santoyo, G., Gómez-Godínez, L.J., Cira-Chávez, L.A., Parra-Cota, F. I., de los Santos-Villalobos, S., 2023. Complete genome sequencing of *Bacillus cabrialesii* TE3^T: a plant growth-promoting and biological control agent isolated from wheat (*Triticum turgidum* subsp. *durum*) in the Yaqui Valley. *Curr. Res. Microb. Sci.* 4, 100193 <https://doi.org/10.1016/j.crmicr.2023.100193>.
- Valenzuela-Aragon, B., Parra-Cota, F.I., Santoyo, G., Arellano-Wattenbarger, G.L., de los Santos-Villalobos, S., 2018. Plant-assisted selection: a promising alternative for in vivo identification of wheat (*Triticum turgidum* L. subsp. *durum*) growth-promoting bacteria. *Plant Soil* 435 (1–2), 367–384. <https://doi.org/10.1007/s11104-018-03901-1>.
- Valenzuela-Ruiz, V., Robles-Montoya, R.I., Parra-Cota, F.I., Santoyo, G., del Carmen, Orozco-Mosqueda, M., Rodríguez-Ramírez, R., de los Santos-Villalobos, S., 2019. Draft genome sequence of *Bacillus paralicheniformis* TRQ65, a biological control agent and plant growth-promoting bacterium isolated from wheat (*Triticum turgidum* subsp. *durum*) rhizosphere in the Yaqui Valley, Mexico. *3 Biotech* 9 (11). <https://doi.org/10.1007/s13205-019-1972-5>.
- Villa-Rodríguez, E., Parra-Cota, F.I., Castro-Longoria, E., López-Cervantes, J., de los Santos-Villalobos, S., 2019. *Bacillus subtilis* TE3: a promising biological control agent against *Bipolaris sorokiniana*, the causal agent of spot blotch in wheat (*Triticum turgidum* L. subsp. *durum*). *Biol. Control* 132, 135–143.
- Villarreal-Delgado, M.F., Villa-Rodríguez, E.D., Cira-Chávez, L.A., Estrada-Alvarado, M. I., Parra-Cota, F.I., de los Santos-Villalobos, S., 2018. El Género *Bacillus* Como Agente de Control Biológico y Sus Implicaciones En La Bioseguridad Agrícola. *Revista Mexicana de Fitopatología* 36, 95–130.
- Wilding, L.P., Smek, N.E., & Hall, G.F. (Eds.) 1983. *Pedogenesis and Soil Taxonomy: The Soil Orders*. Elsevier B.V.
- Wongsanit, J., Teartisup, P., Kerdsueb, P., Tharnpoophasiam, P., Worakhunpiset, S., 2015. Contamination of nitrate in groundwater and its potential human health: a case study of lower Mae Klong river basin, Thailand. *Environ. Sci. Pollution Res.* 22 (15), 11504–11512. <https://doi.org/10.1007/s11356-015-4347-4>.
- Zapata, F., & Axman, H. 1990. Stable and radioactive isotopes. In G. Hardarson (Ed.), *Use of Nuclear Techniques in Studies of Soil-Plant Relationships* (Vol. 2, Issue 2).
- Zarco-Hernandez, J.A., Santiveri, F., Michelena, A., Javier Peña, R., 2005. Durum wheat (*Triticum turgidum*, L.) carrying the 1BL/1RS chromosomal translocation: agronomic performance and quality characteristics under Mediterranean conditions. *Eur. J. Agron.* 22 (1), 33–43. <https://doi.org/10.1016/j.eja.2003.12.001>.