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SERRATIA MARCESCENS STRAIN FA-4 ENHANCES ZINC CONTENT IN RICE GRAINS BY ACTIVATING THE ZINC TRANSLOCATING ENZYMES

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SUMMARY

Zinc deficiency in cereal crops is a significant issue for human health. Rice, being a staple food crop, could cause severe zinc deficiency. The use of zinc-solubilizing bacteria (ZSB) is an ecological tactic to raise bioavailable zinc in the soil that may alleviate yield loss and, subsequently, enhance the nutritional value of rice. In the presented study, treating rice plants with plant growth-promoting rhizobacteria *S. marcescens* FA-4 along with the recommended dose of chemical Zn and half dose of chemical Zn ensued under pot and field conditions at the COMSATS University, Islamabad, Pakistan. The obtained data indicated an augmentation in rice growth, yield, and grain zinc content in response to the *S. marcescens* FA-4 inoculation with and without the chemical Zn application. The *S. marcescens* FA-4 significantly enhanced the grain zinc content (21.4–27.7 mg kg^{-1}) under the pot and (18.7–30.1 mg kg^{-1}) under field conditions, with 1.5 to twofold rise in superoxide dismutase (SOD) and carbonic anhydrase (CA) activity in rice compared with the control. The rice plants treated with zinc solubilizing bacteria, followed by zinc treatments gave higher grain yields of 23.4–34.1 g pot^{-1} and 3.2–3.6 t ha^{-1} in rice cultivars, Basmati 385 and Super Basmati. The *S. marcescens* FA-4 with a half dose of chemical Zn also increased the zinc translocation index (1.4 to 1.7) toward grains. Consistency in the performance of zinc solubilizing bacteria occurred in the pot and field conditions. Hence, a conclusion that the use of zinc solubilizing strains is an efficient approach to enhance the zinc content of rice grains and combat the problem of zinc deficiency in humans.

Keywords: Zinc-solubilizing bacteria, superoxide dismutase, carbonic anhydrase, grain zinc content, rice

Key findings: The rice growth and yield increased in response to the combination of zinc solubilizing *Serratia marcescens* FA-4 and half a dose of chemical Zn. The *Serratia marcescens* FA-4 significantly enhanced the grain zinc content under the pot and field conditions, causing a 1.5 to twofold rise in superoxide dismutase (SOD) and carbonic anhydrase (CA) activity in rice compared with the control.

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INTRODUCTION

Food security is coupled with economic and ecological security based on quantity, as well as, the available food quality. Plant growth-promoting rhizobacteria (PGPR) helps ensure food security by enhancing crop yields through nutrient availability in plants and their quality through enhanced uptake of nutrients—a phenomenon termed 'biofortification.' Many PGPRs have served the biofortification of cereals (Abaid-Ullah *et al*., 2015; Shakeel *et al*., 2015; Mumtaz *et al*., 2017). Among cereals, rice is a staple food crop that more than 560 million people worldwide feed on (Krithika and Balachandar, 2016). Rice is highly prone to zinc (Zn) deficiency. Almost 50% of soils under flooded rice cultivation are Zn deficient, producing less quality and quantity rice yields on such soils. The qualitatively low yield is due to Zn-deficient grains, and their consumption as food leads to malnutrition in humans, particularly in developing countries (Bouis and Welch, 2010; Sharma *et al*., 2013; Krithika and Balachandar, 2016).

The practice of Zn fertilization usually seeks to overcome rice’s zinc deficiency. Sadly, most Zn fertilizers do not meet the plant zinc nutrition requirement (Gandhi and Muralidharan, 2016). It is due to the available form of zinc for an application getting converted into an unavailable form with the physio-chemical properties of soil. The applied zinc, particularly permeating as phosphates, hydroxides, sulfides, and carbonates, lowers the fertilizer use efficiency (1%–5%). Moreover, the bicarbonates, clay content, and high pH fixed the available Zn into an unavailable form for plants. Although a sufficient amount of Zn exists in the soil, crop plants show deficiency due to occurring inaccessible Zn forms (Vanitha *et al*., 2016; Duoc *et al*., 2020; Prathap *et al*., 2022). Resolving this problem can turn to exploiting the soil bacteria with the chemical fertilizers capable in solubilizing Zn and making it available for crop assimilation (Mader *et al*., 2011; Shafgh *et al*., 2019). Numerous Zn solubilizing bacteria (ZSB) have been characterized from different soils to enhance plants’ growth, yield, and zinc content (Hussain *et al*., 2018).

These ZSBs include *Bacillus* sp. (Hussain *et al*., 2015), *Bacillus aryabhattai* (Ramesh *et al*., 2014), *and Azospirillum* and *Pseudomonas* strains (Naz *et al*., 2016). The *Pseudomonas fluorescens, P. striata, P. aeruginosa*, *Burkholderia cenocepacia, Bacillus* sp., *and Gluconacetobacter diazotrophicus* are the most reported bacterial strains that have shown zinc solubilization on a laboratory scale (Pawar *et al*., 2015, Kamran *et al*., 2017). The inoculation of ZSB bacteria improved the Zn uptake of rice (Vaid *et al*., 2014; Shakeel *et al*., 2015), wheat (Kamran *et al*., 2017; Rehman *et al*., 2018), maize (Mumtaz *et al*., 2017), and soybean (Khande *et al*., 2017). The *Bacillus subtilis* and *Bradyrhizobium sp* inoculation increased the root length, shoot length, plant dry weight, chlorophyll content, grain yield, nutrient uptake, total starch, carbohydrates, and protein contents of guar versus non-inoculated plants (El-Sawah *et al*., 2021). Khan *et al*. (2019) applied *Bacillus megaterium*, *Bacillus thuringiensis*, and *Bacillus subtilis* strains in chickpeas and reported a significant effect on their yield, chlorophyll content, and nutrient uptake.

Serratia marcescens (Gram-negative bacteria) can associate with plants as free-living species and as endophytes in the rhizosphere (Gyaneshwar *et al*., 2001). *Serratia marcescens* FA-4 has the potential to solubilize the zinc and increase the yield and grain zinc content of wheat crops (Abaid-Ullah *et al*., 2015). The rice plant uptakes zinc (Zn^{2+}) from the soil by different root membrane transport mechanisms, which include Zn-regulated transporters (ZIP family), phytosiderophores iron (Fe)-regulated transporter, and certain enzymes, viz., copper (Cu)/zinc superoxide dismutase (SOD) and carbonic anhydrase (CA) (Guerinot, 2000; Bashir *et al*., 2010; Mathpa *et al*., 2015). Cakmak (2000) has speculated that Zn deficiency may inhibit the activities of various antioxidant enzymes, and thus, SOD and CA are an indicator of zinc deficiency in plants (Bharti *et al*., 2014). Zn deficiency induces a decline in CA activity, particularly in Zn-inefficient wheat genotypes and rice (Mathpa *et al*., 2015; Singh *et al*., 2019). The plant growth-promoting rhizobacteria (PGPR) colonize the roots and produce numerous compounds, especially organic acids, and siderophores. These compounds solubilize the Zn from the rhizospheric soil and help the plants in Zn uptake. However, studies are required to explain the role of zinc solubilizing bacteria in regulating the activities of enzymes associated with Zn deficiency because understanding the ZSB-plant interactions would help to alleviate Zn deficiency. The current study aims to evaluate the potential of *S. marcescens* FA-4 to increase the growth, yield, and grain zinc translocation of rice by activating the SOD and CA activities.

MATERIALS AND METHODS

Collection of zinc solubilizing strain and plant material

Procuring the zinc solubilizing strain *S. marcescens* FA-4 (Accession No. KJ813007) (used previously for fortifying wheat) was from Applied Microbiology and Biotechnology Laboratory, Department of Biosciences, COMSATS University, Islamabad, Pakistan (Abaid-Ullah *et al*., 2015). The strain was routinely grown on LB agar and preserved in 20% glycerol at -20 °C.

The seeds and seedlings (30 days old) of two rice cultivars, viz*.,* Super Basmati and Basmati 385, came from the National Agriculture Research Center (NARC), Islamabad, Pakistan. Two independent experiments, viz*.,* pot experiments under natural conditions in the net house and field experiments, proceeded for two consecutive years, 2013 and 2014, to assess the effect of ZSB, *S. marcescens* FA-4 on rice. The following treatments in both experiments included T_1 = uninoculated plants (negative control), T_2 = chemical Zn (full recommended dose of $ZnSO_4$ at the rate of 7.5 mg kg^{-1} of soil/15 kg ha^{-1}), T_3 = *S. marcescens* FA-4, T_4 = *S. marcescens* FA-4 + ½ chemical Zn ($ZnSO_4$ at the rate of 3.75 mg kg^{-1} of soil/7.5 kg ha^{-1}), T_5 = *S. marcescens* FA-4 + full chemical Zn ($ZnSO_4$ at the rate of 7.5 mg kg^{-1} /15 kg ha^{-1} of soil). Description of other details of each experiment follows.

Net house experiment

The net house experiment, conducted at COMSATS University, Islamabad, Pakistan, employed a complete randomized design (CRD) with three replications per treatment. The rice cultivars, grown in pots (20 cm × 30 cm) during their natural growing season, i.e., June to October, followed the description from previous studies (Shakeel *et al*., 2015; Rais *et al*., 2017). The filled pots with sterilized soil had physicochemical characteristics, as shown in Table 1. The experiment kept the tubs under net house conditions. Roots of rice seedlings (30 days old) gained surface sterilization by dipping in 0.1% mercuric chloride for 45–60 s, followed by washing (2x) with sterile water. The sterilized seedlings, transplanted in every pot, had two per hole in five holes per pot. Pruning followed the seedling establishment, one seedling maintained in each jar.

Nitrogen (N), phosphorus (P), and potassium (K) fertilizers application had the rate of 20 mg, 30 mg, and 40 mg per kg soil in the form of urea, single super phosphate, and potassium sulfate, respectively. Applying the whole doses of K and P ensued during transplanting, while N application was in three separate doses. Chemical Zn applied in the respective treatments occurred after 15 days of transplanting. The maintained standard moisture level used tube well water throughout the rice crop.

Bacterial inoculation

Zinc solubilizing bacterial strain *Serratia marcescens* FA-4 culturing was in LB broth at 28 °C ± 2 °C and 120 rpm for 24 h. The bacterial inoculum attained centrifuging, with the cell pellet suspended in 0.85% saline solution, to obtain the cell population (10^9 CFU mL^{-1}). Applying 1 mL of this saline suspension (10^9 CFU mL^{-1}) continued near the roots of each seedling by the soil drenching method. The negative control received only 0.85% saline without cell suspension. Repeating the injection after 45 days of the first inoculation used a similar process.

Table 1. Physical and chemical properties of soil used in pot and field experiments.

Parameter	Texture/Value
Texture	Clay loam
pH	7.73
Organic Matter (%)	0.82
Total Nitrogen (%)	0.041
Available Phosphorus (mg kg^{-1})	4.8
Saturation (%)	40
EC (dSm^{-1})	0.31
Zinc mg kg^{-1}	0.54

Values are the mean of three replicates and the test was repeated twice.

Biochemical assessment

The inoculated rice plants underwent assessment for various biochemical parameters, such as, chlorophyll content, superoxide dismutase (SOD), and carbonic anhydrase (CA) activity. The recorded chlorophyll content of rice plants went on at the anthesis stage, with three leaves of different plants in the same treatment randomly selected for measuring chlorophyll content with a SPAD meter (Minolta, Tokyo, Japan) (Ranganathan *et al*., 2006).

Enzymatic activity

The superoxide dismutase (SOD) and carbonic anhydrase (CA) activities in rice flag leaves during the flowering stage were measured, with 14–15 plants randomly selected from each replication and their leaves mixed to make a representative sample. The preserved leaves in liquid nitrogen received storage at -20 °C for measuring the enzymatic activity.

The SOD enzyme extraction followed the method of Asadi *et al*. (2012), with frozen leaves weighed and homogenized in phosphate buffer. The homogenate gained centrifuged at 13,000 rpm for 20 min, then collected the supernatant for enzyme assay. SOD enzyme activity was measured by determining its capacity to stop the photochemical decline of nitro blue tetrazolium chloride (NBT) (Sharma *et al*., 2015). The reaction comprised 20 μ mL^{-1} riboflavin, 75 μ mL^{-1} nitro blue tetrazolium (NBT), 130 m mL^{-1} methionine, 100 μ mL^{-1} ethylene diamine tetra acetic acid (EDTA-Na_2), and 0.025 mL enzyme extract, with distilled water used in control. The reactions illuminated with 4000 lux light intensity took 20 min. The same reactions without illumination served as blank. After illumination for 20 min, the observed absorbance was at 560 nm. Expressing one unit (U) of SOD enzyme activity as the quantity of enzyme necessary to cause 50% inhibition of the NBT photoreduction rate ensued. The SOD enzyme activity of the extract got denoted as SOD U mg^{-1} FW.

Extracting the carbonic anhydrase ground the leaves in 0.01 M Na_2EDTA, 0.1 M Tris-HCl (pH 8.3), and 0.05 M 1,1,1,-trichloro-2,2-bis (p-chlorophenyl) ethane (Mathpal *et al*., 2015). Homogenate centrifuging was at 11,000 g for 20 min, estimating the CA activity in the supernatant (Hacisalihoglu *et al*., 2003). Mixing 1 mL of enzyme extract into the 0.0025 M veronal buffer (pH 8.2) with bromothymol blue, inoculation of 2 mL of cold saturated CO_2 solution went on using a syringe into the veronal buffer. Observing the period of the CO_2 solution inoculation continued until the color change from blue to yellow. Activity units (U) calculation proceeded according to the formula:

$$U = 10 \times (T_b/T_c)-1/\text{ mg fresh weight}$$

Where:
T_b = time of uncatalyzed reaction
T_c = the time of enzyme-catalyzed reaction

Growth parameters, yield, and Zn analysis

The growth parameters recording for plant height, panicle length, and the number of tillers were at the time of plant maturity. The plant height measurement used a meter rod from the tip of the panicle to the ground level of the plant. With all plants uprooted, the number of tillers per pot and per plant got counted. Measuring the biomass and grain yield, each plant underwent threshing individually. Commercial analysis of the zinc content of straw and grains was from the Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan. The Zn translocation index (ZTI) calculation used the following formula (Rengel and Graham, 1996):

$$ZTI = \text{Grain Zn content}/ \text{ Straw Zn content}$$

Field experiment

The field experiment proceeded at the National Agriculture Research Center (NARC), Islamabad, Pakistan, for two consecutive years. Figure 1 depicts the two-year meteorological conditions, while soil physicochemical characteristics appear in Table 1. The experiment, set in randomized complete block design, had a net plot size of 3 m x 3 m with three replications per treatment. Raising rice seedlings were from their sown seeds during the first week of June. The field preparation consisted of five plowings, followed by leveling and planking with help from tractor-drawn implements. Manual transplanting of 35-day-old seedlings transpired in the field, maintaining row × row and plant × plant distances of 20 cm and 20 cm, respectively. The seedling transplanting in standing water of 5–7 cm had the same water level maintained till physiological maturity. The bacteria inoculation by soil drenching ensued, as described earlier. Different blocks, prepared for each replication, avoided mixing bacterial inoculum through water seepage, with each

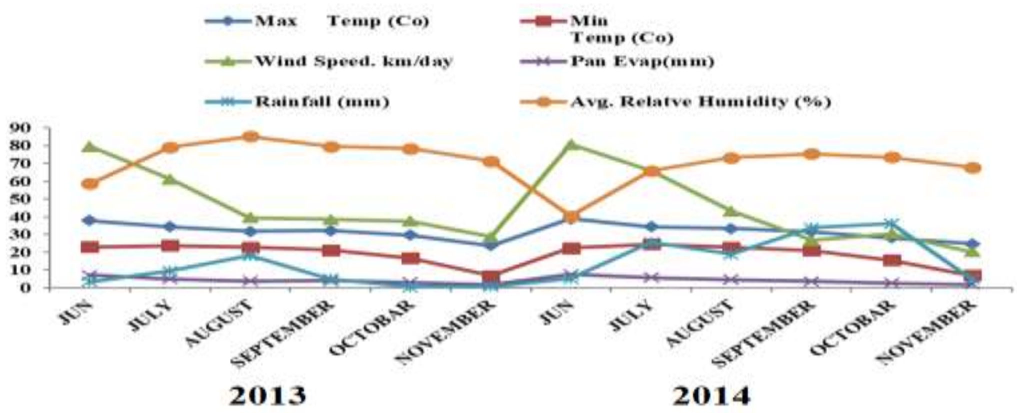

Figure 1. Meteorological conditions throughout the experiment over two years.

block connected with a separate water channel. Nitrogen, phosphorus, and potassium (NPK) fertilizers application had the rate of 80:60:40 kg ha^{-1}. Applying P and K was full as basal dose during transplanting, with half of the N quantity as basal and the other half in two separate doses at the tillering and panicle initiation stages. Chemical Zn ($ZnSO_4$) use in the respective treatments occurred after 15 days of transplanting. All the agronomic operations happened normally and uniformly for all the treatments. Biochemical, agronomic, and yield parameters assessment progressed, as previously described.

Statistical analysis

The statistical analysis used the software, Statistics 8.1., to analyze data by following the specific experimental layout and analysis of variance (ANOVA). Comparing the treatments employed Fisher's least significant differences test (LSD) at a probability level ($P \leq 0.05$) (Steel and Torrie, 1980).

RESULTS

Effect of *S. marcescens* FA-4 on rice growth and yield in the pot experiment

Inoculation of zinc solubilizing *S. marcescens* FA-4 strain improved the growth attributes, i.e., plant height, panicle length, number of tillers, and grain yield for rice Super Basmati and Basmati 385 cultivars compared with uninoculated control. Increases showed in grain yield at 31 and 34 g pot^{-1}, plant height at 91 cm, panicle length at 19.4 and 26.8 cm, number of tillers at 25 and 29 pot^{-1}, and chlorophyll content (31.2 and 31.5) in Super Basmati and Basmati 385, respectively (Tables 2 and 3) when inoculated with *S. marcescens* FA-4 with a recommended dose of chemical fertilizer than uninoculated plants in the negative control (Tables 2 and 3). The growth parameters and yield remained the same in the *S. marcescens* FA-4 with a half dose of chemical Zn. These results showed that zinc solubilizing strain *S. marcescens* FA-4 can potentially save 50% chemical Zn. In both cultivars, all treatments significantly affected various parameters.

Enzymatic activity and Zn translocation in the pot experiment

The examined effect of *S. marcescens* FA-4 on the activities of zinc-requiring enzymes, such as, SOD and CA in rice leaves has the results in Figures 2 and 3. Both rice cultivars had a similar trend in changing the SOD and CA activity. SOD and CA activity in both cultivars enhanced one to twofold in plants inoculated with *S. marcescens* FA-4 alone and combined with a half dose of zinc and a full one compared with the control.

The *S. marcescens* FA-4 enhanced the grain Zn content and zinc translocation toward rice grains of both cultivars efficiently in the existing chemical Zn (Tables 2 and 3). The detected highest zinc translocation index (1.4–1.5) were in Basmati 385 and Super Basmati, respectively, treated with *S. marcescens* FA-4 + chemical Zn in half and complete doses, followed by chemical Zn with ZTI (1.3), as presented in Tables 2 and 3. *S. marcescens* FA-4 inoculated in the absence of zinc caused a higher zinc translocation index (1.2) compared with untreated plants, with a zinc translocation index of 0.9–1.1, and remained lower versus other treatments (Tables 2 and 3).

Table 2. Effect of zinc solubilizing *S. marcescens* FA-4 on rice variety Basmati 385 in a pot experiment.

Treatment	Plant Height (cm)	No. Tiller (pot^{-1})	Chlorophyll Content (SPAD Unit)	Panicle Length (cm)	Grain Yield (Pot^{-1} [g])	Grains Zinc Content (mg kg^{-1})	ZTI
Control (T_1)	51.4 ± 0.58^{d}	17.3 ± 0.33^{c}	12.4 ± 0.15^{d}	6.1 ± 0.28^{d}	22.2 ± 0.32^{c}	16.8 ± 0.55^{c}	0.9 ± 0.00^{d}
Chemical Zn* (T_2)	86.94 ± 0.57^{b}	21.04 ± 0.57^{b}	29.4 ± 0.60^{b}	16.9 ± 0.50^{b}	33.2 ± 0.20^{a}	25.2 ± 0.55^{a}	1.3 ± 0.04^{b}
S. marcescens FA-4 (T_3)	67.94 ± 0.49^{c}	19.34 ± 0.66^{b}	18.9 ± 0.14^{c}	11.0 ± 0.57^{c}	31.5 ± 0.35^{b}	21.4 ± 0.44^{b}	1.2 ± 0.03^{c}
S. marcescens FA-4 + Chemical Zn** (T_4)	90.84 ± 0.83^{a}	24.34 ± 0.88^{a}	31.1 ± 0.17^{a}	19.1 ± 0.68^{a}	34.0 ± 0.41^{a}	26.7 ± 0.51^{a}	1.4 ± 0.04^{a}
S. marcescens FA-4 + Chemical Zn*(T_5)	91.24 ± 0.52^{a}	24.74 ± 0.66^{a}	31.2 ± 0.33^{a}	19.4 ± 0.52^{a}	34.1 ± 0.54^{a}	26.9 ± 0.51^{a}	1.4 ± 0.04^{a}

Values are the mean of three replicates ± SE and values bearing different letters in the same column are significantly different from each other according to Fisher's LSD ($p < 0.05$). The zinc translocation index was calculated by dividing the grain zinc content by the shoot zinc content. Control = non-inoculated.

Table 3. Effect of zinc solubilizing *S. marcescens* FA-4 on rice variety Super Basmati in a pot experiment.

Treatments	Plant Height (cm)	No. Tiller (pot^{-1})	Chlorophyll Content (SPAD Unit)	Panicle Length (cm)	Grain Yield (pot^{-1} [g])	Grains Zinc Content (mg kg^{-1})	ZTI
Control (T_1)	65.4 ± 0.14^{d}	16.7 ± 0.33^{d}	18.7 ± 0.52^{c}	18.7 ± 0.24^{d}	18.9 ± 0.60^{d}	19.9 ± 0.54^{b}	1.1 ± 0.05^{b}
Chemical Zn* (T_2)	77.7 ± 0.61^{b}	27.0 ± 0.57^{b}	30.3 ± 0.40^{a}	24.5 ± 0.30^{b}	29.1 ± 0.57^{b}	26.4 ± 0.60^{a}	1.4 ± 0.01^{a}
S. marcescens FA-4 (T_3)	71.1 ± 0.65^{c}	19.3 ± 0.33^{c}	26.2 ± 0.63^{b}	21.1 ± 0.54^{c}	23.4 ± 0.28^{c}	21.7 ± 0.66^{b}	1.2 ± 0.06^{b}
S. marcescens FA-4 + Chemical Zn** (T_4)	90.7 ± 0.21^{a}	29.0 ± 0.57^{a}	31.2 ± 0.75^{a}	26.6 ± 0.55^{a}	30.8 ± 0.06^{ab}	27.3 ± 0.69^{a}	1.5 ± 0.04^{a}
S. marcescens FA-4 + Chemical Zn* (T_5)	90.9 ± 0.17^{a}	29.3 ± 0.33^{a}	31.5 ± 0.17^{a}	26.8 ± 0.48^{a}	30.9 ± 0.88^{a}	27.7 ± 0.46^{a}	1.5 ± 0.03^{a}

Values are the mean of three replicates ± SE and values bearing different letters in the same column are significantly different from each other according to Fisher's LSD ($p < 0.05$). The zinc translocation index was calculated by dividing the grain zinc content by the shoot zinc content. Control = non-inoculated.

* Recommended dose of $ZnSO_4$ (7.5 mg kg^{-1} of soil), ** Half dose of $ZnSO_4$ (3.75 mg kg^{-1} of soil).

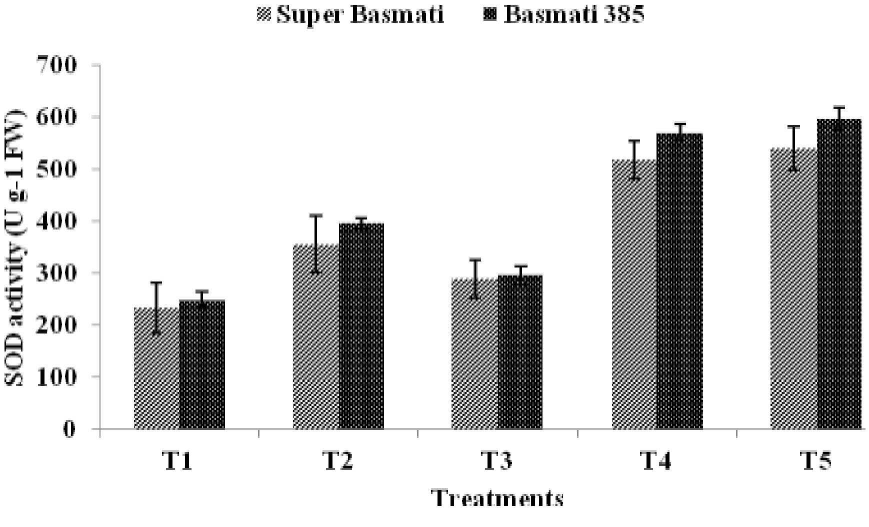

Figure 2. The SOD activity of rice plants treated with *S. marcescens* FA-4 in a pot experiment. Values are the mean of three replicates with letters. Bars represent standard error.

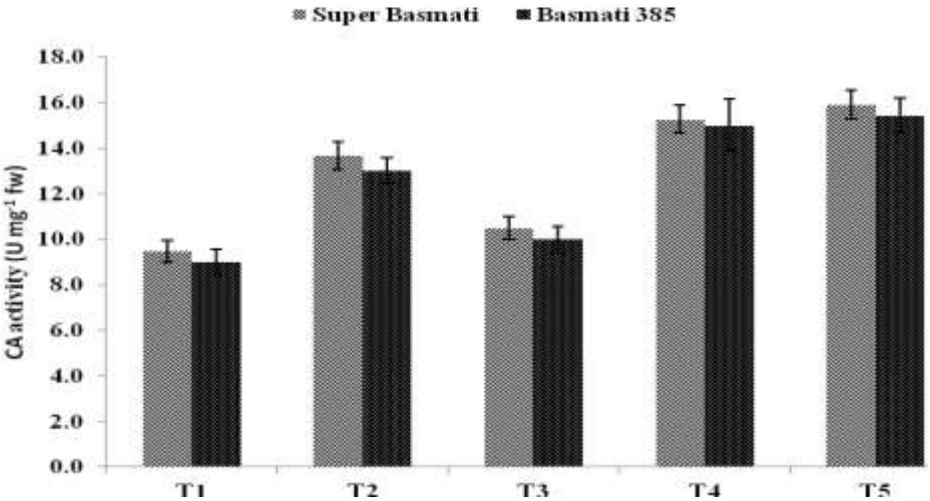

Figure 3. The CA activity of rice plants treated with *S. marcescens* FA-4 in a pot experiment. Values are the mean of three replicates with letters. Bars represent standard error.

Effect of *S. marcescens* FA-4 on rice growth and grain yield in field experiments

In all the treatments, the growth parameters of both rice cultivars Basmati 385 and Super Basmati, i.e., plant height, number of tillers, chlorophyll content, panicle length, and grain yield were a little bit lower and higher in the first and second growing season, respectively (Tables 4 and 5). The *S. marcescens* alone and with half and complete doses of chemical Zn treatments revealed significant effects on plant height, number of tillers, chlorophyll content, panicle length, and grain yield of both cultivars. Their influence was more prominent in both the years of the experiments, though, due to few variations, effects were insignificant between both growing seasons (Tables 4 and 5). Meanwhile, control (uninoculated) treatments had lower plant height, number of tillers, chlorophyll content, panicle length, and grain yield in both cultivars (Basmati 385 and Super Basmati) compared with all treatments during both years (Tables 4 and 5).

Enzymatic activity and Zn translocation in the field experiment

The zinc-requiring enzyme superoxide dismutase (SOD) activity of Basmati 385 was higher in the second year than in the first year of experiment in control (uninoculated) with chemical Zn (at the rate of 15 kg ha^{-1}) treatments, while in *S. marcescens* alone and with half and a full dose of chemical Zn treatments, the SOD activity was lower in the second year compared with the first year (Figure 4). In Super Basmati, the trend of SOD activity was different. It was higher in the first year than in the second in control (uninoculated) treatment and lower in the first year than in year two in chemical Zn (at the rate of 15 kg ha^{-1}), *S. marcescens* alone, and with half and a regular dose of chemical Zn treatments (Figure 4). The zinc solubilizing *S. marcescens* significantly affected the SOD activity alone or in a combination of half and full doses of chemical Zn compared with the control (uninoculated). The SOD activity was maximum (577.8, 437.0 U g^{-1} FW) in *S. marcescens* with a half dose of chemical Zn and minimum (207.4, 196.3 U g^{-1} FW) in control (uninoculated) in both cultivars Basmati 385 and Super Basmati, respectively. The SOD activity was statistically at par in *S. marcescens* with half and a regular dose of chemical Zn (Figure 4).

The carbonic anhydrase (CA) activity of both cultivars Basmati 385 and Super Basmati revealed minimum differences for both years, generally between 1 and 2 U mg^{-1} fresh weight higher in the second than the first growing season (Figure 5). The maximum CA activity (14.5, 13.2 U mg^{-1} fresh weight) occurred in *S. marcescens* with a half dose of chemical Zn in Basmati 385 and Super Basmati, respectively. Similarly, a scarce difference appeared in the grain Zn concentrations and zinc translocation index of Basmati 385 and Super Basmati for both years. The *S. marcescens* enhanced the zinc translocation toward the grain in both cultivars with half and a regular dose of chemical Zn (Tables 4 and 5).

Table 4. Effect of zinc solubilizing *S. marcescens* FA-4 on rice variety Basmati 385 in the field experiment.

Treatments	Plant Height	No. Tiller	Chlorophyll Content	Panicle Length	Grain Yield	Grains Zinc Content	ZTI
	(cm)	Plant^{-1}	SPAD Unit	(cm)	tons ha^{-1}	mg kg^{-1}	
2013							
Control (T$_1$)	104.0 ± 0.34^{e}	7.8 ± 0.20^{b}	27.5 ± 1.24^{c}	22.0 ± 1.52^{a}	3.0 ± 0.08^{c}	15.4 ± 0.52^{d}	0.9 ± 0.07^{d}
Chemical Zn* (T$_2$)	112.5 ± 0.78^{c}	9.3 ± 0.36^{ab}	31.5 ± 0.55^{b}	22.7 ± 0.64^{a}	3.4 ± 0.06^{b}	25.0 ± 0.43^{b}	1.3 ± 0.03^{b}
S. marcescens FA-4 (T$_3$)	109.3 ± 0.52^{d}	9.1 ± 0.55^{ab}	30.1 ± 0.57^{b}	22.3 ± 0.18^{a}	3.3 ± 0.02^{b}	18.7 ± 0.52^{c}	1.1 ± 0.04^{c}
S. marcescens FA-4 + Chemical Zn** (T$_4$)	114.7 ± 0.51^{b}	8.9 ± 0.57^{ab}	31.1 ± 0.48^{b}	23.0 ± 0.57^{a}	3.4 ± 0.05^{b}	26.2 ± 0.15^{ab}	1.5 ± 0.02^{a}
S. marcescens FA-4 + Chemical Zn*(T$_5$)	117.7 ± 1.04^{a}	9.5 ± 0.43^{a}	34.3 ± 0.87^{a}	22.7 ± 0.23^{a}	3.6 ± 0.05^{a}	26.5 ± 0.23^{a}	1.6 ± 0.03^{a}
2014							
Control (T$_1$)	106.0 ± 0.57^{c}	8.2 ± 0.20^{b}	29.3 ± 0.81^{a}	20.0 ± 0.57^{c}	3.2 ± 0.02^{b}	16.1 ± 0.52^{c}	0.9 ± 0.07^{d}
Chemical Zn* (T$_2$)	112.1 ± 0.84^{b}	9.9 ± 0.52^{a}	30.9 ± 1.19^{a}	21.9 ± 0.20^{a}	3.5 ± 0.03^{a}	25.8 ± 0.43^{a}	1.4 ± 0.03^{b}
S. marcescens FA-4 (T$_3$)	107.1 ± 0.57^{c}	8.1 ± 0.57^{b}	28.1 ± 0.97^{a}	20.1 ± 0.49^{bc}	3.2 ± 0.02^{b}	20.4 ± 0.54^{b}	1.2 ± 0.03^{c}
S. marcescens FA-4 + Chemical Zn** (T$_4$)	116.9 ± 0.40^{a}	9.9 ± 0.51^{a}	30.7 ± 0.86^{a}	21.0 ± 0.57^{abc}	3.5 ± 0.01^{a}	26.3 ± 1.01^{a}	1.5 ± 0.03^{ab}
S. marcescens FA-4 + Chemical Zn*(T$_5$)	115.9 ± 0.76^{a}	10.3 ± 0.49^{a}	30.2 ± 1.45^{a}	21.5 ± 0.26^{ab}	3.5 ± 0.02^{a}	26.9 ± 1.27^{a}	1.6 ± 0.04^{a}

Values are the mean of three replicates ± SE and values bearing different letters in the same column (years) are significantly different from each other according to Fisher's LSD ($p < 0.05$). Control = non-inoculated.
* Recommended dose of ZnSO$_4$ (15 kg ha^{-1}), ** Half dose of ZnSO$_4$ (7.5 kg ha^{-1}).

Table 5. Effect of zinc solubilizing *S. marcescens* FA-4 on rice variety Super Basmati in the field experiment.

Treatment	Plant Height	No. Tiller	Chlorophyll Content	Panicle Length	Grain Yield	Grains Zinc Content	ZTI
	(cm)	Plant^{-1}	SPAD Unit	(cm)	tons ha^{-1}	mg kg^{-1}	
2013							
Control (T$_1$)	98.8 ± 1.15^{b}	9.6 ± 0.20^{c}	27.3 ± 1.24^{c}	20.0 ± 1.52^{a}	2.8 ± 0.08^{c}	19.7 ± 0.38^{c}	1.2 ± 0.06^{c}
Chemical Zn* (T$_2$)	109.5 ± 3.33^{a}	11.1 ± 0.36^{a}	33.8 ± 0.55^{a}	19.2 ± 0.64^{a}	3.3 ± 0.06^{b}	29.1 ± 0.67^{a}	1.4 ± 0.04^{ab}
S. marcescens FA-4 (T$_3$)	101.5 ± 0.83^{b}	9.7 ± 0.55^{bc}	30.0 ± 0.57^{b}	19.6 ± 0.18^{a}	3.2 ± 0.02^{b}	23.1 ± 0.43^{b}	1.3 ± 0.01^{bc}
S. marcescens FA-4 + Chemical Zn** (T$_4$)	108.5 ± 0.49^{a}	10.2 ± 0.03^{abc}	32.0 ± 0.57^{ab}	19.4 ± 0.51^{a}	3.2 ± 0.05^{b}	29.3 ± 0.60^{a}	1.6 ± 0.05^{a}
S. marcescens FA-4 + Chemical Zn*(T$_5$)	107.2 ± 1.24^{a}	11.0 ± 0.43^{ab}	34.0 ± 0.87^{a}	21.3 ± 0.23^{a}	3.5 ± 0.05^{a}	29.5 ± 0.52^{a}	1.6 ± 0.09^{a}
2014							
Control (T$_1$)	100.7 ± 0.55^{b}	9.2 ± 0.20^{a}	30.3 ± 0.81^{a}	18.0 ± 0.57^{c}	3.0 ± 0.02^{b}	20.5 ± 0.38^{c}	1.1 ± 0.05^{c}
Chemical Zn* (T$_2$)	109.8 ± 1.55^{a}	9.9 ± 0.52^{a}	30.8 ± 1.19^{a}	21.2 ± 0.20^{a}	3.4 ± 0.03^{a}	29.9 ± 0.67^{a}	1.4 ± 0.04^{b}
S. marcescens FA-4 (T$_3$)	102.7 ± 0.31^{b}	10.1 ± 0.57^{a}	30.8 ± 0.97^{a}	19.0 ± 0.49^{bc}	3.1 ± 0.02^{b}	23.8 ± 1.00^{b}	1.3 ± 0.05^{bc}
S. marcescens FA-4 + Chemical Zn** (T$_4$)	109.7 ± 0.46^{a}	9.9 ± 0.54^{a}	34.0 ± 1.15^{a}	20.8 ± 0.54^{a}	3.4 ± 0.03^{a}	30.0 ± 0.06^{a}	1.7 ± 0.05^{a}
S. marcescens FA-4 + Chemical Zn*(T$_5$)	111.5 ± 0.58^{a}	10.0 ± 0.49^{a}	33.5 ± 1.45^{a}	20.1 ± 0.26^{ab}	3.4 ± 0.02^{a}	30.1 ± 0.53^{a}	1.7 ± 0.08^{a}

Values are the mean of three replicates ± SE and values bearing different letters in the same column (years) are significantly different from each other according to Fisher's LSD ($p < 0.05$). Control = non-inoculated.
* Recommended dose of ZnSO$_4$ (15 kg ha^{-1}), ** Half dose of ZnSO$_4$ (7.5 kg ha^{-1}).

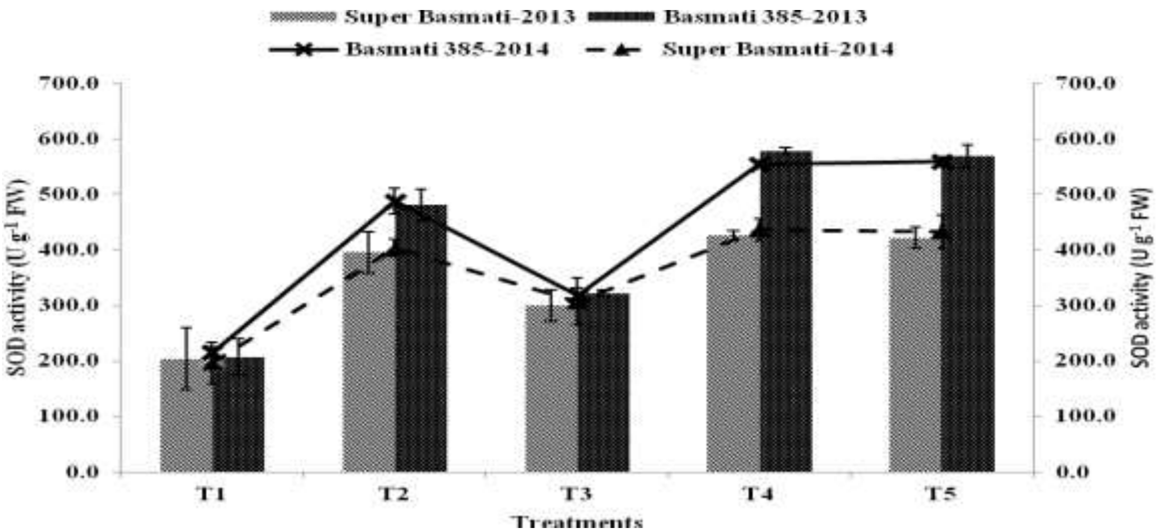

Figure 4. The SOD activity of rice plants treated with zinc solubilizing *S. marcescens* FA-4 in field experiment. Values are mean of three replicates. Bars represent standard errors.

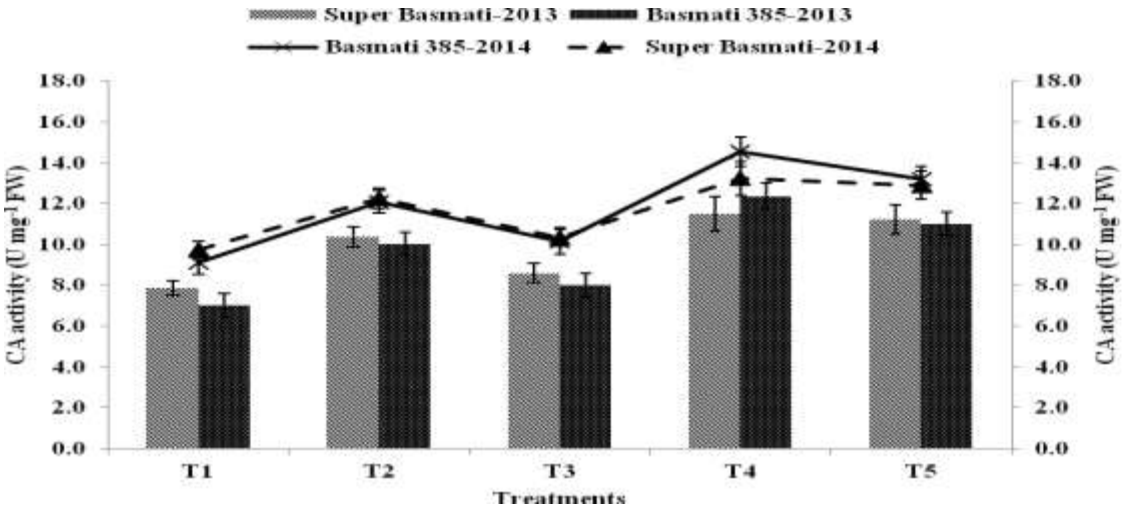

Figure 5. The CA activity of rice plants treated with zinc solubilizing *S. marcescens* FA-4 in the field experiment. Values are the mean of three replicates. Bars represent standard errors.

DISCUSSION

Cereals are recognized significant wellsprings of proteins and dietary minerals, particularly in developing nations. Currently, more than three million people globally suffer from micronutrient malnutrition. Improving the bioavailable nutrients, such as zinc and iron in staple crops, commonly known as biofortication, could be a possible option. Among the approaches directed toward the mineral micronutrient biofortification, enhanced physiological processes, such as uptake from the rhizosphere can have mediation of plant growth-promoting rhizobacteria. Inoculation of ZSB *Serratia marcescens* FA-4 in the presence or absence of chemical Zn proved to enhance plant growth, plant height, number of tillers, chlorophyll content, and yields, while increasing the zinc content in rice plants. The zinc solubilizing rhizobacteria *S. marcescens* FA-4 producing the siderophore, exhibited antifungal activity and synergistic interactions with other ZSB *S. liquefaciens* (FA-2) and *Bacillus thuringiensis* (FA-3) in an earlier study (Abaid-Ullah *et al*., 2015).

Biologically suitable qualities may have played a vital role in improving plant biomass, grain yield, and zinc content. Therefore, a positive correlation between plant biomass, grain yield, and zinc content was notable. The findings also strengthened the earlier studies in which plant height, number of tillers, and grain yield of rice crops gained improvement by PGPR inoculum (Shakeel *et al*., 2015; Kamran *et al*., 2017). Interestingly, the study results indicated *S. marcescens* FA-4 with the half and regular recommended doses of chemical Zn enhanced growth and yield compared with applying a recommended amount of chemical Zn alone. The treatments bearing microbial inputs comprised only a half dose of chemical Zn that showed a savings of 50% chemical Zn, possibly due to PGPR traits supporting the plants to make deeper roots and hunt nutrients even from nutrient-deficient soils.

The zinc uptake from the rhizosphere is the initial phase during the accumulation process in the plant before translocation to seeds. The plant growth-promoting rhizobacteria play a significant role in nutrient solubilization from soil and increasing their accessibility to plants. The research's PGPR strain also showed PGPR traits, i.e., IAA production and P solubilization (Abaid-Ullah *et al*., 2015), which may have helped the rice plant show better uptake of micro and macronutrients. In this investigation, zinc solubilizing bacteria *S. marcescens* FA-4 inoculation improved the Zn content of rice plants, representing a promising role in enhancing the translocation of micronutrients. These findings also agree with earlier reports that this strain enhanced Zn uptake in wheat crops (Abaid-Ullah *et al*., 2015). Better flux into shoots could give extra micronutrients for grain biofortification and activate native homeostasis mechanisms that enhance root uptake capability. It is a fact that the antioxidative system of plants removes reactive oxygen species (ROS), which are produced in plants from the electron transport chain and molecular oxygen due to the leakage of electrons (Dumanović *et al*., 2021).

Table 6. Pearson's correlation among zinc-requiring enzyme contents, grain yield, and zinc translocation index toward the grain of rice in pot.

Genotypes and traits	SOD	CA	Grain yield	Zinc translocation index (ZTI)
Super Basmati				
SOD	1.0			
CA	0.98**	1.0		
Grain yield	0.94**	0.97**	1.0	
Zinc translocation index (ZTI)	0.95**	0.98**	0.99**	1.0
Basmati 385				
SOD	1.0			
CA	0.99**	1.0		
Grain yield	0.95**	0.97**	1.0	
Zinc translocation index (ZTI)	0.95**	0.97**	0.99**	1.0

Asterisks indicate significance as follows *P* ≤ 0.05, **Highly Significance, *Significance.

Table 7a. Pearson's correlation among zinc-requiring enzyme contents, grain yield, and zinc translocation index toward the grain of rice in the field during 2013.

Genotypes and traits	SOD	CA	Grain yield	Zinc translocation index (ZTI)
Super Basmati				
SOD	1.0			
CA	0.75^{**}	1.0		
Grain yield	0.61**	0.61**	1.0	
Zinc translocation index (ZTI)	0.64**	0.47ns	0.51*	1.0
Basmati 385				
SOD	1.0			
CA	0.83**	1.0		
Grain yield	0.62**	0.71**	1.0	
Zinc translocation index (ZTI)	0.92**	0.89**	0.69**	1.0

Asterisks indicate significance as follows *P* ≤ 0.05, **Highly Significance, *Significance.

Table 7b. Pearson's correlation among zinc-requiring enzyme contents, grain yield, and zinc translocation index toward the grain of rice in the field during 2014.

Genotypes and traits	SOD	CA	Grain yield	Zinc translocation index (ZTI)
Super Basmati				
SOD	1.0			
CA	0.83**	1.0		
Grain yield	0.71**	0.68**	1.0	
Zinc translocation index (ZTI)	0.72**	0.74**	0.76**	1.0
Basmati 385				
SOD	1.0			
CA	0.82**	1.0		
Grain yield	0.94**	0.77**	1.0	
Zinc translocation index (ZTI)	0.93**	0.84**	0.86**	1.0

Asterisks indicate significance as follows $P \leq 0.05$, **Highly Significance, *Significance.

The ROS formation and removal under many types of stress lack balance resulting in excess, which causes oxidation of biomolecules like nucleic acids, chlorophyll, proteins, and lipids. In this aspect, the superoxide dismutase (SOD) enzyme plays a critical role by acting as the first line of defense to scavenge the ROS (Alscher *et al*., 2002; Mishra and Sharma, 2019). This enzyme has a cupro zinc protein property, which sustains zinc homeostasis by protecting the plant from oxidative stress. PGPR making a close association with plants not only change the rhizosphere processes but also affects the plant physiology, particularly enhancing the activity of antioxidant enzymes. This latest study showed an effective PGPR that enhances yield and grain zinc content. Moreover, a positive correlation occurred between the zinc application with zinc solubilizing strain, the activity of zinc-requiring enzymes, grain yield, and zinc translocation index (Table 6, 7a, and 7b). This investigation also provides the yield increased in both half and a regular dose of zinc with zinc solubilizing strain.

Yield enhancement may be due to proper enzymes functioning under sufficient zinc supply. An increase in grain yield may be because Zn is a cofactor of enzymes that are directly involved in the photosynthesis process (i.e., rubisco and carbonic anhydrase), which may have prompted the photosynthates in the grain, thus expanding the grain yield. The study follows that of Bharti *et al*. (2014) in which an observed expansion in the grain yield accounts for both soil and foliar use of zinc. Another reason for yield increment is the appropriate working of both enzymes. In the current examination, augmentation appeared in the carbonic anhydrase and superoxide dismutase enzyme action with the dynamic addition of zinc level along with *S. marcescens* FA-4 inoculation. This may be that zinc is required as a cofactor in influencing CA and SOD (Cu-Zn SOD). Given this reason, a fall could occur under Zn deficit conditions (Negative control) and an enhancement under zinc supply (half dose of chemical Zn + *S. marcescens* FA-4). Although, the regular dose of chemical Zn + *S. marcescens* FA-4 was the best supplementation that has indicated greater augmentation, which could refer to the proper use of zinc at the cell or tissue level. Hence, this study derives support from the findings of former researchers who stated an augmented SOD activity in response to zinc (Frei *et al*., 2010; Singh *et al*., 2019). Copper/zinc SOD could play a straight part in zinc tolerance (Hacisalihoglu and Kochian, 2003).

The increase in yield could refer to the better working of SOD, which helps scavenge ROS. ROS scavenging is essential in the formation of assimilates. Heavy metal stresses cause the fast development of ROS and disrupt the cell's redox homeostasis. The ROS cause oxidative injury to primary biomolecules, such as proteins, lipids, and nucleic acids, impeding plant growth and development (Sharma and Dubey, 2007; Gao *et al*., 2008). The antioxidant plants enzymes, such as guaiacol peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD), control H_2O_2 and O_2 concentrations in the cell to search for ROS and keep the cell away from oxidative damage (Gratão *et al*., 2005; Sharma and Dubey 2007). Zinc can contribute to the structure of SOD isozyme. Therefore, the combined application of chemical Zn and Zn solubilizing strain *S. marcescens* FA-4 enhanced SOD activity. Tavallali *et al*. (2010) also found the same results that SOD enzyme activity increased in response to Zn. Hence, from these results, proposing SOD enzyme activity as an indicator of zinc nutritional status for plant parts and grains is valid.

CONCLUSIONS

The combination of zinc solubilizing bacteria *S. marcescens* FA-4 with chemical zinc exhibited consistency in improving rice growth and yield and enhancing grain zinc content under pot and field conditions. Furthermore, the zinc solubilizing strain with zinc application enhanced the activity of zinc-requiring enzyme, which enabled the plant to accumulate more chlorophyll content enhancing the grain yield and zinc translocation toward the grain. These findings suggest that using this strain with chemical zinc can be a good inoculant for improving rice grain productivity and nutritional quality, consequently decreasing malnutrition. The integrated use of this strain with other biofortification strategies could be another choice for future study. However, more studies are necessary to explore further the zinc translocation toward rice grain.

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