Jalal *et al. Chem. Biol. Technol. Agric.* (2023) 10:77
https://doi.org/10.1186/s40538-023-00440-5

Chemical and Biological Technologies in Agriculture

RESEARCH

Open Access

Nano-zinc and plant growth-promoting bacteria is a sustainable alternative for improving productivity and agronomic biofortification of common bean

Arshad Jalal[1], Emariane Satin Mortinho[1], Carlos Eduardo da Silva Oliveira[1], Guilherme Carlos Fernandes[1], Enes Furlani Junior[2], Bruno Horschut de Lima[1], Adônis Moreira[3], Thiago Assis Rodrigues Nogueira[4], Fernando Shintate Galindo[5] and Marcelo Carvalho Minhoto Teixeira Filho[1*]

Abstract

Background and aims Nano-zinc (Zn) fertilizer is an easily adaptable and environmentally safe alternative option that can effectively improve growth, yield and biofortification of common bean. Plant growth-promoting bacteria (PGPBs) could promote plant growth and nutrients availability in sustainable manner. Therefore, this study aimed to investigate the influence of foliar nano-Zn application in association with seed co-inoculations of PGPBs on growth, yield, biofortification and Zn use efficiencies in common bean cultivation. Two field experiments were performed with seven co-inoculations of PGPBs and three foliar nano-Zn doses applied 50% at R5 and 50% at R8 stages of common bean to determine plant height, shoot dry matter, grain yield, Zn concentration and uptake in shoot and grains, Zn partitioning index, daily Zn intake and Zn use efficiencies for agronomic biofortification.

Results The combined foliar nano-Zn application and co-inoculation of *R. tropici* + *B. subtilis* enhance grain yield, leaf chlorophyll index, total protein content, grain Zn concentration and uptake, daily Zn intake, Zn use efficiency, applied Zn recovery and Zn utilization efficiency in common beans in 2019 and 2020 cropping seasons. Foliar nano-Zn application at a dose of 1.5 kg ha^{-1} increased plant height, shoot dry matter, shoot Zn uptake, Zn partitioning and agro-physiological efficiency under co-inoculation with *R. tropici* + *B. subtilis* in both cropping years.

Conclusions The treatments with foliar nano-Zn application at a dose of 1.5 ha^{-1} and co-inoculation with *R. tropici* + *B. subtilis* improved performance, chlorophyll index, protein content, grain yield, and Zn efficiencies that can lead to better biofortification of common bean in tropical savannah. Therefore, it is recommended that applying nano-Zn via foliar along with co-inoculation of PGPBs could be the better option for productivity and biofortification of common bean.

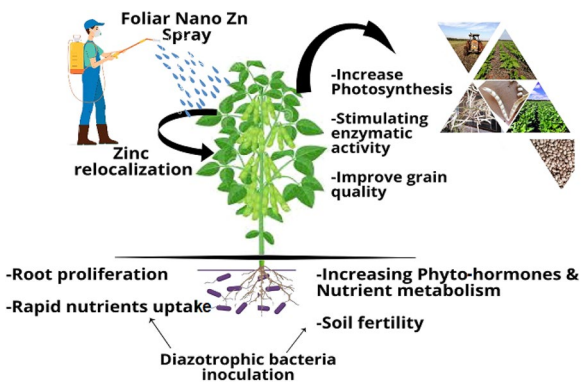
*Correspondence:
Marcelo Carvalho Minhoto Teixeira Filho
mcm.teixeira-filho@unesp.br
Full list of author information is available at the end of the article

Highlights

1. Foliar nano-zinc (Zn) fertilization can improve agronomic biofortifcation and producitvity of common beans.
2. Plant growth-promoting bacteria (PGPBs) can sustainably increase nutrient use efficiency and zinc content in edible tissues.
3. The combined application of nano-Zn and PGPBs can potentially alleviate food and nutritional security crises.
4. The sustianbale mechanisms of co-application of nano-Zn and PGPBs need further investigation.

Keywords Agronomic biofortification, Foliar nano-zinc, Zinc use efficiencies, Zinc uptake, Zinc partitioning, PGPBs

Graphical Abstract



Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important legume crop and being a part of daily diet for human consumption around the world. Brazil is one of the largest global producer of common bean, ranked third in terms of grain production (3.2 million metric tons) and second in terms of cultivated area (2.9 million hectares) during three major sowing seasons [1]. It is one of the most consumed grain food in Brazilian nutrition for being a source of important protein, vitamins, minerals and amino acids [2]. Agricultural production systems and practices are responsible to ensure food and nutrition security to increasing population without environmental risks, which linking soil, plants and animals to human health for maintaining sustainable agriculture [3]. Climatic extremes and variabilities are disturbing sustainable agriculture production, availability, utilization, and stability of food in both developed and developing countries, leading to global hunger [4]. In addition, crop cultivation is accompanied by intensive use of synthetic fertilizers to meet food demand of increasing global population which contribute to the deficiency of most common nutrients including iron (Fe), zinc (Zn), selenium (Se) and iodine (I) in human blood plasma [5].

Zinc (Zn) malnutrition is a global dietary concern and most vulnerable to agriculture soil, crop production and nutritional quality of staple field crops. Zinc is one of the most persistent deficiency in tropical regions that lead to serious health issues especially in low income countries [6]. Plant-based Zn deficiency could impair a series of human metabolic reactions and affect more than 2 billion people, being ranked as the second most common deficiency and health concern [6, 7]. The inadequate Zn supply has affected around 17% of the global population and was ranked as 5th health risk factor with a prevalence deficiency in least developed or developing countries [8, 9]. To feed the raising population of the world with both sufficient and nutritious food is itself one of sustainable development goal of United Nation (https://sdgs.un.org/goals/goal3). Thus, it is consider that agronomic biofortification is one the best and prompt alternative to enrich grain crops with targeted deficient nutrient in the soil and fulfill nutritional requirments [10, 11].

Biofortification with nano-fertilizer could enhance nitrogen metabolism, productivity and nutrition into the edible tissues of targeted plant crops to address global malnutrition [12]. Biofortification could better be define by the time of foliar spray, choice and growth stage of crop as well as source, formulation and particle size of

applied fertilizers [13, 14]. Foliar spray of nano-Zn fertilizer has gained more attention and interest in agriculture sector for increasing crop nutrition and productivity in ecofriendly manner [11, 15]. Nano-zinc oxide (ZnO) is widely use Zn fertilizer that could quickly absorb via stomata and cuticles, mobilize and translocated to chloroplast thus contributing to plant nutrition and production [16, 17]. The efficacy of nano-ZnO could be recognized by their physical characteristics, composition and size of particle that potentially increase nutrient use efficiency and redudce dependency on synthetic fertilizers, promoting Zn concentration in stem and edible tissues, and other growth characteristics of different crops [18, 19]. However, excessive use of synthetically produced nanoparticles may retarder plant growth and productivity, depending on the surface and size of particulate [20]. Therefore, more greener and sustainable alternative could be adapted to to improve crop production and nutritional status as well as minimizing devestating environmental impacts of current agriculture techniques [21].

Plant growth-promoting bacteria (PGPBs) application is one of the sustainable and greener approach that are enhancing food quantity without compromising quality and productivity [22]. These PGPBs are able to improve nutrient use efficiency and tolerance against biotic and abiotic stresses by contributing to root architecture, improving soil fertility, and enhancing solubilization of macro- and micronutrient that all together can lead to higher plant productivity [23]. Several genus of PGPBs including *Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Rhizobium* sp., *Acinetobacter* sp. and *Klebsiella* sp. are being reported and studied for improving nutrient use efficiency and productivity, reducing the use of syhthetic fertilizers in different crops [24–27]. Plant growth-promoting bacteria support plant health and performance by production and regulation of phyto-hormones [28, 29], solubilization of nutrients [30, 31], biosynthesis of siderophores and antibiotics [32, 33] to resist biotic and abiotic stresses. The interaction of PGPBs with soil and plant tissues enhance fitness of the plant to drastic environemntal conditions by regulating plants metabolic and physiological triats [34].

Sustainable nutrient management in field crops is challenging issue in tropical savannah. Despite this, Brazil is being spotted in the list of Zn deficient countries (http://www.harvestplus.org). Several strategies have being adapted to increase nutrient use efficiency and productivity; however, there still exist research gap on the use of nano-Zn foliar spray in association with PGPBs for improving nutrition, productivity and biofortification of common bean. In this context, the hypothesis of the current study was that PGPBs and nano-Zn foliar may improve nutrition, productivity, Zn use efficiencies and biofortification of common bean edible tissues. Hence, the study aimed to determine the best performing PGPBs seed co-inoculation with nano-Zn foliar application on growth, yield, concentration of Zn in shoot and grains, Zn use efficiencies and estimated Zn intake in common beans under tropical savannah of Brazil.

Materials and methods

Description of experimental site

Two field experiments with a test crop common bean were conducted during May–August of 2019 and 2020, respectively, at the Research and Extension Farm of School of Engineering, São Paulo State University (UNESP) in Selvíria, state of Mato Grosso do Sul, Brazil. The geographical coordinates of the site are 20° 22′ S latitude, 51° 22′ W longitude, and an altitude of 335 m (Fig. 1). The experimental soil is classified as Rhodic Haplustox [35] and Red Dystrophic according Brazilian soil classification system [36], being cultivated with cereal–legume cropping system for last 30 years, and no-tillage for last 13 years.

The climate of the region is characterized as Aw-Köppen with rainy summer, dry winter and humid tropical with relative humidity 70–80% [37]. The climatic data during current experiment in both cropping seasons is summarized in Fig. 2.

Soil analysis

Twenty random soil samples were collected with the help of cup-auger from a soil layer of 0.00–0.25 m before initiation of common bean experiments during both cropping seasons. The samples were mixed to make a composite sample and determined for chemical characterizations [38]. The physio-chemical characterizations of soil are summarized in Table 1.

Experimental design and treatments

The experiments were designed in a randomized complete block design in a 7×3 factorial scheme and four replications. The treatments were consisted of seven different inoculations with PGPBs (1—No inoculation, 2—*Rhizobium tropici*, 3—*R. tropici* + *Azospirillum brasilense,* 4—*R. tropici* + *Bacillus subtilis*, 5—*R. tropici* + *Pseudomonas fluorescens,* 6—*R. tropici* + *A. brasilense* + *B. subtilis* and 7—*R. tropici* + *A. brasilense* + *P. fluorescens*) via seeds, and three foliar nano-zinc oxide spray (0.0, 1.5 and 3.0 kg ha^{-1}), applied 50% at R5 and 50% at R8 stage of common bean according to International Center for Tropical Agriculture—CIAT [40].

Seeds of common bean were manually inoculated by mixing each inoculant of PGPBs and seeds in a plastic bag an hour before to sowing. Common bean seeds were inoculated with *R. tropici*, using commercial peat

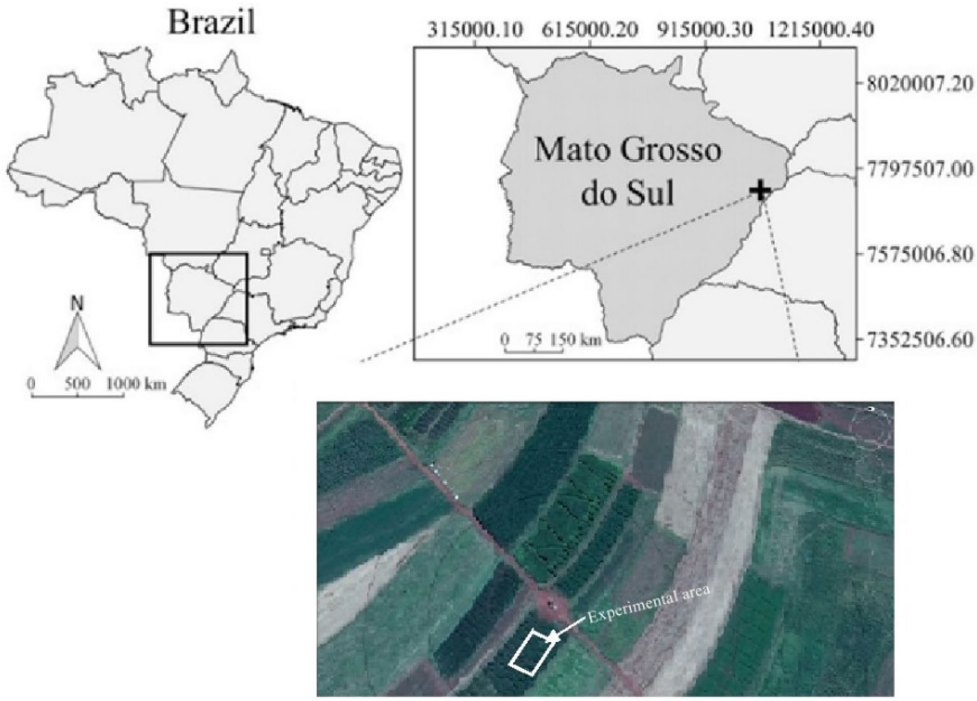


Fig. 1 Geographical location of field trail at Research and Extension Farm, UNESP—Ilha Solteira at Selvíria, Mato Grosso do Sul, Brazil (20°22′ S, 51°22′ W, altitude of 335 m) during 2019 and 2020 cropping seasons. The map was composed using geographic information system (QGIS) and Google Earth program (The QGIS Development Team 2021) and Open Source Geospatial Foundation project. http://qgis.osgeo.org. Accessed on: 12th September, 2022. Projection System WGS 84/UTM 200DC [EPSG: 4326]. This image was taken from Google Earth program, Google Company (2021). Map data: Google, Maxar Technologies

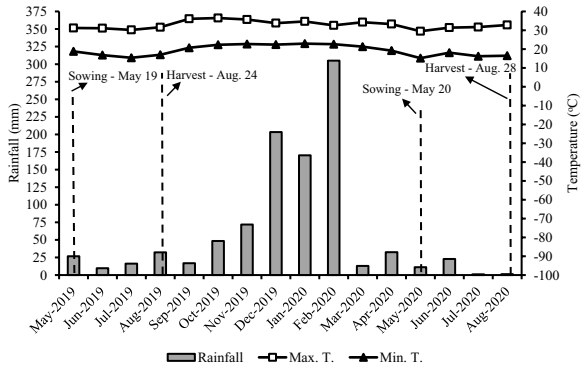


Fig. 2 Rainfall, and maximum and minimum temperatures during common bean cultivation, acquired from the weather station of Research and Extension Farm of UNESP from May to August 2019 and 2020

inoculant, strain SEMIA 4080 with 2×10^9 colony forming units (CFU) g^{-1} at a dose of 200 g per 100 kg seeds. A 10% sugar solution was used to facilitate inoculant adhesion with seeds. *R. tropici* is a commercially registered inoculant with Ministry of Agriculture and Livestock, Brazil for common bean cultivation. *A. brasilense* strains Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084) seed inoculation was carried out at a dose of 200 mL liquid inoculant per 24 kg seeds with guarantee of 2×10^8 CFU mL^{-1}. Inoculations with *B. subtilis* strain (CCTB04), guarantee of 1×10^8 CFU mL^{-1} and *P. fluorescens* strain (CCTB03), guarantee of 2×10^8 CFU mL^{-1} at dose of 150 mL ha^{-1} per 24 kg seeds were manually performed by following the procedures of inoculants providing company (Total Biotechnology®), Curitiba, Brazil. It has been reported that *A. brasilense* strains Ab-V5 and Ab-V6 are carrying *fix* and *nif* genes to enhance nutrients availability and recycling, also has a role in biological nitrogen fixation, auxin production and induce plant tolerance against biotic and abiotic stresses [41–43]. *B. subtilis* is gram positive bacterium carrying nonribosomal peptide synthetases and a beta-glucanase as phytopathogens resistant and zntR as Zn transportator possessing plant growth-promotion while resisting phyto-pathogens and heavy metals absorption [44–46]. *P. fluorescens* is considered the most efficient biocontrol agent due to the synthesis of antibiotics, volatile organic compounds to resist soil pathogens, produce gluconic acid, solubilize nutrients and fixing N [47, 48].

Table 1 Soil physio-chemical characteristics of field site before experiment initiation during 2019 and 2020 common bean growing seasons

Properties	Units	Values	
		2019	2020
Clay†	g kg^{-1}	433	
Sand†	g kg^{-1}	471	
Silt†	g kg^{-1}	90	
pH ($CaCl_2$)	–	5.2	5.1
Organic matter	mg dm^{-3}	18	21
P	mg dm^{-3}	38	42
K	$mmol_c$ dm^{-3}	1.7	1.9
Ca	$mmol_c$ dm^{-3}	21	22
Mg	$mmol_c$ dm^{-3}	15	13
B	mg dm^{-3}	0.14	0.35
Cu	mg dm^{-3}	3.4	3.6
Fe	mg dm^{-3}	25	29
Zn	mg dm^{-3}	0.9	1.1
Mn	mg dm^{-3}	38.1	37.1
S–SO_4	mg dm^{-3}	4.0	9.2
H + Al	$mmol_c$ dm^{-3}	34	30
CEC	$mmol_c$ dm^{-3}	75.7	67.9
V	%	50	52

CEC: cation exchange capacity; V: base saturation

† Determined according to methodology of Teixeira et al. [39]

Nano-Zn folair spray was carried out from a liquid Zn source (Nano-R1 zinco) of ALLPLANT™ fertilizers industry, São Paulo-Brazil, being registered with Ministry of Agriculture, Brazil. This nano-Zn is characterized as fluid suspension with 50% p/p Zn, 1000 g L^{-1} solubility and 2.0 density and successfully studied for improving plant growth and productivity [11, 49]. All the doses of foliar nano-Zn were applied in two split applications (50% at R5 and 50% at R8 stage of common bean according to International Center for Tropical Agriculture—CIAT) [40]. The application was performed through manual sprayer pump with water capicity of 6.0 L (300 L ha^{-1} of volume spray). The field was thoroughly visited soon after foliar nano-Zn spray and no leaf damage was observed.

Field management

The field site was sprayed with herbicides 2,4-D + glyphosate (670 + 1800 g ha^{-1} of a.i.) for controlling pre-experiment emerged weeds. Common bean cultivar (IPR—Campos Gerais belonging to commercial Carioca group), erect and inderterminate type-II with and an avreage life cycle of 88 days [50]. Two field experiments of common bean were conducted in the first half of May, 2019 and 2020 under a no-tillage system. Each plot was consisted of 5 m long 6 lines with plot size of 2.7 m × 5 m, totalizing 13.5 m^2. The rows were spaced by 0.45 m and regulated 12 seeds m^{-1}. The treatments in both experiments were performed at the same phenological stages.

All the treatments were applied with a basal dose of NPK according to initial soil analysis (Table 1). A total amount of nitrogen (N: 40 kg ha^{-1}) from a source of urea, phosphorus (P_2O_5: 80 kg ha^{-1}) from triple superphosphate and potassium (K_2O: 40 kg ha^{-1}) from ammonium sulphate applied at plantation. Despite this, a recommneded dose of 90 kg ha^{-1} of N was applied in top dressing after 5 weeks of plantation. The region of the experiment was interpreted as boron deficient according Campinas Agronomic Institute—IAC [38]. Therefore, the entire field was sprayed with 1 kg boron ha^{-1} from the source of boric acid through a tractor spryer at folwering stage of common bean. The crop was irrigated with a central-pivot sprinkler irrigation system (14 mm water volume on a shift of 72 h) and all cultural practices were performed, when necessary. The common bean were manully harvested on 24th and 28th August, 2019 and 2020, respectively.

Assessments and evaluations

The assessments and evalautions were carried out in all four replicates. Plant height was determined at physiological maturity by measuring plant length from ground surface to upper apex of plants. Plants from four central rows were harvested, labeled, dried and weighed with analytical balance for shoot dry matter. Each sample was mechanically threshed and transferred into kg ha^{-1} at 13% humidity to quantify grain yield of common bean.

Leaf chlorophyll index was measured using a non-destructive, hand-held chlorophyll Falker meter (ClorofiLOG®—model CFL—1030 Falker, Porto Alegre, Brazil). Total protein content was derived from the nitrogen content (Kjeldahl method) of the current experiment multiplied with Jones's factor, which was specified as 6.25 for common beans [51].

Zinc concentrations in shoot and grains were determined with nitroperchloric digestion and quantified by atomic absorption spectrophotometry (FAAS—Model Varian SpectrAA-55B, Varian, California, USA), following the procedure of Malavolta et al. [52]. In addition, uptake of Zn in shoot and grains were estimated from the ratio of Zn concentration in shoot and grains, and shoot dry matter and grain yield, respectively. Zinc partitioning index (ZPI) was derived from grains and shoot Zn concentration [53], while estimated Zn intake with common bean grains in Brazil was calculated from the biofortified common bean grains of the present study [54]:

$$\text{ZPI} = \text{Grain Zn concentration} - \text{Shoot Zn concentration} \times 100 \quad (1)$$

$$\text{Zinc intake} = [\text{Zn}] \times \text{C} \quad (2)$$

where Zn intake (g person^{-1} day^{-1}) is daily Zn intake estimation person^{-1}, [Zn] (g kg^{-1}) is Zn concentration in biofortified grains in the present study and C (kg person^{-1} day^{-1}) is the mean consumption of common bean grains per person in Brazil, which is ~ 142.2 g person^{-1} day^{-1} [55].

Zinc use efficiency (ZnU), agro-physiological efficiency (APE), applied Zn recovery (AZnR) and utilization efficiency were derived in the fractions aquired from Fageria et al. [56] via the following equations:

$$\text{ZnU} = \frac{\text{GYF} - \text{GYC}}{\text{Applied Zn dose}} \quad (3)$$

$$\text{APE} = \frac{\text{GYF} - \text{GYC}}{\text{Grain} + \text{Shoot ZnUF} - \text{Grain} + \text{Shoot ZnUC}} \quad (4)$$

$$\text{AZnR (\%)} = \frac{\text{Grain} + \text{Shoot ZnUF} - \text{Grain} + \text{Shoot ZnUC}}{\text{Applied Zn dose}} \frac{\text{GYF} - \text{GYC}}{} \quad (5)$$

$$\text{UE} = \text{PE} \times \text{RAZn} \quad (6)$$

where GYF = grain yield with nano-Zn foliar spray, GYC = grain yield in without nano-Zn foliar, ZnF = with nano-Zn foliar spray, ZnC = without nano-Zn foliar spray, ZnUF = grain + shoot Zn uptake in nano-Zn sprayed treatments, ZnUC = grain + shoot Zn uptake in without nano-Zn foliar treatments and PE = physiological efficiency.

Statistical analysis

The entire data were tested for normality with Shapiro–Wilk test and Levene's homoscedasticity test ($p < 0.05$) which showed that data were to be normally distributed ($W \geq 0.90$). Then, data were subjected to analysis of variance (F test). Foliar nano-Zn doses, PGPBs inoculations and their interactions were considered fixed and replication was random effect in the model. When a main effect or interaction was observed significant by F test ($p \leq 0.05$), then Tukey test ($p \leq 0.05$) was used for means comparison of nano-Zn spray and Scott Knott test ($p \leq 0.05$) for PGPBs inoculations using ExpDes package of R software [57].

The Pearson correlation analysis ($p \leq 0.05$) was calculated and heatmap was created using corrplot package of "color" and "cor.mtest" functions to calculate coefficients and evaluate relationship among growth, yield, nutritional, biochemical and metabolic attributes of maize using R software [57].

Results

Growth and grain yield of common bean

Plant height of common bean was positively influenced by nano-Zn foliar spray and co-inoculation with plant growth-promoting bacteria (PGPBs) in 2019, while their effect in 2020 was not significant (Table 2). Nano-Zn foliar application at a dose of 1.5 kg ha^{-1} increased plant height by 6.4% as compared to control in first cropping season. In addtion, co-inoculation with *R. tropici* + *B. subtilis* was observed with increasing plant height by 9.7% as compared to without inoculation treatments in 2019 crop season. The interaction of nano-Zn foliar spray and inoculations with PGPBs were not significant in both cropping seasons (Table 2).

Shoot dry matter of common bean was significantly influenced by nano-Zn foliar spray and inoculation with PGPBs, whereas their interaction was not significant in both crop seasons (Table 2). Shoot dry matter was increased by 2.8% with application of 1.5 kg ha^{-1} of foliar nano-Zn in 2019, while an increase of 2.7% was observed with 3 kg ha^{-1} of foliar nano-Zn application, which was statistically at per with 1.5 kg ha^{-1} of foliar nano-Zn in 2020 as compared without nano-Zn foliar application treatments. In addtion, co-inoculation with *R. tropici* + *B. subtilis* increased shoot dry matter of common bean by 11.8% and 9.2% in first and second crop seasons, respectively, in comparison of without inoculation treatments (Table 2).

The effect of of nano-Zn foliar doses and inoculations with PGPBs and their interaction were significant for grain yield of common bean in both 2019 and 2020 cropping seasons (Table 2). The treatments with co-inoculation of *R. tropici* + *B. subtilis* at a dose of 1.5 kg ha^{-1} of nano-Zn foliar application were observed with higher grain yield as compared to no-inoculation and other inoculations treatments in 2019 and 2020 common bean cropping seasons (Fig. 3A, B). The treatments with nano-Zn foliar application and inoculations with PGPBs were observed with greater grain yield as compared to without inoculation and Zn application. The treatments with 1.5 kg ha^{-1} of nano-Zn foliar application was observed with greater grain yield under all inoculations treatments as compared to other Zn doses (Fig. 3A, B). The treatments without inoculations of PGPBs and nano-Zn foliar

Table 2 Plant height, shoot dry matter and grain yield of common bean as a function of plant growth-promoting bacteria and nano-Zn foliar doses in 2019 and 2020 cropping seasons

	Plant height (cm)		Dry matter (kg ha^{-1})		Grain yield (kg ha^{-1})	
	2019	2020	2019	2020	2019	2020
Inoculations (I)						
Control (no-inoculation)	93 ± 4.25 d	93 ± 4.47	3861 ± 238 e	3908 ± 165 d	3521 ± 424	4076 ± 329
R. tropici	95 ± 5.25 d	92 ± 7.58	3982 ± 137 d	3982 ± 151 d	3761 ± 177	4199 ± 240
R. tropici + *A. brasilense*	98 ± 4.83 c	93 ± 8.29	4081 ± 109 c	4070 ± 181 c	3902 ± 332	4347 ± 290
R. tropici + *B. subtilis*	102 ± 3.37 a	97 ± 6.92	4318 ± 102a	4265 ± 148 a	4196 ± 244	4602 ± 145
R. tropici + *P. fluorescens*	100 ± 3.35 b	93 ± 7.13	4157 ± 188 b	4120 ± 172 b	3999 ± 277	4452 ± 279
R. tropici + *A. brasilense* + *B. Subtilis*	98 ± 4.42 c	91 ± 3.76	4036 ± 168 c	4052 ± 187 c	3860 ± 427	4340 ± 373
R. tropici + *A. brasilense* + *P. fluorescens*	96 ± 3.48 c	91 ± 4.77	3948 ± 121 d	4018 ± 135c	3708 ± 251	4298 ± 230
Nano-Zn folair spray (kg ha^{-1})						
0.0	94 c	90.6	4007 b	3961 b	3530	4153
1.5	100 a	93.8	4119 a	3146 a	4097	4533
3.0	98 b	94.3	4041 b	4071 a	3921	4305
F values						
I	13**	1.3^{ns}	67**	9.9**	38**	17**
Nano-Zn	31**	2.8^{ns}	23**	15.9**	161**	50**
I × nano-Zn	0.24^{ns}	1.0^{ns}	1.4^{ns}	0.15^{ns}	3.2**	2.5*
CV (%)	3	7	2	3.1	3	3.3

Means in the column followed by similar letters are statistically not different by Tukey test ($p \leq 0.05$) for foliar nano-Zn dose and Scott Knott test for PGPBs ($p \leq 0.05$). ** and *—significant at $p < 0.01$ and $p < 0.05$, respectively, while ^{ns}—non-significant by F test. CV = Coefficient of variance. ($n = 4$ replicates)

application was observed with the lowest grain yield in 2019 and 2020 in relation to other treatments (Fig. 3A, B).

Chlorophyll index and protein content

Leaf chlorophyll index (LCI) was significantly influenced by co-inoculation and nano-Zn foliar application, while their interactive effect was not significant in both cropping seasons (Table 3). All the treatments with inoculation and co-inoculations increased LCI in both cropping seasons, when compared with control. Co-inoculation with *R. tropici* + *B. subtilis* increased LCI by 16.9% and 15.2% as compared to no-inoculation in first and second cropping season. Foliar nano-Zn application at a dose of 1.5 kg ha^{-1} increased LCI by 24.0% and 14.9% as compared to without nano-Zn application in 2019 and 2020 cropping season, respectively.

Inoculation and co-inoculation with PGPBs increased total protein in 2019 and 2020 cropping seasons. The foliar nano-Zn application increased protein content only in the second cropping season, while the interactive effect of inoculations and foliar nano-Zn application in both seasons was not significant (Table 3). Total protein was increased by 16.32% with co-inoculation of *R. tropici* + *A. brasilense* in 2019, which was statistically not different from the rest of co-inoculation treatment except *R. tropici* + *A. brasilense* + *B. subtilis* as compared to control. In addition, co-inoculation with *R. tropici* + *B. subtilis* increased crude protein by 16.27% in 2020 cropping season, which was statistically not different from the treatments with co-inoculation as compared to control. Foliar nano-Zn application at a dose of 1.5 kg ha^{-1} increased total protein by 3.1% and 6.2% as compared to without foliar nano-Zn application in 2019 and 2020 cropping season, respectively.

Shoot and grain Zn nutrition, and Zn daily intake

Shoot Zn concentration of common bean was increased with nano-Zn foliar doses and co-inoculations with PGPBs, while the interactions were not significant in both 2019 and 2020 (Table 4). Nano-foliar Zn application at the dose of 1.5 kg ha^{-1} increased shoot Zn conentration of common bean by 31.3% and 14.4% in 2019 and 2020 cropping season in relation to the treatments without nano-Zn foliar application. Shoot Zn concentration was increased by 38.2% and 23.6% with co-inoculation of *R. tropici* + *B. subtilis* in 2019 and 2020 common bean crop seasons, respectively, as compared to un-inoculated treatments.

The interaction of nano-foliar Zn doses and inoculation with PGPBs was significant for grain Zn concentration in both 2019 and 2020 common bean crop seasons (Table 4). The treatments applied with 1.5 kg ha^{-1} foliar nano-Zn in combination with co-inoculation of *R.*

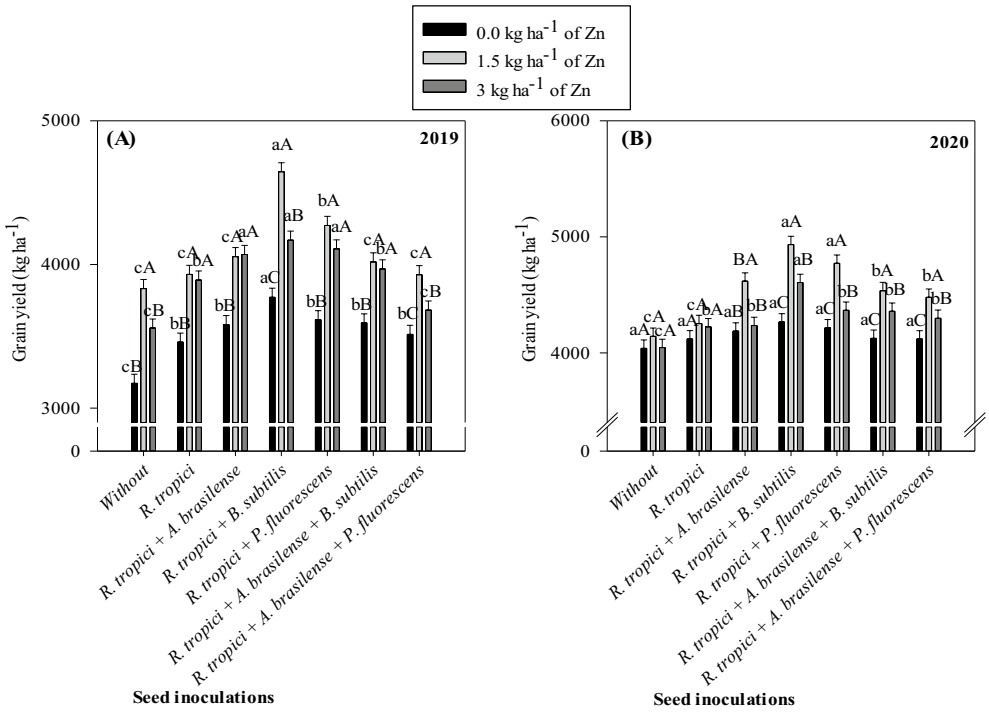


Fig. 3 Grain yield of common bean in 2019 (**A**) and 2020 (**B**) cropping seasons as a function of foliar nano-Zn application and co-inoculations of plant growth-promoting bacteria. The upper case letters are used for the interactions of PGPBs inoculations within each dose of foliar nano-Zn application, whereas lower case letters are used for the interaction of foliar nano-Zn doses within each inoculation treatment. The identical alphabetic letters are statistically similar with each other as analyzed by Tukey test for foliar Zn doses ($p < 0.05$) and Scott–Knott test for PGPBs inoculations ($p < 0.05$) in 2019 and 2020, respectively. Error bars indicate standard error of the means ($n = 4$ replicates)

tropici + *B. subtilis* were observed with higher grain Zn concentration in 2019 and 2020 cropping seasons as compared with all other treatments (Fig. 4A, B). The treatments with 1.5 kg ha^{-1} foliar nano-Zn was observed with higher grain concentration regardless of the inoculations. The treatments with no foliar nano-Zn application and without inoculation was observed with lowest grain Zn concentration in 2019, while 3 kg ha^{-1} of foliar nano-Zn and without inoculation treatments were noted with lowest grain Zn concentration in 2020 as compared to other treatments (Fig. 4A, B).

Nano-Zn foliar doses and inoculations with PGPBs had positively improved shoot Zn uptake in 2019 and 2020 cropping seasons. The interaction of nano-Zn foliar doses and inoculations with PGPBs for shoot Zn uptake was significant in first season while non-significant in the second season (Table 4). Shoot Zn uptake was improved by 35.8% and 19.6% with foliar nano-Zn at a dose of 1.5 kg ha^{-1} in first and second cropping seasons, respectively, in relation to without nano-Zn fertilization. The treatments with co-inoculation of *R. tropici* + *B. subtilis* improved shoot Zn uptake by 55.0% and 34.6% in 2019 and 2020 resepctively as compared to without inoculation treatments. In addition, the intreactive effect of 1.5 kg ha^{-1} nano-Zn foliar application and co-inoculation of *R. tropici* + *B. subtilis* was observed with higher shoot Zn uptake in first common bean growing season as compared with other treatments (Fig. 4C). In general, the treatments with foliar nano-Zn application and inoculations of PGPBs were observed with higher shoot Zn uptake as compared to control. The treatments without foliar application of nano-Zn and without inoculation were observed with low shoot Zn uptake as compared to other treatments (Fig. 4C).

The single and interaction effects of nano-Zn foliar doses and inoculations with PGPBs were positive for grain Zn uptake in both studied years (Additional file 1: Table S1). Grains Zn uptake was improved with foliar nano-Zn at a dose of 1.5 kg ha^{-1} in association with co-inoculation of *R. tropici* + *B. subtilis* in both common bean copping seasons (Fig. 4D, E). Interesting, the treatments with foliar nano-Zn application and inoculations with PGPBs were observed with higher grain Zn uptake as compared to control. In addition, treatments without foliar nano-Zn application and without inoculation were observed with low grain Zn uptake in 2019 (Fig. 4D), while foliar nano-Zn at a dose of 3 kg ha^{-1}

Table 3 Leaf chlorophyll index and total protein of common bean as a function of plant growth-promoting bacteria and nano-Zn foliar doses in 2019 and 2020 cropping seasons

Treatments	Chlorophyll index (g kg^{-1})		Protein content (g kg^{-1})	
	2019	2020	2019	2020
Inoculations (I)				
Control (no-inoculation)	32.2 ± 3.6 b	33.0 ± 3.1 b	164.2 ± 22.0 c	168.2 ± 19.2 b
R. tropici	36.2 ± 7.9 a	35.3 ± 4.7 a	189.3 ± 17.9 a	195.5 ± 13.0 a
R. tropici + *A. brasilense*	34.9 ± 5.4 a	36.3 ± 4.4 a	191.0 ± 12.5 a	191.3 ± 15.6 a
R. tropici + *B. subtilis*	37.6 ± 3.6 a	38.0 ± 1.9 a	184.73 ± 11.6 a	195.53 ± 8.9 a
R. tropici + *P. fluorescens*	37.4 ± 2.7 a	36.7 ± 2.3 a	183.5 ± 8.9 a	192.6 ± 8.0 a
R. tropici + *A. brasilense* + *B. Subtilis*	35.0 ± 4.9 a	36.0 ± 2.5 a	175.5 ± 9.9 b	189.7 ± 11.1 a
R. tropici + *A. brasilense* + *P. fluorescens*	36.3 ± 2.9 a	35.4 ± 2.6 a	182.8 ± 8.2 a	194.6 ± 9.0 a
Nano-Zn foliar spray (kg ha^{-1})				
0.0	31.4 c	33.1 c	178.8 a	183.9 c
1.5	38.9 a	38.0 a	184.3 a	195.2 a
3.0	36.6 b	36.4 b	181.6 a	189.9 b
F values				
I	3.8*	5.04**	7.02**	23.17**
Nano-Zn	37.4**	31.4**	1.51^{ns}	18.34**
I × nano-Zn	0.85^{ns}	0.58^{ns}	0.12^{ns}	2.04^{ns}
CV (%)	9.4	6.6	6.6	3.79

Means in the column followed by similar letters are statistically not different by Tukey test ($p \leq 0.05$) for foliar nano-Zn dose and Scott Knott test for PGPBs ($p \leq 0.05$). ** and *—significant at $p < 0.01$ and $p < 0.05$, respectively, while ^{ns}—non-significant by F test. CV = Coefficient of variance. ($n = 4$ replicates)

Table 4 Zinc concentration and accumulation in shoot and grains tissues of common beans as a function of plant growth-promoting bacteria and nano-Zn foliar doses in 2019 and 2020 cropping seasons

Treatments	Shoot Zn concentration (mg kg^{-1})		Grain Zn concentration (g ha^{-1})		Shoot Zn uptake (g ha^{-1})	
	2019	2020	2019	2020	2019	2020
Inoculations (I)						
Control (no-inoculation)	30.6 ± 6.7 d	39.9 ± 5.0 d	43.6 ± 5.1	51.7 ± 3.7	118 ± 34.5	156 ± 26.5 d
R. tropici	32.5 ± 5.3 c	42.0 ± 3.9 c	44.9 ± 2.5	54.6 ± 2.3	130 ± 25.1	167 ± 21.6 c
R. tropici + *A. brasilense*	36.6 ± 6.7 b	44.7 ± 4.9 b	47.6 ± 4.7	56.3 ± 3.8	149 ± 31.2	182 ± 25.8 b
R. tropici + *B. subtilis*	42.3 ± 4.5 a	49.3 ± 3.6 a	51.9 ± 2.1	60.6 ± 3.2	183 ± 21.1	210 ± 19.6a
R. tropici + *P. fluorescens*	38.3 ± 5.4 b	45.8 ± 5.2 b	48.0 ± 5.4	58.2 ± 3.6	159 ± 28.5	189 ± 28.7 b
R. tropici + *A. brasilense* + *B. Subtilis*	34.3 6.8 c	42.1 ± 4.5 c	45.6 ± 4.5	55.1 ± 4.0	139 ± 34.6	171 ± 25.9 c
R. tropici + *A. brasilense* + *P. fluorescens*	32.7 ± 5.2 c	40.8 ± 2.8 d	44.1 ± 2.3	54.6 ± 2.6	129 ± 25.2	164 ± 14.1 c
Nano-Zn folair spray (kg ha^{-1})						
0.0	30.7 c	41.0 c	44.2	54.3	123	163 c
1.5	40.3 a	46.9 a	49.5	57.7	167	195 a
3.0	34.9 b	42.6 b	45.8	55.7	141	174 b
F values						
I	31*	31*	43.5**	44**	57**	39**
Nano-Zn	105*	62*	88.9**	38**	129**	71**
I × nano-Zn	1.6^{ns}	0.74^{ns}	4.9**	2.3*	3*	0.9^{ns}
CV (%)	7	4.7	3.3	2.6	7	6

Means in the column followed by similar letters are statistically not different by Tukey test ($p \leq 0.05$) for foliar nano-Zn dose and Scott Knott test for PGPBs ($p \leq 0.05$). ** and *—significant at $p < 0.01$ and $p < 0.05$, respectively, while ^{ns}—non-significant by F test. CV = Coefficient of variance. ($n = 4$ replicates)

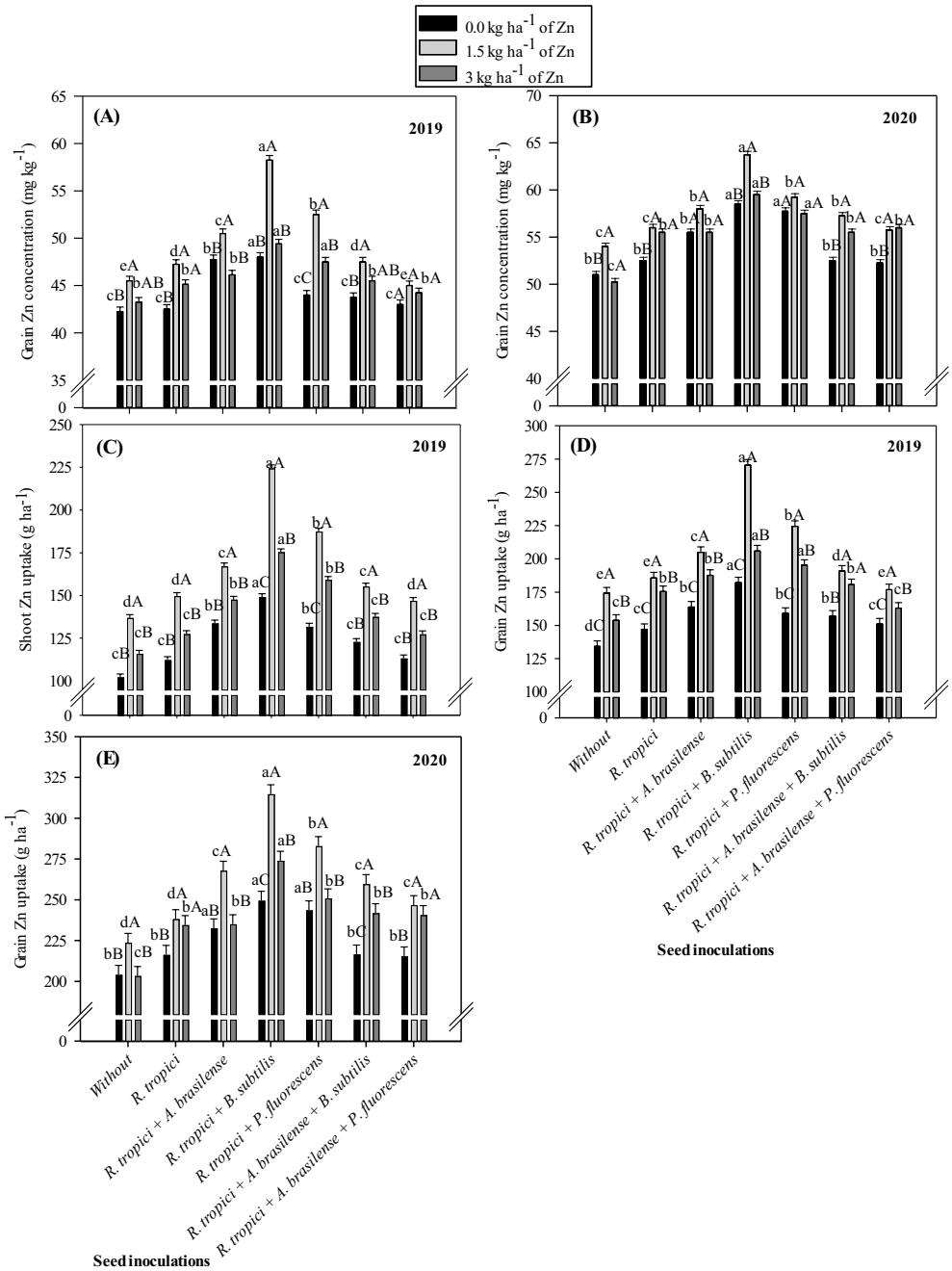


Fig. 4 Grain zinc (Zn) concentration in 2019 (**A**) and 2020 (**B**), shoot Zn uptake in 2019 (**C**), grain Zn uptake in 2019 (**D**) and 2020 (**E**) common bean cropping seasons as a function of foliar nano-Zn application and co-inoculations of plant growth-promoting bacteria. The upper case letters are used for the interactions of PGPBs inoculations within each dose of foliar nano-Zn application, whereas lower case letters are used for the interaction of foliar nano-Zn doses within each inoculation treatment. The identical alphabetic letters are statistically similar with each other as analyzed by Tukey test for foliar Zn doses ($p < 0.05$) and Scott–Knott test for PGPBs inoculations ($p < 0.05$) in 2019 and 2020, respectively. Error bars indicate standard error of the means ($n = 4$ replicates)

and without inoculation was noted with low Zn uptake in common bean grains in 2020 as compared to other treatments (Fig. 4E). In general, the treatments without inoculations of PGPBs were observed with low grain Zn uptake regardless of the foliar nano-Zn applications in both cropping seasons (Fig. 4D, E).

Foliar nano-Zn doses and inoculation with PGPBs had positively increased partitioing of Zn to common bean grains, while their interaction was not significant in 2019 and 2020 cropping seasons (Additional file 1: Table S1). Foliar nano-Zn application at a dose of 1.5 kg ha^{-1} increased Zn partitioning index by 7.0% and 11.3% in 2019 and 2020, respectively, as comapred to control. The treatments with co-inoculation of *R. tropici* + *B. subtilis* were observed with 13.9% and 11.4% higher Zn partitioning to common bean grains in 2019 and 2020, respectively, as compared to without inoculations treatments.

The interactions and single effect of foliar nano-Zn doses and inoculation with PGPBs were significant for estimated daily Zn intake in Brazil with consumption of common bean grains in 2019 and 2020 (Additional file 1: Table S1). The treatments with foliar nano-Zn application at a dose of 1.5 kg ha^{-1} in association with co-inculation of *R. tropici* + *B. subtilis* were observed with greater daily Zn intake in first and second cropping seasons, respectively, in relation to control (Fig. 5A, B). The treatments with foliar nano-Zn application and inoculation with PGPBs were observed with greater daily Zn intake as compared to control treatments. The treatments in the absence of foliar nano-Zn application were noted with low daily Zn intake regaless of the inoculation in both studied years. However, least Zn intake with daily consumption of common bean grains was observed without foliar nano-Zn fertilization and inoculation in 2019 cropping season (Fig. 5A). In addition, foliar nano-Zn at a dose of 3 kg ha^{-1} and without inoculation was observed with lowest daily Zn intake in 2020 crooping season, which was statistically similar with co-application of 0.0 kg ha^{-1} foliar nano-Zn and without inoculation, inoculation with *R. tropici*, co-inoculation with *R. tropici* + *A. brasilense* + *B. subtilis* and *R. tropici* + *A. brasilense* + *P. fluorescens* (Fig. 5B).

Zinc use efficiencies

Foliar nano-Zn and inoculation with PGPBs, and their interactions were significant for Zn use efficiency in both 2019 and 2020 cropping seasons (Additional file 1: Table S2). The co-application of foliar nano-Zn at a dose of 1.5 kg ha^{-1} along with inoculation of *R. tropici* + *B. subtilis* increased Zn use efficiency as compared, when compared with 3 kg ha^{-1} foliar nano-Zn and inoculation

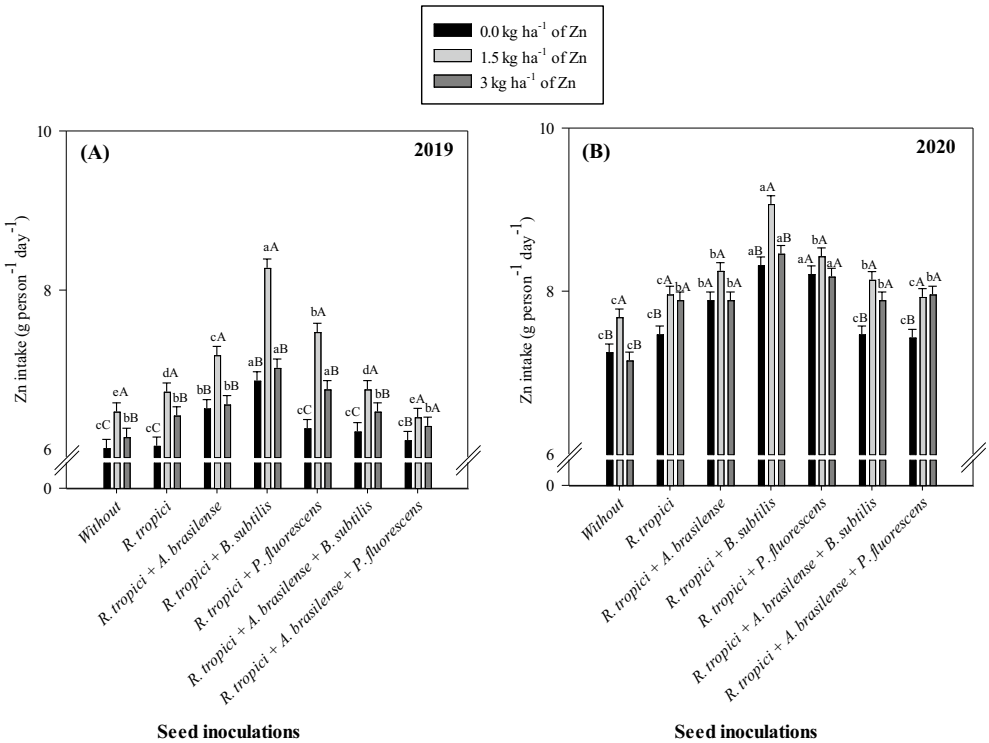


Fig. 5 Daily zinc (Zn) intake with common bean grains in 2019 (**A**) and 2020 (**B**) cropping seasons as a function of foliar nano-Zn application and co-inoculations of plant growth-promoting bacteria. The upper case letters are used for the interactions of PGPBs inoculations within each dose of foliar nano-Zn application, whereas lower case letters are used for the interaction of foliar nano-Zn doses within each inoculation treatment. The identical alphabetic letters are statistically similar with each other as analyzed by Tukey test for foliar Zn doses ($p < 0.05$) and Scott–Knott test for PGPBs inoculations ($p < 0.05$) in 2019 and 2020, respectively. Error bars indicate standard error of the means ($n = 4$ replicates)

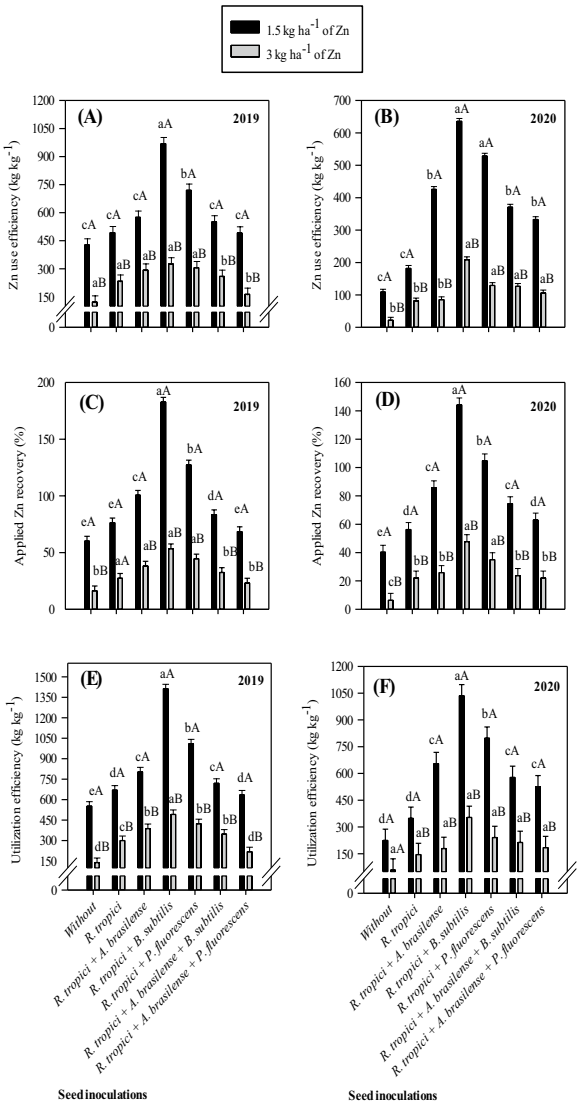


Fig. 6 Zinc (Zn) use efficiency (**A**, **B**), applied Zn recovery (**C**, **D**), and utilisation efficiency (**E**, **F**) in 2019 and 2020 common bean cropping seasons, respectively, as a function of foliar nano-Zn application and co-inoculations of plant growth-promoting bacteria. The upper case letters are used for the interactions of PGPBs inoculations within each dose of foliar nano-Zn application, whereas lower case letters are used for the interaction of foliar nano-Zn doses within each inoculation treatment. The identical alphabetic letters are statistically similar with each other as analyzed by Tukey test for foliar Zn doses ($p < 0.05$) and Scott–Knott test for PGPBs inoculations ($p < 0.05$) in 2019 and 2020, respectively. Error bars indicate standard error of the means ($n = 4$ replicates)

other PGPBs in both cropping seasons (Fig. 6A, B). The lowest Zn use efficiency in both common bean cropping seasons was observed at 3 kg ha^{-1} foliar nano-Zn application, under no-inoculation treatments (Fig. 6A, B).

Agro-physiological efficency (APE) of common bean was positively influenced by foliar nano-Zn doses and inoculation with PGPBs only in 2019, while the treatments effect and interaction were not significant in 2020 cropping season (Additional file 1: Table S2). The treatments with foliar nano-Zn at a dose of 3 kg ha^{-1} increased APE by 19% in 2019 cropping season. The treatments with inoculation of *R. tropici* were observed with higher APE, which was statistically similar with triple inoculation of *R. tropici* + *A. brasilense* + *B. subtilis* and *R. tropici* + *A. brasilense* + *P. fluorescens,* co-inoculation of *R. tropici* + *A. brasilense,* and without inoculation treatments in relation to other inoculations (Additional file 1: Table S2).

The interactions and treatment effect of foliar nano-Zn and inoculation with PGPBs were significant for applied Zn recovery (AZnR) in 2019 and 2020 (Additional file 1: Table S2). The treatments with foliar nano-Zn at a dose of 1.5 kg ha^{-1} along with co-inoculation of *R. tropici* + *B. subtilis* in both cropping seasons as compared to other treatments (Fig. 6C, D). Interestingly, AZnR was increased with 1.5 kg ha^{-1} foliar nano-Zn application under inoculation, co-inoculation and without inoculation treatments as compared with 3 kg ha^{-1} foliar nano-Zn application. The lowest AZnR in the first cropping season was observed without inoculation and foliar nano-Zn application at a dose of 3 kg ha^{-1} as compared to other treatments (Fig. 6C, D).

Foliar nano-Zn doses, inoculation with PGPBs and their interaction had positively improved Zn utilisation efficiency (UE) of common bean in both studied years (Additional file 1: Table S2). The highest Zn utilization efficiency was observed with foliar nano-Zn at a dose of 1.5 kg ha^{-1} in combination with co-inoculation of *R. tropici* + *B. subtilis* in both 2019 and 2020 cropping seasons as compared to other treatments (Fig. 6E, F). In general, the treatments with 1.5 kg ha^{-1} foliar nano-Zn application performed better within all inoculation, co-inoculation and without inoculation treatments as compared with 3 kg ha^{-1} foliar nano-Zn application. The lowest UE was noted with 3 kg ha^{-1} foliar nano-Zn application under without inoculation treatments as compared to other treatments (Fig. 6E, F).

Pearson's correlation

There were positive and significant correlations between zinc use efficiency and plant height, shoot dry matter, shoot–grain Zn concentration and accumulation, Zn partitioning index, Zn intake in Brazil under common beans cultivation in 2019 regardless the treatments applied (Fig. 7A). A positive correlation was observed between Zn partitioning index and shoot and grain Zn accumulation, applied Zn recovery, shoot dry matter, and grain yield of common beans in 2020 cropping season (Fig. 7B).

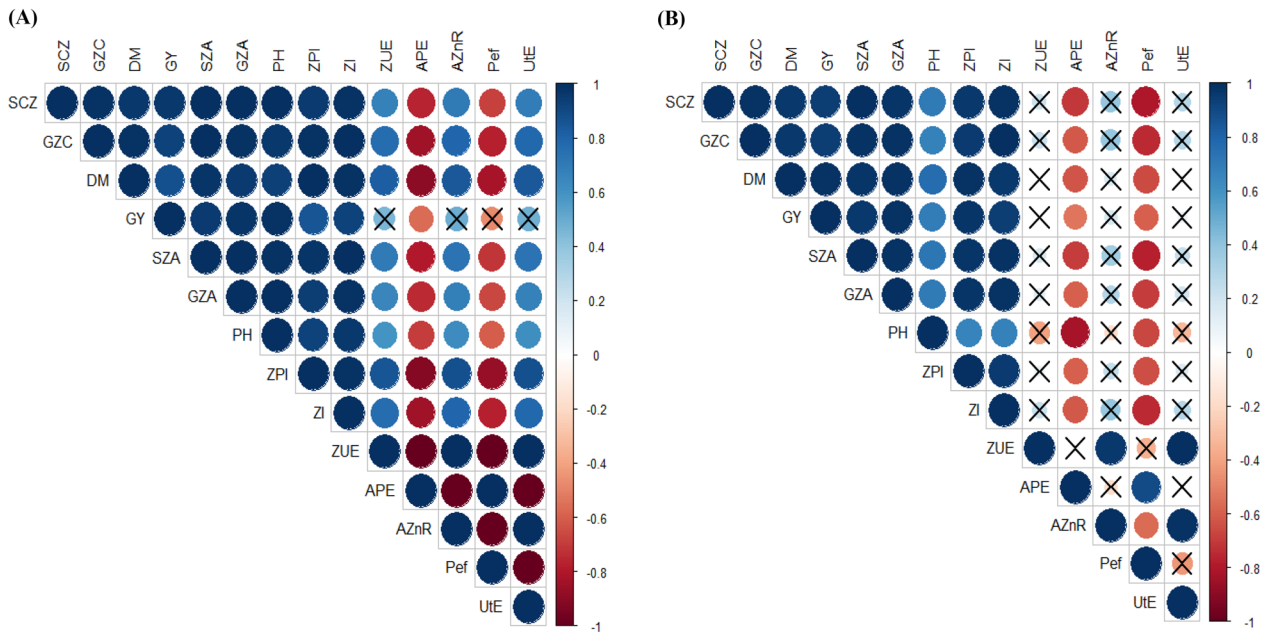


Fig. 7 Heat-map color scale indicating Pearson's correlation among evaluated attributes of common beans in response to plant growth-promoting bacteria and foliar nano-ZnO applications in 2019 (**A**) and 2020 (**B**) cropping seasons. * × = indicates a non-significant relationship ($p \leq 0.05$). *PH* plant height, *DM* shoot dry matter, *NLC* number of row per cob, *NGC* number of grains per cob, *HGs* 100-grains weight, *GY* grain yield, *SZnA* shoot Zn accumulation, *GZnA* grain Zn accumulation, *ZnUE* Zn use efficiency, *AZnR* applied Zn recovery, *APE* agro-physiological efficiency, *UE* utilization efficiency

Discussion

Several sustainable strategies have been adapted to maintain growth, productivity and quality of crop plants under harsh tropical conditions [43]. In this context, nano-Zn fertilizers have attracted the attention by providing nutrients to plants in more technical manner to minimize leaching and adsorption as well as improving fertilizer efficiency and grain tissue assimilation [11, 58]. Plant growth-promoting bacteria (PGPBs) are being used as sustainable and eco-friendly approach to enhance Zn availability to plants through several direct and indirect mechanisms [29, 30, 33], leading to better grains biofortification [59, 60]. Thus, the positive interaction between foliar nano-Zn and inoculation/co-inoculation with PGPBs (Fig. 7) endorsed the hypothesis of the present study.

The present results indicated that foliar nano-Zn and co-inoculation with *R. tropici* + *B. subtilis* were observed with taller plants and greater shoot dry matter (Table 2), and higher grain yield of common bean (Fig. 3). The increase might be driven by the direct mechanisms (nutrients solubilization and availaibility, and phytohormones production) of PGPBs that could enhance nutrients availability by either fixation, solubilization or alteration of hormonal activities and Zn involvement in maintaining cell division, elongation and photosynthesis and regulatory co-factor in protein synthesis [25, 61]. Previous studies have reported that inoculation of PGPBs either alone or with Zn could alter different enzymatic activities of soil and plant to enhance plant growth, yield and biofortification of edible tissues [27, 62]. Plant growth-promoting bacteria could increase vegetative growth of crop plants that contribute to higher productivity at later reproductive stage [63]. In addition, Zn has critical role in regulation of cell multiplication and elongation as well as several biochemical function of plant that could ultimately increase shoot dry matter and productivity of different crops [64–66]. The co-application of Zn and PGPBs could increase pod formation through pollen development that could contribute to better Zn use efficiency and consequently greater growth and yield of different crops [67–69].

Zinc is considered as one of the key element of photosynthesis that can help in the synthesis of chlorophyll and protein, which might increase biomass accumulation in common beans [70, 71]. The integrated use of PGPBs and zinc fertilizer has also been reported for the improvement of biochemical and yield attributes of crops [43, 67]. Hence, the current results exhibited that combined use of PGPBs and nano-Zn fertilizer increased leaf chlorophyll index and total protein of common bean (Table 3). Zinc is involved in the improvement of different biochemical processes of plant, such as improving chlorophyll content and protein synthesis that may results in greater growth

and development of crops [64, 67]. Several studies reported that different bacterial strains could stimulate the optimization of stomatal conductance and nutrient transportation, which may increase chlorophyll and protein content in plants [72, 73].

Zinc deficiency is an alarming issue in agricultural soils that caused stagnation in agronomic biofortification and productivity [65, 66]. However, Zn foliar spray could be an efficient alternative strategy to cope Zn edaphic deficiency by ameliorating its bioavailability in edible tissue, leading to biofortification [11]. In this context, the present study indicated that treatments with foliar Zn and inoculation of PGPB were observed with higher Zn concentration and uptake in shoot and grains of common bean (Table 4). Foliar nano-Zn and co-inoculation with *R. tropici* + *B. subtilis* were noted with higher shoot and grain Zn concentration (Table 4, Fig. 4A, B) and also shoot and grain Zn uptake (Fig. 4C, D, E). This might be due to the influence of applied inoculants in the roots rhizosphere that may help the host plant to articulate root architecture and stimulate nutrients availability for better uptake [74]. In addition, PGPB have the ability to produce organic acids, chelating agents and siderophores that could not only promote plant growth and productivity but also increasing Zn assimilation to the edible tissue to sustain biofortification [75, 76]. The co-application of PGPB with foliar/soil Zn could reduce phytic acid concentration in the edible tissue, thus contributing to higher Zn concentration in embryo, aleurone, endosperm and whole grains of cereal [77].

Zinc partitioning index and daily Zn estimated intake in common bean grains were improved with foliar nano-Zn fertilization along with co-inoculation of PGPBs (Additional file 1: Table S1 and Fig. 5A, B). It might be due to the positive interaction of plants and microbes that could biosynthesis several compounds including phenolic acid, siderophores, organic acids and phyto-hormones to contribute different biochemical and metabolic functions of plants [77, 78]. In addition, foliar Zn has also been reported a rapid strategy that could improve translocation and remobilization of applied Zn into newly generated grains to deal with human malnutrition [64, 79]. It has also reported in previous studied that Zn and PGPB contributed to the biosynthesis of nodules and leg-hemoglobin, where root nodulation could improve transportation of Zn in sucrose from leaves to nodules and leg-hemoglobin could optimize plant growth [26, 80].

The combined application of Zn with PGPBs has the ability to increase Zn efficiency by dissolving carbonates and oxides of Zn as compared to individual Zn fertilization [81]. Zinc efficiencies are being defined by the availability of Zn in edible tissues in Zn deficient soils [56], where foliar Zn fertilization is considered a better option for biofortification and higher yield [11]. Therefore, the present results indicated that combined application of foliar nano-Zn with co-inoculations of PGPBs improved Zn use efficiency (Fig. 6A, B), agro-physiological efficiency (Additional file 1: Table S2), applied Zn recovery (Fig. 6C, D), and utilization efficiency (Fig. 6E, F) in tropical savannah of Brazil. The possible reason of higher Zn efficiencies might be due to higher Zn concentration and uptake in shoot and grains (Table 4 and Additional file 1: Table S1) and greater biomass and grain yield (Table 2). Plant growth-promoting bacteria adapt several mechanism including solubilization of nutrients, nitrogen fixation, organic acids production and enzymatic activities that could possibly increase nutrient absorption and relocalization to stem and grains tissues [31, 72]. It has also previously reported that Zn in combination with diazotrophic bacteria could improve bioavailability and translocation of Zn to shoot and grains, leading to higher Zn use efficiencies [26, 27]. Hence, current results are forefront step to understand influence of foliar nano-Zn fertilization and inoculation with PGPBs on growth, yield and biofortification of common bean, emphasizing on the integrated use of foliar ZnO and PGPBs to ameliorate Zn accumulation, yield and Zn use efficiencies in tropical savannah.

Conclusions

Foliar nano-zinc (Zn) fertilization is an efficient and rapid alternative option of nutrients delivery that enhance plant performance and this nutrient use efficiency in a sustainable manner. The multifaceted functions of plant growth-promoting bacteria (PGPBs) to increase productivity and nutrition of crop plants while maintaining sustainability of agriculture is not deniable. Hence, it has derived from the current results that foliar nano-Zn application along with co-inoculation of PGPBs improved plant growth, shoot dry matter, grain yield, leaf chlorophyll index, total protein and grain nutrition of common bean. Zinc partitioning index and estimated daily Zn intake as well as Zn efficiencies including use efficiency, agro-physiological efficiency, applied Zn recovery and utilization efficiency were also increased with foliar nano-Zn and co-inoculation of PGPBs in common bean cultivation. It has concluded that foliar nano-Zn application at a dose of 1.5 ha^{-1} along with co-inoculation of *R. tropici* + *B. subtilis* was observed the most effective for improving performance and grain yield, Zn nutrition and its efficiencies, leading to biofortification of common bean grains in tropical savannah. Therefore, it is recommended that applying nano-Zn via foliar along with co-inoculation of PGPBs could improve productivity and nutrition of common bean in more sustainable manner.

The present study showed integrated use of synthetic fertilizers with PGPBs; however, we believe that it is not remain an ambitious to highlight that PGPBs/bio-stimulants have the potential to replace synthetic fertilizers in near future. The molecular and physiological behavior of these PGPBs would attract more attention of agriculturists, which will bring the technology out of developing stage and can highly improve sustainability and agriculture under harsh environmental conditions.

Abbreviations

PGPBs	Plant growth-promoting bacteria
ZnO	Nano-zinc oxide
CFU	Colony forming units
ZPI	Zinc partitioing index
ZnUE	Zinc use efficiency
APE	Agro-physiological efficiency
AZnR	Applied Zn recovery
UE	Utilisation efficiency
DTPA	Diethylene triamine penta-acetic acid
CEC	Cation exchange capacity
V	Bases saturation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00440-5.

Additional file 1: Table S1. Zinc partitioning index (ZPI) and estimated Zn intake in Brazil in common bean grains as a function of plant growth -promoting bacteria and nano Zn foliar doses in 2019 and 2020 cropping seasons. **Table S2.** Zinc use efficiencies (Zinc use efficiency, agro-physiological efficiency, physiological efficiency, utilization efficiency and applied zinc recovery of common beans as a function of plant growth-promoting bacteria and nano Zn foliar doses in 2019 and 2020 cropping seasons.

Acknowledgements

The authors thanks São Paulo State University (UNESP) for providing technology and support as well as CNPq and TWAS for financial support.

Author contributions

Conceptualization: AJ and MCMTF; methodology: AJ, ESM; formal analysis and investigation: AJ, CEdSO, BHdL and GCF; writing—original draft preparation: AJ; writing—review and editing: MCMTF, FSG and AM; funding acquisition: AJ and MCMTF; resources: AJ, CEdSO; supervision: MCMTF. All authors have read and agreed to the published version of this manuscript.

Funding

This research received funding from The World Academy of Science (TWAS) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), first author's doctoral fellowship (CNPq/TWAS Grant Number: 166331/2018-0, and productivity research grant (award number: 311308/2020-1) of the corresponding author.

Data availability

The data sets generated during this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Meets ethical standards applicable to the research discipline.

Consent for publication

All authors agree to the publication of the work.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Plant Protection, Rural Engineering and Soils (DEFERS), São Paulo State University (UNESP), Ilha Solteira, SP 15385-000, Brazil. [2]Department of Plant Science, Food Technology and Socio-Economics, São Paulo State University (UNESP), Ilha Solteira, SP 15385-000, Brazil. [3]Embrapa Soja, Londrina 86085-981, Brazil. [4]Department of Agricultural Production Sciences, São Paulo State University (UNESP), Jaboticabal, SP 14884-900, Brazil. [5]Faculty of Agricultural Sciences and Technology–Campus Dracena, Department of Plant Production, São Paulo State University (UNESP), Dracena, Brazil.

Received: 4 May 2023 Accepted: 12 July 2023
Published online: 11 August 2023

References

1. CONAB - Companhia Nacional de Abastecimento. Bean crop 2020/2021. 2022. https://www.conab.gov.br/info-agro/safras/graos. Accessed 23 May 2022.
2. Rezende AA, Pacheco MTB, Silva VSND, Ferreira TAPDC. Nutritional and protein quality of dry Brazilian beans (*Phaseolus vulgaris* L.). Food Sci Technol. 2017;38:421–7. https://doi.org/10.1590/1678-457X.05917.
3. Yan Z, Xiong C, Liu H, Singh BK. Sustainable agricultural practices contribute significantly to one Health. J Sustain Agric Environ. 2022. https://doi.org/10.1002/sae2.12019.
4. FAO. The State of Food Security and Nutrition in the World 2018. Building Climate Resilience for Food Security and Nutrition. Rome: FAO. 2018. http://www.fao.org/3/I9553EN/i9553en.pdf.
5. Bouis H. Reducing mineral and vitamin deficiencies through biofortification: progress under HarvestPlus. In: Biesalski HK, Birner R, editors. Hidden hunger: strategies to improve nutrition quality, vol. 118. Basel: Karger Publishers; 2018. p. 112–22.
6. Silva VM, Nardeli AJ, de Carvalho Mendes NA, de Moura RM, Wilson L, Young SD, Broadley MR, White PJ, Dos Reis AR. Agronomic biofortification of cowpea with zinc: Variation in primary metabolism responses and grain nutritional quality among 29 diverse genotypes. Plant Physiol Biochem. 2021;162:378–87. https://doi.org/10.1016/j.plaphy.2021.02.020.
7. Haider MU, Hussain M, Farooq M, Ul-Allah S, Ansari MJ, Alwahibi MS, Farooq S. Zinc biofortification potential of diverse mungbean [*Vigna radiata* (L.) Wilczek] genotypes under field conditions. PLoS ONE. 2021;16(6):e0253085. https://doi.org/10.1371/journal.pone.0253085.
8. Moreira A, Moraes LA, dos Reis AR. The molecular genetics of zinc uptake and utilization efficiency in crop plants. Plant Micronutr Use Effic. 2018. https://doi.org/10.1016/B978-0-12-812104-7.00006-X.
9. WHO. World Health Organization 2021. The state of food security and nutrition in the world 2018: building climate resilience for food security and nutrition. 2021. https://www.fao.org/3/i9553en/i9553en.pdf Accessed 19 Sept 2022.
10. Mengist MF, Milbourne D, Griffin D, McLaughlin MJ, Creedon J, Jones PW, Alves S. Zinc uptake and partitioning in two potato cultivars: implications for biofortification. Plant Soil. 2021;463:601–13. https://doi.org/10.1007/s11104-021-04874-4.
11. Jalal A, Galindo FS, Freitas LA, de Oliveira CES, de Lima BH, Pereira ÍT, Ferraz GF, de Souza JS, da Costa KN, Nogueira TAR, Teixeira Filho MCM. Yield, zinc efficiencies and biofortification of wheat with zinc sulfate application in soil and foliar nanozinc fertilisation. Crop Pasture Sci. 2022;73:749–59. https://doi.org/10.1071/CP21458.
12. Yusefi-Tanha E, Fallah S, Rostamnejadi A, Pokhrel LR. Zinc oxide nanoparticles (ZnONPs) as a novel nanofertilizer: influence on seed yield and antioxidant defense system in soil grown soybean (*Glycine max* cv. Kowsar). Sci Total Environ. 2020;738:140240–1. https://doi.org/10.1016/j.scitotenv.2020.140240.
13. Afshar RK, Chen C, Zhou S, Etemadi F, He H, Li Z. Agronomic and economic response of bread wheat to foliar zinc application. Agronomy J. 2020;112(5):4045–56.
14. Fernández V, Brown PH. From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. Front Plant Sci. 2013. https://doi.org/10.3389/fpls.2013.00289.

15. Manivannan N, Aswathy S, Malaikozhundan B, Boopathi T. Nano-zinc oxide synthesized using diazotrophic *Azospirillum* improves the growth of mung bean, *Vigna radiata*. Int Nano Lett. 2021;11(4):405–15.
16. Su Y, Ashworth V, Kim C, Adeleye AS, Rolshausen P, Roper C, White J, Jassby D. Delivery, uptake, fate, and transport of engineered nanoparticles in plants: a critical review and data analysis. Environ Sci Nano. 2019;6(8):2311–31.
17. Weisany W, Mohammadi M, Tahir NA, Aslanian N, Omer DA. Changes in growth and nutrient status of maize (*Zea mays* L.) in response to two zinc sources under drought stress. J Soil Sci Plant Nutri. 2021;21(4):3367–77.
18. Wang XP, Li QQ, Pei ZM, Wang SC. Effects of zinc oxide nanoparticles on the growth, photosynthetic traits, and antioxidative enzymes in tomato plants. Biol Plant. 2018;62(4):801–8.
19. Munir T, Rizwan M, Kashif M, Shahzad A, Ali S, Amin N, Zahid R, Alam MFE, Imran M. Effect of zinc oxide nanoparticles on the growth and Zn uptake in wheat (*Triticum aestivum* L.) By seed priming method. Dig J Nanomater Biost. 2018;13(1):315–23.
20. Subbaiah LV, Prasad TN, Krishna TG, Sudhakar P, Reddy BR, Pradeep T. Novel effects of nanoparticulate delivery of zinc on growth, productivity, and zinc biofortification in maize (*Zea mays* L.). J Agric Food Chem. 2016;64(19):3778–88.
21. Drobek M, Frąc M, Cybulska J. Plant biostimulants: Importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress—a review. Agron. 2019;9(6):335.
22. Chouhan GK, Verma JP, Jaiswal DK, Mukherjee A, Singh S, de Araujo PAP, Liu H, Abd-Allah EF, Singh BK. Phytomicrobiome for promoting sustainable agriculture and food security: opportunities, challenges, and solutions. Microbiol Res. 2021;248:126763.
23. Rouphael Y, Colla G. Editorial: biostimulants in agriculture. Front Plant Sci. 2020;11:1–7. https://doi.org/10.3389/fpls.2020.00040.
24. Rossi L, Fedenia LN, Sharifan H, Ma X, Lombardini L. Effects of foliar application of zinc sulfate and zinc nanoparticles in coffee (*Coffea arabica* L.) plants. Plant Physiol Biochem. 2019;135:160–6. https://doi.org/10.1016/j.plaphy.2018.12.005.
25. Routray S, Kumari S, Borah B, Shelat H, Veena Khanna JPS. A review on Rhizobia and PGPRs interactions in legumes. J Pharm Innov. 2021;10(7):1448–57.
26. Ullah A, Farooq M, Nadeem F, Rehman A, Hussain M, Nawaz A, Naveed M. Zinc application in combination with zinc solubilizing *Enterobacter* sp MN17 improved productivity, profitability, zinc efficiency, and quality of desi chickpea. J Soil Sci Plant Nutri. 2020;20(4):2133–44.
27. Jalal A, Galindo FS, Boleta EHM, Oliveira CEDS, Reis ARD, Nogueira TAR, Moretti Neto MJ, Mortinho ES, Fernandes GC, Teixeira Filho MCM. Common bean yield and zinc use efficiency in association with diazotrophic bacteria co-inoculations. Agron. 2021;11(5):959.
28. Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, Prigent-Combaret C. Plant growth-promoting rhizobacteria and root system functioning. Front Plant Sci. 2013;4:356.
29. Yahaghi Z, Shirvani M, Nourbakhsh F, Pueyo JJ. Uptake and effects of lead and zinc on alfalfa (*Medicago sativa* L.) seed germination and seedling growth: role of plant growth promoting bacteria. S Afr J Bot. 2019;124:573–82. https://doi.org/10.1016/j.sajb.2019.01.006.
30. Patel JK, Archana G. Diverse culturable diazotrophic endophytic bacteria from Poaceae plants show cross-colonization and plant growth promotion in wheat. Plant Soil. 2017;417(1):99–116. https://doi.org/10.1007/s11104-017-3244-7.
31. Hakim S, Naqqash T, Nawaz MS, Laraib I, Siddique MJ, Zia R, Mirza MS, Imran A. Rhizosphere engineering with plant growth promoting microorganisms for agriculture and ecological sustainability. Front Sustain Food Syst. 2021;5:16. https://doi.org/10.3389/fsufs.2021.617157.
32. Goswami D, Thakker JN, Dhandhukia PC. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. Cogent Food Agric. 2016;2(1):1127500. https://doi.org/10.1080/23311932.2015.1127500.
33. Lambrese Y, Guiñez M, Calvente V, Sansone G, Cerutti S, Raba J, Sanz MI. Production of siderophores by the bacterium *Kosakonia radicincitans* and its application to control of phytopathogenic fungi. Bioresour Technol Rep. 2018;3:82–7. https://doi.org/10.1016/j.biteb.2018.06.003.
34. Ravanbakhsh M, Kowalchuk GA, Jousset A. Root-associated microorganisms reprogram plant life history along the growth–stress resistance tradeoff. The ISME J. 2019;13(12):3093–101. https://doi.org/10.1038/s41396-019-0501-1.
35. Soil Survey Staff. *Keys to soil taxonomy*—USDA. 12th ed. Washington, DC: Natural Resources Conservation Service; 2014.
36. Dos Santos HG, Jacomine PT, Dos Anjos LHC, De Oliveira VÁ, Lumbreras JF, Coelho MR, De Almeida JA, de Araujo Filho JC, De Oliveira JB, Cunha TJF. Brazilian soil classification system, 5th ed. rev. and exp. Embrapa, Brasília, DF, Brazil. 2018. http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1094001
37. Alvares CA, Stape JL, Sentelhas PC, Gonçalves JDM, Sparovek G. Köppen's climate classification map for Brazil. Meteorol Z. 2013;22(6):711–28.
38. van Raij B, Andrade JC, Cantarella H, Quaggio JA. Chemical analysis for fertility evaluation of tropical soils, 285. Campinas: IAC; 2001. (**in Portuguese**).
39. Teixeira PC, Donagemma GK, Fontana A, Teixeira WG. Manual of soil analysis methods. Rio de Janeiro: Centro Nacional de Pesquisa de Solos, Embrapa; 2017. (**in Portuguese**).
40. de Fernández CF, Gepts PL, López M. Etapas de desarrollo de la planta de frijol comúm (*Phaseolus vulgaris* L.). Cali: Centro Internacional de Agricultura Tropical (CIAT); 1986.
41. Fukami J, Abrantes JLF, Del-Cerro P, Nogueira MA, Ollero FJ, Megías M, Hungria M. Revealing different strategies of quorum sensing in *Azospirillum brasilense* strains Ab-V5 and Ab-V6. Arch Microbiol. 2018;200:47–56. https://doi.org/10.1007/s00203-017-1422-x.
42. Fukami J, Ollero FJ, Megías M, Hungria M. Phytohormones and induction of plant-stress tolerance and defense genes by seed and foliar inoculation with *Azospirillum brasilense* cells and metabolites promote maize growth. AMB Express. 2017;7:153–63. https://doi.org/10.1186/s13568-017-0453-7.
43. Galindo FS, Bellotte JLM, Santini JMK, Buzetti S, Rosa PAL, Jalal A, Teixeira Filho MCM. Zinc use efficiency of maize-wheat cropping after inoculation with *Azospirillum brasilense*. Nutr Cycl Agroecosyst. 2021;120:205–21. https://doi.org/10.1007/s10705-021-10149-2.
44. Chaoprasid P, Nookabkaew S, Sukchawalit R, Mongkolsuk S. Roles of *Agrobacterium tumefaciens* C58 ZntA and ZntB and the transcriptional regulator ZntR in controlling $CD_2^+/Zn_2^+/Co_2^+$ resistance and the peroxide stress response. Microbiol. 2015;161:1730–40. https://doi.org/10.1099/mic.0.000135.
45. Muñoz-Moreno CY, Cruz-Rodríguez Y, Vega-Arreguín J, Alvarado-Rodríguez M, Gómez-Soto JM, Alvarado-Gutiérrez A, Fraire-Velázquez S. Draft genome sequence of *Bacillus subtilis* 2C–9B, a strain with biocontrol potential against chili pepper root pathogens and tolerance to Pb and Zn. Genome Announc. 2018;6:e01502-e1517. https://doi.org/10.1128/genomeA.01502-17.
46. Rekha K, Baskar B, Srinath S, Usha B. Plant-growth-promoting rhizobacteria *Bacillus subtilis* RR4 isolated from rice rhizosphere induces malic acid biosynthesis in rice roots. Can J Microbiol. 2017;18:1–8. https://doi.org/10.1139/cjm-2017-0409.
47. David BV, Chandrasehar G, Selvam PN. Pseudomonas fluorescens: a plant-growth-promoting rhizobacterium (PGPR) with potential role in biocontrol of pests of crops. In: Crop improvement through microbial biotechnology. Amsterdam: Elsevier; 2018. p. 221–243.
48. Jing X, Cui Q, Li X, Yin J, Ravichandran V, Pan D, Fu J, Tu Q, Wang H, Bian X, et al. Engineering *Pseudomonas protens* Pf-5 to improve its antifungal activity and nitrogen fixation. Microb Biotechnol. 2020;13:118–33.
49. Nakao AH, Costa NR, Andreotti M, Souza MFP, Dickmann L, Centeno DC, Catalani GC. Características agronômicas e qualidade fisiológica de sementes de soja em função da adubação foliar com boro e zinco. Cult Agron. 2018;27(3):312–27 (**in Portugese**).
50. IAPAR - Instituto Agronômico do Paraná. Principais características das cultivares de feijão com sementes disponíveis no mercado. 2019. https://www.idrparana.pr.gov.br/system/files/publico/negocios/folders/feijao/IPR-Campos-Gerais.pdf. Accessed 17 Sept 2020. (**in Portugese**)
51. Shevkani K, Singh N. Relationship between protein characteristics and film-forming properties of kidney bean, field pea and amaranth protein isolates. Int J Food Sci Technol. 2015;50(4):1033–43. https://doi.org/10.1111/ijfs.12733.
52. Malavolta E, Vitti GC, Oliveira SA. Evaluation of the nutritional status of plants: principles and applications. 2nd ed. Piracicaba: Associação Brasileira para Pesquisa da Potasse e do Fosfato; 1997. (**in Portugese**).

53. Rengel Z, Graham RD. Uptake of zinc from chelate-buffered nutrient solutions by wheat genotypes differing in zinc efficiency. J Exp Bot. 1996;47:217–26. https://doi.org/10.1093/jxb/47.2.217.
54. de Lima Lessa JH, Araujo AM, Ferreira LA, da Silva Júnior EC, de Oliveira C, Corguinha APB, Martins FAD, de Carvalho HWP, Guilherme LRG, Lopes G. Agronomic biofortification of rice (*Oryza sativa* L.) with selenium and its effect on element distributions in biofortified grains. Plant Soil. 2019;444:331–42. https://doi.org/10.1007/s11104-019-04275-8.
55. IBGE. Directorate of Research: Coordination of Work and Income [Family Budget Survey 2017–2018: First results]. IBGE, Rio de Janeiro, Brazil. 2019. https://agenciadenoticias.ibge.gov.br/agencia-sala-de-imprensa/2013-agencia-de-noticias/releases/28646-pof-2017-2018-brasileiro-ainda-mantem-dieta-a-base-de-arroz-e-feijao-mas-consumo-de-frutas-e-legumes-e-abaixo-do-esperado. Accessed 12 Sept 2022 (**in Portugese**)
56. Fageria NK, dos Santos AB, Cobucci T. Zinc nutrition of lowland rice. Commun Soil Sci Plant Anal. 2011;42:1719–27. https://doi.org/10.1080/00103624.2011.584591.
57. R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. 2015. https://www.R-project.org/
58. Salama DM, Osman SA, Abd El-Aziz ME, Abd Elwahed MS, Shaaban EA. Effect of zinc oxide nanoparticles on the growth, genomic DNA, production and the quality of common dry bean (*Phaseolus vulgaris*). Biocatal Agric Biotechnol. 2019;18:101083. https://doi.org/10.1016/j.bcab.2019.101083.
59. Jalal A, da Silva Oliveira CE, Freitas LA, Galindo FS, Lima BH, Boleta EH, Da Silva EC, do Nascimento V, Nogueira TA, Buzetti S, Teixeira Filho MCM. Agronomic biofortification and productivity of wheat with soil zinc and diazotrophic bacteria in tropical savannah. Crop Pasture Sci. 2022;73(7–8):817–30. https://doi.org/10.1071/CP21457.
60. Jalal A, Oliveira CEDS, Fernandes HB, Galindo FS, Silva ECd, Fernandes GC, Nogueira TAR, De Carvalho PHG, Balbino VR, Lima BHd, Teixeira Filho MCM. Diazotrophic bacteria is an alternative strategy for increasing grain biofortification, yield and zinc use efficiency of maize. Plants. 2022;11(9):1125. https://doi.org/10.3390/plants11091125.
61. Swarnalakshmi K, Yadav V, Tyagi D, Dhar DW, Kannepalli A, Kumar S. Significance of plant growth promoting rhizobacteria in grain legumes: Growth promotion and crop production. Plants. 2020;9(11):1596.
62. Tanveer Y, Yasmin H, Nosheen A, Ali S, Ahmad A. Ameliorative effects of plant growth promoting bacteria, zinc oxide nanoparticles and oxalic acid on *Luffa acutangula* grown on arsenic enriched soil. Environ Pollut. 2022;300:118889. https://doi.org/10.1016/j.envpol.2022.118889.
63. Sousa SM, Oliveira CA, Andrade DL, Carvalho CG, Ribeiro VP, Pastina MM, Gomes EA. Tropical *Bacillus* strains inoculation enhances maize root surface area, dry weight, nutrient uptake and grain yield. J Plant Growth Regul. 2021;40:867–77. https://doi.org/10.1007/s00344-020-10146-9.
64. Doolette CL, Read TL, Howell NR, Cresswell T, Lombi E. Zinc from foliar-applied nanoparticle fertiliser is translocated to wheat grain: a 65Zn radiolabelled translocation study comparing conventional and novel foliar fertilisers. Sci Total Environ. 2020;749:142369. https://doi.org/10.1016/j.scitotenv.2020.142369.
65. Jalal A, Shah S, Teixeira Filho MCM, Khan A, Shah T, Ilyas M, Rosa PAL. Agro-biofortification of zinc and iron in wheat grains. Gesunde Pflanzen. 2020;72(3):227–36.
66. Jalal A, Shah S, Teixeira Filho MCM, Khan A, Shah T, Hussain Z, Younis M, Ilyas M. Yield and phenological indices of wheat as affected by exogenous fertilization of zinc and iron. Braz J AgricSci. 2020;15(1):1–8.
67. Jalal A, Oliveira CEDS, Bastos A, Fernandes G, de Lima B, de Carvalho P, Galindo FS, Gato I, Teixeira Filho MCM. Nanozinc and plant growth-promoting bacteria improve biochemical and metabolic attributes of maize in tropical Cerrado. Front Plant Sci. 2022;13:1046642.
68. Jalal A, Oliveira CEDS, Fernandes GC, da Silva EC, Costa KND, Souza JSD, Leite GDS, Biagini ALC, Galindo FS, Teixeira Filho MCM. Integrated use of plant growth-promoting bacteria and nano-zinc foliar spray is a sustainable approach for wheat biofortification, yield and zinc use efficiency. Front Plant Sci. 2023;14:1593.
69. Ullah A, Farooq M, Hussain M. Improving the productivity, profitability and grain quality of kabuli chickpea with co-application of zinc and endophyte bacteria *Enterobacter* sp. MN17. Arch Agron Soil Sci. 2019;25:1–6.
70. Mahdieh M, Sangi MR, Bamdad F, Ghanem A. Effect of seed and foliar application of nano-zinc oxide, zinc chelate, and zinc sulphate rates on yield and growth of pinto bean (*Phaseolus vulgaris*) cultivars. J Plant Nutri. 2028;41(18):2401–12. https://doi.org/10.1080/01904167.2018.1510517.
71. Palacio-Márquez A, Ramírez-Estrada CA, Gutiérrez-Ruelas NJ, Sánchez E, Ojeda-Barrios DL, Chávez-Mendoza C, Sida-Arreola JP. Efficiency of foliar application of zinc oxide nanoparticles versus zinc nitrate complexed with chitosan on nitrogen assimilation, photosynthetic activity, and production of green beans (*Phaseolus vulgaris* L.). Sci Hortic. 2021;288:110297. https://doi.org/10.1016/j.scienta.2021.110297.
72. Abadi VAJM, Sepehri M, Khatabi B, Rezaei M. Alleviation of zinc deficiency in wheat inoculated with root endophytic fungus *Piriformospora indica* and rhizobacterium *Pseudomonas putida*. Rhizosphere. 2021;17:100311. https://doi.org/10.1016/j.rhisph.2021.100311.
73. Reis MNO, Vitorino LC, Lourenço LL, Bessa LA. Microbial Inoculation Improves Growth, Nutritional and Physiological Aspects of *Glycine max* (L.) Merr. Microorganisms. 2022;10(7):1386. https://doi.org/10.3390/microorganisms10071386.
74. Singh D, Prasanna R. Potential of microbes in the biofortification of Zn and Fe in dietary food grains. A review. Agron Sustain Dev. 2020;40(2):1–21. https://doi.org/10.1007/s13593-020-00619-2.
75. Upadhayay VK, Singh AV, Khan A, Sharma A. Contemplating the role of zinc-solubilizing bacteria in crop biofortification: an approach for sustainable bioeconomy. Front Agron. 2022. https://doi.org/10.3389/fagro.2022.903321.
76. Verma S, Kumar M, Kumar A, Das S, Chakdar H, Varma A, Saxena AK. Diversity of Bacterial Endophytes of Maize (*Zea mays*) and Their Functional Potential for Micronutrient Biofortification. Curr Microbiol. 2022;79:6. https://doi.org/10.1007/s00284-021-02702-7.
77. Rehman A, Farooq M, Naveed M, Ozturk L, Nawaz A. *Pseudomonas*-aided zinc application improves the productivity and biofortification of bread wheat. Crop Pasture Sci. 2018;69(7):659. https://doi.org/10.1071/cp17441.
78. Khoshru B, Mitra D, Mahakur B, Sarikhani MR, Mondal R, VermaD PK. Role of soil rhizobacteria in utilization of an indispensable micronutrient zinc for plant growth promotion. J Crit Rev. 2020;21:4644–54.
79. Firdous S, Agarwal BK, Chhabra V, Kumar A. Zinc allocation and its re-translocation in wheat at different growth stages. Plant Arch. 2020;20:8653–9.
80. Kryvoruchko I. Zn-use efficiency for optimization of symbiotic nitrogen fixation in chickpea (*Cicer arietinum* L.). Turk J Bot. 2017;41:423–41. https://doi.org/10.3906/bot-1610-6.
81. Saravanan VS, Madhaiyan M, Thangaraju M. Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. Chemosphere. 2007;66(9):1794–8.

Publisher's Note