

Deciphering the Mechanisms of Endophyte-Mediated Biofortification of Fe and Zn in Wheat

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Abstract An investigation was carried out to understand the mechanism(s) underlying enhanced Fe or Zn uptake in low Fe–Zn accumulator wheat genotype 4HPYT-414, due to inoculation of siderophore-producing and zinc-solubilizing endophytes—*Arthrobacter sulfonivorans* DS-68 and *Arthrobacter* sp. DS-179. Root anatomical features, using transmission electron microscopy (TEM), qualitative and quantitative aspects of production of organic acids and sugars in root exudates, and expression of *TaZIP* genes were analysed to relate to endophyte-mediated higher concentrations of Fe and Zn in the roots and shoots of wheat plants. TEM studies revealed that the endodermis, cortical region, root hair extension, xylem and xylem vessels, pericycle and vascular bundles were more pronounced and thicker in inoculated treatments, as compared to control. The organic acid profile of root exudates revealed five types of organic acids, with citric acid being predominant. Inoculation of *A. sulfonivorans* and *Arthrobacter* sp. brought about 5- and eightfold increases in the amounts of acids,

respectively, as compared to control, particularly citric acid, succinic acid and acetic acid. Among the four *TaZIP* genes targeted, expression was achieved only for *TaZIP3* and *TaZIP7* genes, which showed 1–2 fold increases in the inoculated treatments. The results clearly indicated that the endophyte-mediated overexpression of *TaZIP3* and *TaZIP7* genes in roots and shoots, and the observed anatomical and exudate changes were acting synergistically in facilitating higher Fe and Zn translocation in roots and shoots.

Keywords Anatomical features · Gene expression · Root exudates · Organic acids · *TaZIP* · TEM

Introduction

Bacteria capable of solubilizing insoluble sources of Zn have been reported to enhance uptake of zinc by 21% in soybean (Sharma and others 2012); similarly there are reports on the use of siderophore-producing bacteria to enhance the uptake and transport of Fe to the grains (de Santiago and others 2011; Rana and others 2011). Bacterial and fungal endophytes are a less investigated group, although a few reports have implicated them in the biofortification of grains of wheat and rice, particularly Fe and Zn (Ramesh and others 2014; Abaid-Ullah and others 2015). Different mechanisms have been reported for this enhanced uptake of Fe or Zn on the inoculation of endophytes. Chelation of iron by siderophore-producing microorganisms, production of organic acids in the root exudates leading to changes in the soil pH, proton extrusion by rhizospheric microorganisms, production of phytohormones such as auxin, ethylene and gibberellic acid, have all been implicated in the enhanced uptake of iron and zinc (Fasim and others 2002; Li and others 2010; Desai and Archana 2011;

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Hayat and others 2010; Hindt and Guerinot 2012; Ivanov and others 2012; Kobayashi and Nishizawa 2012; Chen and others 2014). The root exudates of plants significantly influenced the solubilization and mobilization of soil nutrients by modulating the biological and chemical processes operating in the rhizosphere (Zhang and others 2010). Reports on modifications in the root exudation patterns due to inoculation of microorganisms (Malinowski and others 2004; da Silva Lima and others 2014) have revealed that besides sugars, amino acids, phenolics, and organic acids are also important constituents of root exudates from different plants. The increase in the production of organic acids due to microbial inoculation results in the lowering of soil pH around the roots contributing to the solubilization of different insoluble sources of nutrients like P, K and Zn (Goswami and others 2014; Meena and others 2015; Sirohi and others 2015).

It is well established that inoculation of plant growth-promoting rhizobacteria or endophytes significantly enhances the root surface area, root volume, number of root tips and root biomass (Vacheron and others 2014; Delaplace and others 2015). Further, it is also known that plants with prolific root systems have better nutrient use efficiency and crop stand (Dong and others 1995; Genc and others 2007; Lynch 2007). Besides root architecture, anatomical features of the root are also influenced by the soil microflora. Inoculation of rhizobacteria *Burkholderia pyrrocinia* (R-46) + *Pseudomonas fluorescens* (R-55) and *Trichoderma asperellum* was reported to enhance the diameter of vascular cylinders, number of protoxylem and metaxylem vessel elements and expansion of root cortex, aerenchyma, and so on (Rêgo and others 2014). In the present investigation, endophytic bacteria, isolated from wheat roots were used, which were also able to grow under free-living conditions. As these bacteria are originally endophytes, and not rhizobacteria, our investigation represents a first time report related to the inoculation of endophytes and their effects on the root anatomy.

Significant variations in the uptake of nutrients and accumulation in root, shoot and grains have been documented (Chatzistathis and others 2009); among which most of the variation can be attributed to the critical role of metal transporters in the translocation of nutrients from shoots to grains. There are several metal transporters in plants including zinc-regulated transporter (*ZRT*), iron-regulated transporter (*IRT*), the cation diffusion facilitator (*CDF*) family and the zinc–iron transporter-like protein (*ZIP*) family that are involved in the translocation of zinc and iron (Colangelo and Guerinot 2006). *ZIP* family proteins have been reported in rice, wheat, maize and *Arabidopsis thaliana* (Grotz and others 1998; Ishimaru and others 2005; Krämer and others 2007; Xu and others 2010). The overexpression of these proteins leads to the accumulation of excess

amounts of zinc in the cells of wild emmer wheat (Durmaz and others 2011). It needs to be clarified whether inoculation of rhizospheric microorganisms or endophytes leads to the overexpression of transporter genes and consequently enhanced accumulation of iron or zinc in the grains. Our earlier findings revealed that inoculation of siderophore-producing (*Arthrobacter sulfonivorans* DS-68 and *Enterococcus hirae* DS-163) and zinc-solubilizing endophytes (*Bacillus subtilis* DS-178, *Arthrobacter* sp. DS-179) can enhance the uptake of iron and zinc, respectively, in wheat genotypes with different nutrient use efficiencies (Singh 2016; Singh and others 2017). The present study was carried out using a hydroponic system to determine the quantitative and qualitative variations in organic acids and sugars, changes in the root anatomy and also expression of *ZIP* family proteins, due to endophyte inoculation.

Materials and Methods

Microorganisms and Wheat Genotypes

The endophytes, *A. sulfonivorans* DS-68 and *Arthrobacter* sp. DS-179, were isolated and identified earlier from wheat roots, and found to be efficient in the fortification of Fe and Zn in wheat genotypes. These details are given in our previous studies (Singh 2016; Singh and others 2017). Both these bacterial strains were maintained on nutrient agar slants and stored at 4 °C, until use.

Seeds of the low Fe- and Zn-accumulating wheat genotype-4HPYT-414 were obtained from the Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi.

Pot Experiment Under Hydroponic Conditions

A hydroponic experiment was carried out in the National Phytotron Facility, ICAR-Indian Agricultural Research Institute, New Delhi. Wheat seeds were surface sterilized using 0.1% HgCl₂ and 70% alcohol. The broth cultures of both endophytes were grown separately to reach 10⁹ CFU mL⁻¹. The sterilized seeds were coated with inoculum by soaking the seeds in broth for 30 min. Seeds coated only with nutrient broth were used as the control treatment. Seeds were germinated on 0.3% agar. After 4 days, the seedlings were transferred into the Hoagland solution (1X) containing hydroponic system. A total of 12 replications were maintained for each of the three treatments. After 30 days of growth, a set of three replicates from each treatment were used for (a) root exudate studies, (b) estimation of Fe and Zn in roots and shoots, (c) root anatomical studies and (d) expression of *ZIP* transporter genes.

Estimation of Zinc and Iron in Plants

Plants were harvested from three replicates after 30 days of sowing, which represents the period of critical root initiation. The plant parts were ground to a fine powder; samples (0.5 g) were digested with 10 mL of di-acid mixture (nitric acid and perchloric acid) in 4:1 ratio at 300 °C using hot plate, until it became colourless. Digested samples were transferred to volumetric flasks (50 mL), the volume was made up to 50 mL with distilled water and subjected for analysis of zinc and iron using an atomic absorption spectrophotometer (Analytik Jena ZEE nit 700), along with standards (Lindsay and Norvell 1978).

Transmission Electron Microscopy (TEM) of Root System

Plants were harvested from three replicates after 30 days of sowing. Small pieces (1 mm³) of roots from each treatment and controls were sectioned and fixed with 4% glutaraldehyde in 0.1 M sodium cacodylate at pH 7.4. The samples were rinsed with the same buffer, post-fixed in osmium tetroxide and dehydrated with acetone. Samples were embedded in epoxy resin Spurr and thin transverse section (60 nm) were sliced using an ultramicrotome with a diamond knife. The sections were mounted in copper grids of 200 mesh and stained with 2.5% uranyl acetate and lead citrate. Observations and images were captured using a transmission electron microscope (JEOL, JEM 1011, USA) available at the Microscopy Centre, All India Institute of Medical Sciences, New Delhi, India and Division of Plant Pathology, ICAR-IARI, New Delhi. Magnification ($\times 30,000$) was uniformly used, which is equivalent to 1 μ in the images.

Extraction and Analysis of Root Exudates Through HPLC

Root exudates (50 mL) were collected from the remaining three replicates of each treatment, filtered and evaporated to dryness under vacuum at 50 °C followed by resubilization in methanol. The quantity of organic acids and sugars in root exudates was determined using HPLC (high performance liquid chromatography; binary pump model 515, 2414 refractive index (RI) and 2998 photodiode array (PDA) detector; Supelco C-18 column having 300 \times 4 mm \times 5 μ m; Waters Pvt. Ltd.) Samples obtained from each treatment were pooled individually and 10 μ L of each sample was injected with the help of autosampler. Samples were analysed using 5 mM H₂SO₄ as the mobile phase with an Aminex HPX-87H column for sugars and organic acids and a Supelco C-18 column (300 \times 4 mm \times 5 μ m; Bio-Rad Laboratories, Hercules, CA

column, 50 °C) for phenolics with a flow rate of 0.5 mL/min. Photodiode array and refractive index detectors were used to record the signals of organic acids and sugars, respectively, and quantified using the values of standards injected.

Validation of qRT-PCR for ZIP Gene Expression in Roots and Shoots of Wheat

The root and shoot samples were taken and total RNA was isolated using the Trizol RNA isolation method (Rio and others 2010). The expression of unigenes (*ZIP1*, *ZIP3*, *ZIP5* and *ZIP7*) with potential roles in zinc and iron uptake were chosen for validation using qRT-PCR. Three independent biological samples of each were used in the analysis. cDNA was synthesized from 2 μ g of total RNA using the Superscript[®] III first strand cDNA synthesis system (Invitrogen, USA), as per the manufacturer's protocol. The gene specific primers were designed from the available *TaZIP* gene sequences in NCBI database and listed in Table 1. qRT-PCR was performed using an Agilent Mx3000P[™] PCR platform and KAPA SYBR FAST qPCR kit Master Mix (2X) Universal (Kapa Biosystems, Woburn, MA) as per the manufacturer's instructions. The relative expression levels of the selected unigenes normalized to the expression level of actin were calculated from the cycle threshold values using the 2^{- $\Delta\Delta$ Ct} method. This experiment was carried out using three independent biological replicates and three technical replicates of each biological replicate.

Statistical Analysis

The experimental data were presented as the mean value of three replications of each individual experiment. Results obtained from each experiment were statistically analysed using two-way analysis of variance (ANOVA). Mean values of obtained data between treatments were compared with Tukey's least significant difference (LSD) test ($P \leq 0.05$).

Table 1 Primers used for quantitative real-time reverse transcription PCR

Target gene	RT-PCR-primers sequence
<i>TaZIP1</i>	ZIP1-F-AGGTAAGGGCAACCATCATC ZIP1-R-GTCCACCAGGGACATGTAGA
<i>TaZIP3</i>	ZIP3-F-ATGGCCACAATGGTGATAGA ZIP3-R-CTGACAACAATTGCCCTCACC
<i>TaZIP5</i>	ZIP5-F-TCTTCTTCTCTGGTCAAGGCT ZIP5-R-TCCGTCCTTGTTCAAGTGG
<i>TaZIP7</i>	ZIP7-F-AGCGACGAAGACATCACTGT ZIP7-R-ATCTGTGATACCACGACGGA

Results

Estimation of Zn and Fe

The accumulation of Fe and Zn in the roots and shoots of the low Fe-Zn accumulator wheat genotype (4HPYT-414) increased significantly due to inoculation of siderophore-producing (*A. sulfonivorans* DS-68) and zinc-solubilizing endophytes (*Arthrobacter* sp. DS-179), respectively (Table 2). In general, the percent increase in the accumulation of Fe or Zn, over uninoculated control, due to inoculation of endophytes was higher in shoots as compared to roots except for Zn accumulation in the *Arthrobacter* sp. DS-179 inoculated treatment. *Arthrobacter* sp. DS-179, a zinc solubilizer was also able to enhance the uptake of Fe, although the percent increase over the uninoculated control was 50% lower than the values recorded for *A. sulfonivorans* DS-68. Likewise, *A. sulfonivorans* DS-68, a siderophore producer, was also able to enhance the uptake of Zn in roots and shoots, but the percent increase was less than half of that recorded in the treatment with *Arthrobacter* sp. DS-179 (Table 2).

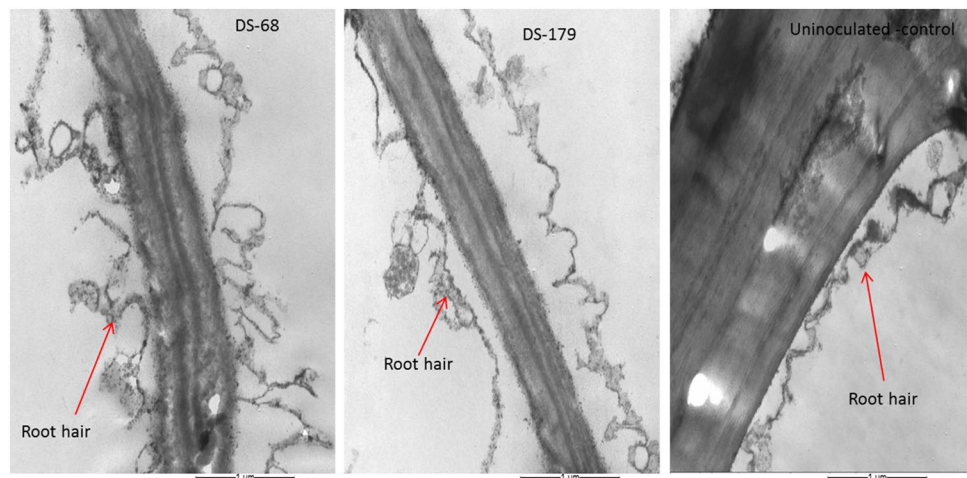
Table 2 Influence of inoculation of endophytes on zinc and iron uptake in root and shoot of wheat

Treatments	Fe concentration (mg/kg DW)		Zn concentration (mg/kg DW)	
	Root	Shoot	Root	Shoot
Control	90.0 ± 26.46 ^c	47.5 ± 13.23 ^c	62.2 ± 17.16 ^c	40.4 ± 15.28 ^c
DS-68	185.0 ± 24.66 ^a	115.0 ± 18.93 ^a	93.5 ± 19.16 ^b	64.5 ± 16.07 ^b
DS-179	136.5 ± 32.15 ^b	76.5 ± 10.41 ^b	126.5 ± 30.55 ^a	96.5 ± 20.82 ^a

Data are the average of three replicates ±SD. Means with different letters in the same column differ significantly at $P \leq 0.05$ (Tukey’s LSD)

DW dry weight

Fig. 1 Transmission electron microphotographs showing anatomical changes in root hairs, due to inoculation of endophytes. Scale bar represents 1 μm



Transmission Electron Microscopy (TEM) of Roots

Variations were observed in some of the anatomical features of roots due to inoculation of endophytes. The endodermis and cortical region were more pronounced and thicker in the inoculated treatments as compared to control (Fig. 2). Other features which were typically more elaborate in the inoculated treatments were root hair extensions (Fig. 1), pericycle and vascular bundles (Fig. 2), xylem and xylem vessels (Fig. 3) and sieve tubes (Fig. 4). The formation of a callose band in the sieve tubes showed a reduction in thickness in the inoculated treatments (Fig. 4).

Analysis of Organic Acids and Sugars

The data on the secretion of organic acids and sugars by roots of wheat genotype are presented in Table 3 and Supplementary Figs. 1 and 2. The organic acid profile showed the presence of five types of organic acids in the root exudates, with citric acid being the predominant acid produced. There was a qualitative and quantitative change in the organic acid profile of root exudates of inoculated, as compared to the uninoculated treatment. Quantitatively distinct variations were observed in the amount of organic acids produced in the exudates due to inoculation. The amount of total organic acids was five and eightfold higher due to

Fig. 2 Transmission electron microphotographs depicting anatomical changes due to inoculation of endophytes in endodermis (E), cortex (C), pericycle (P), vascular bundle (V). Scale bar represents 1 μ m

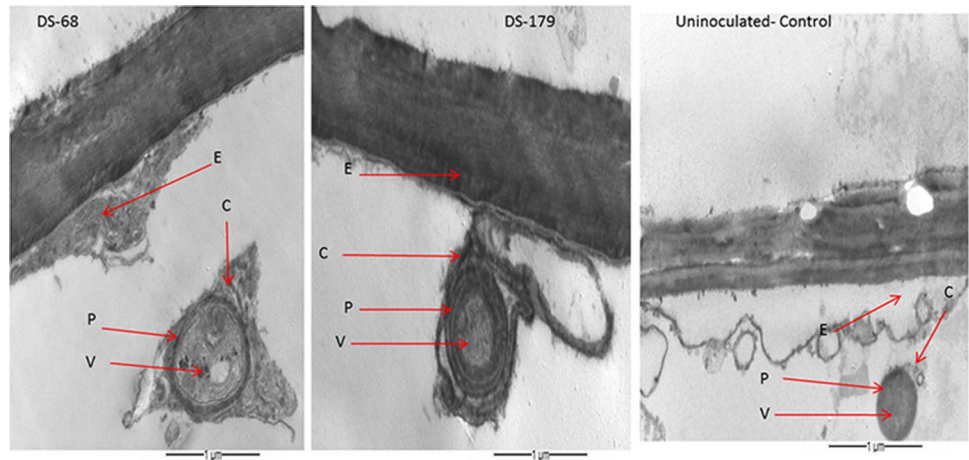


Fig. 3 Transmission electron microphotographs showing anatomical modifications in the xylem vessels and number of vessels, due to inoculation of endophytes. Scale bar represents 1 μ m

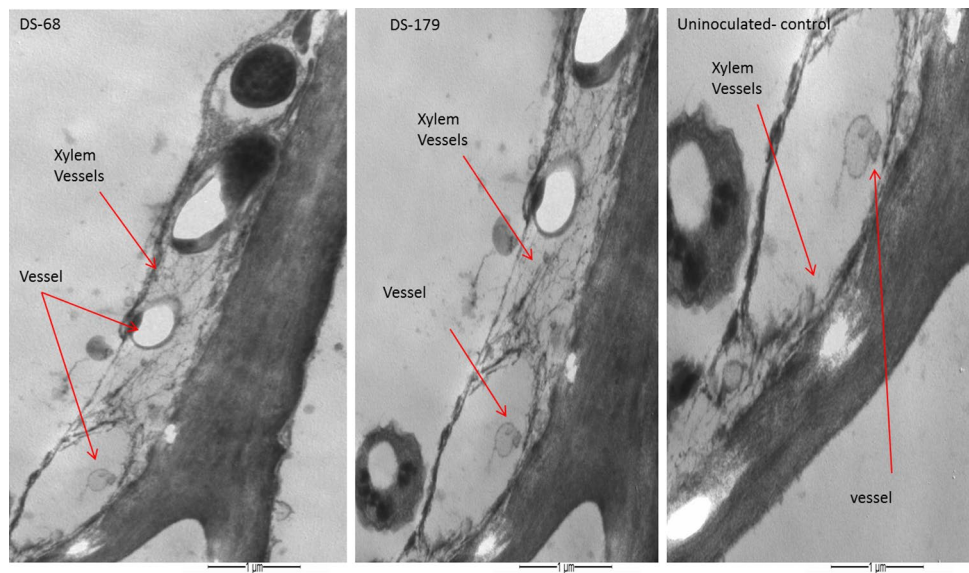


Fig. 4 Transmission electron microphotographs showing anatomical modifications in the sieve tubes of phloem tissue, due to inoculation of endophytes. Scale bar represents 1 μ m

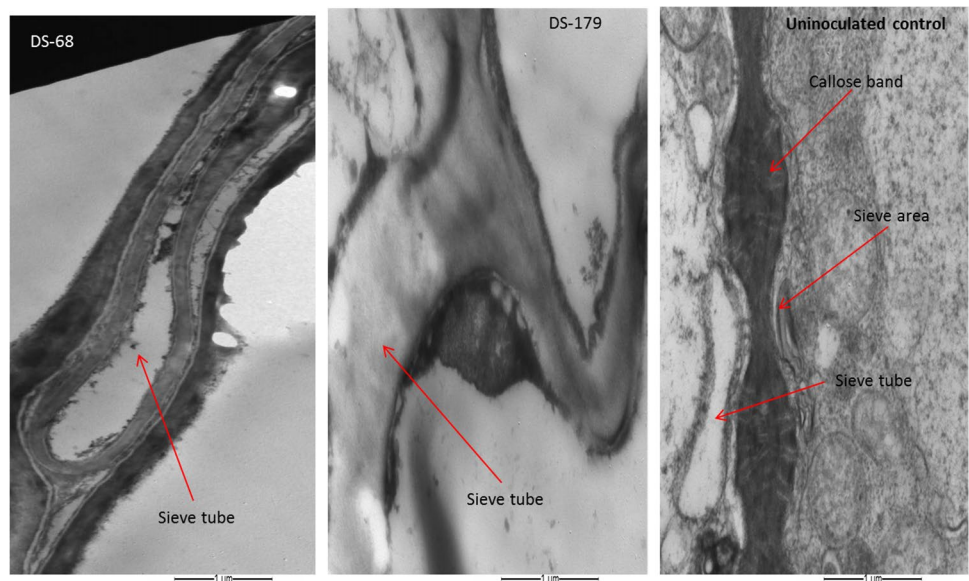


Table 3 Organic acid and sugar profile of root exudates of wheat as influenced by inoculation of endophytes

Treatments	Concentrations (g/L)							
	OA	CA	MA	SA	AA	TA	Glu	Suc
Control	0.00	8.31	0.64	0.23	0.14	9.32	5.6	0.01
DS-68	0.035	43.70	0.85	0.98	3.00	48.56	10.63	0.03
DS-179	0.36	62.35	0.80	1.93	7.57	73.04	9.05	0.03

OA designated oxalic acid, CA citric acid, MA malic acid, SA succinic acid, AA acetic acid, TA total acid, Glu glucose, Suc sucrose

inoculation of *A. sulfonivorans* DS-68 and *Arthrobacter* sp. DS-179, respectively (Table 3), as compared to uninoculated control. Inoculation of endophytes resulted in the production of oxalic acid in root exudates. The amount of citric acid increased by 425 and 650% due to inoculation of *A. sulfonivorans* DS-68 and *Arthrobacter* sp. DS-179 over control. Succinic acid was sixfold higher due to inoculation of endophytes as compared to uninoculated control. The acetic acid concentration also increased several fold in the root exudates of inoculated treatments (Table 3). In the root exudates, the concentrations of glucose and sucrose were significantly higher in the inoculated treatments ($P \leq 0.05$).

Validation of qRT-PCR for ZIP Gene Expression in Roots and Shoots of Wheat

Four ZIP genes were targeted for expression studies using gene specific primers. However, only ZIP3 and ZIP7 genes could be expressed in wheat genotype 4HPYT-414 samples. In the shoots, inoculation of zinc-solubilizing *Arthrobacter* sp. DS-179 and siderophore-producing *A. sulfonivorans* DS-68 endophytes led to 1.91- and 1.00-fold increase in TaZIP3 transcripts, respectively, with a corresponding increase in the TaZIP7 transcript of 4.0- and 2.6-fold, respectively. The expression levels of TaZIP3 and TaZIP7 transcripts in roots due to *Arthrobacter* sp. DS-179 inoculation were 1.7- and 0.61-fold higher than the uninoculated control, respectively. The corresponding values for enhanced expression of the two genes in *A. sulfonivorans* DS-68-treated seedlings were 1.5- and 2.17-fold, respectively (Table 4).

Discussion

The present investigation was carried out to understand the mechanisms involved in siderophore-producing and zinc-solubilizing endophytic bacteria-mediated uptake and accumulation of iron and zinc in a selected low Fe- and Zn-accumulating wheat cultivar. Important traits such as root anatomical features, qualitative and quantitative production of organic acids and sugars in root exudates, and expression

Table 4 Enhancement in expression levels (expressed as fold increase) of TaZIP3 and TaZIP7 genes, due to inoculation of endophytes

Treatments	Gene expression level over the control [#] (times fold)	
	TaZIP3	TaZIP7
Shoot	<i>Arthrobacter sulfonivorans</i> DS-68	1.0 ± 0.4
	<i>Arthrobacter</i> sp. DS-179	1.9 ± 0.04
Root	<i>Arthrobacter sulfonivorans</i> DS-68	1.5 ± 0.2
	<i>Arthrobacter</i> sp. DS-179	1.7 ± 0.2

[#]Control refers to uninoculated treatment; data are the average of three replicates ± SD

of TaZIP genes were analysed in a hydroponic experiment with wheat plants.

Plant growth-promoting microorganisms, free living or endophytic, are known to elicit hormone and signalling pathways related to metabolic pathways which modulate macro- and micronutrient uptake and translocation in plants (Abaid-Ullah and others 2015; Hayat and others 2010). In the present investigation, the inoculation of both siderophore-producing and zinc-solubilizing endophytes significantly enhanced the uptake of iron and zinc in the roots and shoots of wheat. Reports are available on bacteria-mediated increases in the concentrations of iron or zinc in rice (Wang and others 2014), wheat (Abaid-Ullah and others 2015; Rana and others 2012), chickpea (Khalid and others 2015), soybean (Ramesh and others 2014) and maize (Prasanna and others 2015). Hydroponic studies using nutrient solutions with wheat plants by other researchers (Glinska and others 2016; Ai-Qing and others 2011) revealed a similar trend in the concentrations of Fe and Zn in roots and shoots, as recorded in our investigation in which seedlings inoculated with endophytes were evaluated. Different mechanisms have been proposed for this increase of iron or zinc in roots, shoots and grains of different crop plants (Neumann and others 1999; Lambers and others 2006; Tariq and others 2007; Wei-Hong and others 2007; Kobayashi and Nishizawa 2012; Momayezi and others 2012). These mechanisms may be involved independently or simultaneously. The results of our study reveal

that the inoculation of endophytes had a significant influence on the root anatomical features, sugar and organic acid profiles of root exudates, and the expression of *TaZIP3* and *TaZIP7*, illustrative of several mechanisms in play.

Agriculturally important microorganisms tend to modify the root architecture and anatomy so as to allow efficient nutrient uptake from the soil (López-Bucio and others 2007; Ortíz-Castro and others 2008). In the present study, expansion of the root cortex and endodermis, elaborate development of root hair extensions, pericycle, xylem vessels, sieve tubes and vascular bundles were observed due to inoculation of both *A. sulfonivorans* DS-68 and *Arthrobacter* sp. DS-179 individually, which were originally endophytes, but able to grow also as free-living bacteria. Similar results have been reported in rice roots due to inoculation of plant growth-promoting rhizobacteria such as *B. pyrrocinia* and *P. fluorescens* and a fungus—*T. asperellum* (Rêgo and others 2014). Kramer and others (1980) also correlated the thickening of the root cortex and transfer cells and the number and diameter of xylem vessels with enhanced iron uptake in *Helianthus annuus*. The better developed pericycle in the roots of inoculated plants correlates very well with the increase in root surface area and root volume. Because the pericycle is the region from which lateral roots differentiate, the increased size of its cells and circumference contributes to root development and consequently to better water and nutrient uptake (Kotula and others 2009). Future research needs to look at the effect of dual inoculation, whether it is additive and which parameters are affected differentially.

The root exudates influence not only the population and diversity of microorganisms in the rhizosphere, but also regulate the uptake of metal ions through various processes such as chelation, dissolution and redox reactions (Clemens and others 2002; Kuang and others 2002). Some of the nutrients like phosphorus, potassium, zinc and iron when applied to the soil as chemical fertilizers get fixed, and are not available to the plants. The organic acids produced by the plants or the microorganisms are considered to be important in changing unavailable forms of these nutrients to available forms. Several reports involving plant–microbe interaction studies focus on the increase in plant biomass, yield and uptake of nutrients (Ji and others 2014; Shao and others 2015; Zahid 2015). Some of these studies also indicate changes in the exudation pattern in the microbe-inoculated treatments, which favours the dissolution and uptake of nutrients (Raja and others 2006; Chen and others 2014; da Silva Lima and others 2014). In the present investigation, the siderophore-producing endophytes modulated the organic acid profile, both qualitatively and quantitatively. The root exudates from the control treatment were devoid of oxalic acid whereas it was detected in the root exudates of endophyte-inoculated plants. The exudates from the

inoculated treatment showed the presence of five organic acids namely, citric acid, malic acid, oxalic acid, succinic acid and acetic acid. Among them, the proportion of citric acid was 87% of the total organic acids. Earlier studies report the exudation of citric acid, malic acid and oxalic acids from the roots of different crop plants (Jones 1998; Zheng and others 1998; Winkelmann 2007; Li and others 2009; Carvalhais and others 2011). A several-fold increase in the total organic acids in the root exudates of endophyte-treated plants correlated very well with the enhanced uptake of zinc and iron, suggesting it to be one of the major mechanisms facilitating biofortification in plants.

The third mechanism we investigated was searching for exchange of signals between the endophytes and plants so as to trigger overexpression of genes of the *ZIP* family. Among the four *TaZIP* genes targeted, expression of only *TaZIP3* and *TaZIP7* was observed in the wheat treatments. This clearly indicates the significance of endophyte-mediated overexpression of these two genes in the roots and shoots of the 30-day-old wheat seedlings or conversely the upregulation of *ZIP* genes both in roots and shoots of the wheat plants suggests the involvement of endophytes. The zinc-solubilizing *Enterobacter cloacae* strain ZSB14 has been reported to upregulate *OsZIP1* and *OsZIP5* expression and downregulate *OsZIP4* expression in rice genotype (Krithika and Balachandar 2016). In addition to the transporter of the *ZIP* family, there are other transporter proteins that are involved in the translocation of zinc from roots to shoots and shoots to grains. These need to be investigated in future research.

This study can be taken as a starting point to look for the expression of different transporter genes and to identify the signalling molecules exchanged between the endophyte and the plant which leads to enhanced uptake of iron and zinc in the plant.

Conclusion

Endophytes help in the biofortification of Zn and Fe in the wheat genotype that was investigated employing different mechanisms. Endophyte-inoculated plants showed changes in root morphology and anatomy, conducive for better uptake of nutrients. The organic acid profiles of root exudates from endophyte-inoculated treatments and uninoculated control treatments were markedly different. Higher concentrations of organic acids in the root exudates of treated plants can facilitate the dissolution and uptake of Zn and Fe. The upregulation of *TaZIP3* and *TaZIP7* genes in endophyte-treated plants also suggested signalling between the two partners. Based on the three mechanisms studied, it could be surmised that future research should focus on the role of interactions of these endophytes with

plant-mediated signalling mechanisms or the plant microbiome, thereby facilitating the enrichment of micronutrients in plant parts.

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