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REGULAR ARTICLE

Beneficial role of endophytes in biofortification of Zn in wheat genotypes varying in nutrient use efficiency grown in soils sufficient and deficient in Zn

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Abstract
Background and aim Most of the food grains show deficiency of zinc. The study was carried out to evaluate the role of endophytes in the fortification of Zn in wheat genotypes with different nutrient use efficiency and in soils deficient and sufficient for Zn.
Methods Two zinc solubilizing endophytes (*Bacillus subtilis* DS-178 and *Arthrobacter* sp. DS-179) were used to inoculate low and high Zn accumulating genotypes in soils sufficient and deficient in Zn.
Results The data on different root morphological parameters, yield and accumulation of Zn indicated distinct variations among genotypes; soil types and also among the endophytes inoculated, un-inoculated and chemical fertilizer treatments. In general, the amount of Zn in grains due to inoculation of endophytes was 2 folds higher as compared to un-inoculated control. The low and high Zn accumulating genotypes responded in an almost identical manner to endophyte inoculation, irrespective of the soil types.
Conclusion Zn solubilizing endophytes can enhance the translocation and enrichment of Zn to grains in wheat genotypes, irrespective of their different nutrient use efficiency (Zn). This approach can be integrated into the modern strategies for biofortification.

Keywords Endophytes · Wheat genotypes · Root morphology · Translocation · Zn

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Introduction

Deficiency of micronutrients, particularly Zn in soil is a major limiting factor in achieving the yield potential of various crops (Ismail et al. 2007). This is because micronutrients are an absolute requirement for plant growth and many metabolic activities of the plant like photosynthesis, respiration, chlorophyll synthesis, protein synthesis and reproduction (Hänsch and Mendel 2009; Römheld and Marschner 1991; Welch and Shuman 1995). Cereal crops, particularly rice and wheat represent the largest proportion of routine dietary food in countries that have reported most of the micronutrient deficiency incidences (Bouis et al. 2011; Cakmak et al. 2010). In India and other developing nations, deficiency of Zn in the diet is a contributing factor to the development of malnutrition symptoms (White and Broadley 2009). The grains of these cereal crops need to be fortified with Zn to lessen these incidences.

Artificial application of micronutrients as chemical fertilizers is one of the strategies used to enhance the uptake of micronutrients in the grains. However the major drawback with this strategy is the fact that micronutrient use efficiency in crops is only 2–5% of the applied dose (Xiaohong et al. 2008). Additionally, there are other harmful effects on microbial diversity, soil fertility, nutrient equilibrium and environment (Lockhart et al. 2013; Wu and Ma 2015). Another strategy is to utilize the genotypic variations among the cultivars and select and breed for the ones with high micronutrient use efficiency (Rengel 2001; Velu et al. 2014). The third and the least applied strategy involves utilizing the potential of microorganisms to enhance the nutrient use efficiency of genotypes and fortify the grains of cereal crops. Although there are several reports on the utilization of rhizospheric microorganisms to enhance the acquisition of micronutrients (Gosal et al. 2010; Rana et al. 2012; Sharma et al. 2012), literature on the use of endophytic microorganisms is limited (Ren et al. 2012; Wang et al. 2014). In general, endophytes have been implicated in plant growth promotion and in biocontrol of several pathogenic fungi (Sura-de Jong et al. 2015; Goudjal et al. 2014; Melnick et al. 2011). Endophytes are considered more effective than rhizospheric microorganisms in influencing the plant growth or disease suppression as they are present inside the plant tissues and interact closely with the plant as compared to rhizospheric microorganisms (Reiter et al. 2002; Weyens et al. 2013). Earlier reports on the use of endophytes or rhizospheric microorganisms are silent on the response of different genotypes with differential nutrient use efficiency to endophytes. Plant species and heavy metal pollution participate in the shaping of the dynamic bacterial community of endophytes associated with hyperaccumulator (Chen et al. 2014). The microbiome of bacterial endophytes increases plant growth and plays a beneficial role; several bacteria secrete secondary metabolites for nutrient uptake, which play a role in biofilm formation, or as virulence factors or interfere in hormonal signalling (Farinati et al. 2011). Production and modulation of auxins and ethylene play a crucial role in development of plant (Brader et al. 2014). Bacterial strains from selenium-supplemented wheat showed high tolerance to elevated selenium concentration and exhibited potential plant-growth-promoting capabilities through auxin and siderophore production, phytate mineralization, and phosphate solubilization (Durán et al. 2014). Endophytes are also beneficial to their host through secretion of a wide range of natural substances that could be harnessed for potential use in medicine, agriculture or industry (Ryan et al. 2008). In our earlier studies, we have identified low and high Zn accumulating genotypes of wheat for contrasting soils sufficient and deficient in Zn (Singh 2016). Interestingly, the genotypes identified as low or high accumulators in deficient soils were different from those in sufficient soils. Therefore, in the present study an attempt was made to unravel the role of endophytes in the fortification of Zn in wheat genotypes in Zn sufficient and deficient soils. The experiment has been carried out in two broad series: (i) Genotypes identified as low and high accumulators in Zn sufficient soils were evaluated in Zn sufficient soils and (ii) Genotypes identified as low and high accumulators in Zn deficient soils were evaluated in Zn deficient soils. The experimental set up was designed to answer the following questions (i) Do the genotypes with different nutrient use efficiency respond in an identical manner to the inoculation of endophytes? (ii) Does the performance of endophytes vary in enhancing the uptake of Zn in soils deficient or sufficient for Zn?

Materials and methods

Soil characterization

Two soils with contrasting physico-chemical characteristics were collected from the farms of ICAR—Indian Agricultural Research Institute (IARI), New Delhi and Krishi Vigyan Kendra (KVK), Hisar. The soils were analysed for available nitrogen (Subbiah and Asija 1956), phosphorus (Olsen 1954), potassium (Standfold and English 1949), zinc and iron by DTPA extraction method (Lindsay and Norvell 1978). The samples were also tested for pH (Jackson 1965), EC (Jackson 1965) and organic carbon (Walkley and Black 1934) according to the standard protocols and these details are given in Table 1.

Microorganisms

Two endophytes *Bacillus subtilis* DS-178 and *Arthrobacter* sp. DS-179, efficient for fortification of Zn in grains of low accumulator wheat genotype, identified in an earlier study (Singh 2016) were maintained on Nutrient agar and stored at 4 °C until use.

Table 1 Physico-chemical properties of IARI and Hisar soils

Parameters	Soil source	
	IARI	Hisar
pH	7.57 ± 0.21	8.70 ± 0.10
EC (dS m^{-1})	0.22 ± 0.02	0.31 ± 0.03
Organic C (%)	0.50 ± 0.03	0.11 ± 0.03
Available N (kg ha^{-1})	206.67 ± 12.58	126.67 ± 15.28
Available P (kg ha^{-1})	22.89 ± 2.22	10.43 ± 0.71
Available K (kg ha^{-1})	363.00 ± 8.19	176.00 ± 10.15
Zn (mg kg^{-1})	1.43 ± 0.10	0.15 ± 0.03
Fe (mg kg^{-1})	4.75 ± 0.14	1.34 ± 0.11

Wheat genotypes

Four wheat genotypes, namely 4HPYT-414 (low Zn accumulator in Zn sufficient soil), K-65 (high Zn accumulator in Zn sufficient soil), 4HPYT-404 (low Zn accumulator in Zn deficient soil), CIM-412 (High Zn accumulator in Zn deficient soil), categorized earlier based on the preliminary experimentation (Singh 2016) were used for this study.

Pot experiment

Two pot experiments using IARI soil (Zn sufficient soil) and Hisar soil (Zn deficient soil) were carried out in glass house at ICAR- Indian Agricultural Research Institute, New Delhi. The soil was sterilized for three consecutive days and 6 kg soil was filled in each pot. Inocula of endophyte strains were prepared by growing in nutrient broth (50 mL) at 30 °C, with shaking at 150 rpm for 18 h, such that the inoculum contained 10^9 cfu mL^{-1}. The seeds were coated with inocula by dipping in the broth for 30 min. Eight seeds of each genotype were sown in pot and following germination thinned to five plants in each pot. Six replicates were maintained for each treatment. All the pots received the recommended dose of NPK (120:60:40 kg ha^{-1}) fertilizers.

The experiment was designed using the following treatments –

(1) Two series of soils: Deficient (Hisar) and sufficient (IARI, New Delhi) for Zn
(2) Genotypes: Four a. Low accumulator of zinc in deficient soil ((LADS): 4HPYT-404; b. Low accumulator of zinc in sufficient soil (LASS): 4HPYT-414; c. high accumulator of zinc in deficient soil (HADS): CIM-412 and d. high accumulator of zinc in sufficient soil (HASS): K-65.
(3) Four treatments: (i) Only recommended dose of fertilizers (RDF); (ii) RDF + $ZnSO_4$ (40 kg ha^{-1}); iii) RDF + inoculation of *Bacillus subtilis* DS-178 and (iv) RDF + inoculation of *Arthrobacter* sp. DS-179. The total treatments maintained were eight and are presented in Tables 2 and 3.

Analysis of root morphology

Root studies were carried out by harvesting plants from three replicates after 60 days of sowing. The soil adhering to the roots were washed gently following the method of Costa et al. (2000). Root length, surface area, volume, number of root tips and average diameter were measured using a Hewlett Packard scanner and analysed using WIN RHIZO Programme V. 2002C software (Regent Instruments Inc. Ltd. Quebec, Canada). Values of root length represent the sum total of all roots analysed through root scanner, including the main root.

Estimation of zinc in plants and yield

The plants from remaining three replicates were harvested at maturity. The observations on the number of grains per spike, seed weight of 100 grains and grain yield/pot were recorded. The concentration of Zn was analysed in root, shoot and grain. The plant parts were ground to 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 using atomic absorption spectrophotometer (Lindsay and Norvell 1978).

Statistical analysis

The experimental data presented represent 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 ($P < 0.05$). Means in a column followed by

Table 2 Response of low and high Zn accumulating wheat genotypes#, in terms of root parameters to bacterial endophytes inoculation in Zn sufficient (IARI) and deficient (Hisar) soils

Type of soil	Treatments	Root length (cm)	Total Root Volume (cm^3)	Average Root Diameter (mm)	Root length (cm)	Total Root Volume (cm^3)	Average Root Diameter (mm)
Zinc deficient		4HPYT- 404 genotype (Low Zn accumulator)			CIM-412 genotype (High Zn accumulator)		
	RDF	516.6 ± 76.3^{b}	0.41 ± 0.05^{c}	0.31 ± 0.02^{b}	600.0 ± 100.0^{b}	0.57 ± 0.03^{b}	0.36 ± 0.03^{b}
	RDF + $ZnSO_4$	700.0 ± 100.0^{b}	0.54 ± 0.04^{bc}	0.41 ± 0.01^{a}	800.0 ± 100.0^{b}	0.67 ± 0.16^{b}	0.48 ± 0.04^{a}
	RDF+ DS-178	1033.3 ± 152.7^{a}	0.75 ± 0.17^{ab}	0.45 ± 0.03^{a}	1083.3 ± 76.4^{a}	1.00 ± 0.10^{a}	0.52 ± 0.02^{a}
	RDF+ DS-179	1066.6 ± 152.7^{a}	0.91 ± 0.10^{a}	0.46 ± 0.03^{a}	1116.6 ± 104.1^{a}	1.14 ± 0.08^{a}	0.56 ± 0.04^{a}
Zinc sufficient		4HPYT-414 genotype (Low Zn accumulator)			K-65 genotype (High Zn accumulator)		
	Only RDF	878.5 ± 78.9^{c}	1.70 ± 0.09^{c}	0.44 ± 0.03^{b}	1083.0 ± 93.74^{c}	2.04 ± 0.11^{b}	0.47 ± 0.02^{b}
	RDF + $ZnSO_4$	1200.0 ± 100.0^{b}	2.15 ± 0.19^{b}	0.50 ± 0.02^{b}	1316.6 ± 28.87^{b}	2.26 ± 0.25^{b}	0.58 ± 0.04^{a}
	RDF+ DS-178	1555.3 ± 50.8^{a}	3.24 ± 0.19^{a}	0.61 ± 0.03^{a}	1599.6 ± 33.5^{a}	3.38 ± 0.14^{a}	0.62 ± 0.04^{a}
	RDF+ DS-179	1604.6 ± 26.3^{a}	3.53 ± 0.14^{a}	0.63 ± 0.04^{a}	1732.6 ± 104.8^{a}	3.73 ± 0.07^{a}	0.60 ± 0.02^{a}

DS-178, *Bacillus subtilis*, DS-179*, Arthrobacter* sp.

a same superscript letter indicates no significant difference using ANOVA LSD test.

Results

Soil characteristics

The physicochemical characteristics of soils collected from farms of IARI and KVK (Hisar) are presented in Table 1. Both the soils have contrasting characteristics for the parameters tested. The available zinc content in IARI and Hisar soils was 1.43 and 0.15 mg/kg of soil respectively. Based on the content of zinc in the soil, IARI soil was designated as sufficient, whereas Hisar soil was designated as deficient for zinc.

Influence of bacterial endophytes inoculation on root parameters of different genotypes of wheat

The data on different root morphological parameters indicate distinct variations among low and high Zn

Table 3 Response of low and high Zn accumulating wheat genotypes in terms of fortification of Zn in roots, shoots and grains to bacterial endophytes inoculation in Zn sufficient (IARI) and deficient (Hisar) soils

Soil Type	Treatments	Zinc content mg kg^{-1}					
		Low accumulator wheat genotype			High accumulator wheat genotype		
		4HPYT-414 genotype			K-65 genotype		
		Root	Shoot	Grain	Root	Shoot	Grain
IARI Soil	RDF	154.50 ± 16.89^{b}	97.02 ± 15.00^{c}	24.25 ± 5.03^{c}	275.00 ± 15.00^{b}	178.33 ± 10.40^{b}	60.63 ± 7.50^{c}
	RDF + $ZnSO_4$	230.00 ± 20.00^{a}	138.00 ± 23.09^{bc}	33.12 ± 5.00^{bc}	396.67 ± 15.27^{a}	238.67 ± 19.15^{a}	81.14 ± 7.57^{b}
	RDF+ DS-178	205.67 ± 25.16^{ab}	155.00 ± 13.22^{ab}	50.00 ± 8.00^{ab}	355.00 ± 21.80^{a}	223.67 ± 15.82^{a}	98.00 ± 8.77^{b}
	RDF+ DS-179	257.83 ± 36.17^{a}	197.26 ± 20.00^{a}	66.24 ± 10.51^{a}	360.00 ± 26.10^{a}	239.57 ± 20.21^{a}	119.72 ± 5.03^{a}
		4HPYT-404 genotype			CIM-412 genotype		
Hisar Soil	RDF	120.00 ± 12.29^{b}	40.00 ± 5.00^{b}	15.00 ± 2.08^{b}	180.00 ± 10.00^{b}	96.00 ± 6.24^{c}	30.20 ± 5.51^{c}
	RDF + $ZnSO_4$	171.67 ± 20.00^{a}	65.33 ± 10.02^{a}	21.30 ± 4.04^{b}	250.00 ± 10.00^{a}	127.50 ± 8.54^{b}	43.18 ± 7.94^{bc}
	RDF+ DS-178	170.00 ± 17.56^{a}	55.33 ± 10.02^{ab}	23.33 ± 3.21^{ab}	213.33 ± 20.21^{ab}	105.33 ± 5.03^{c}	50.17 ± 4.01^{ab}
	RDF+ DS-179	190.00 ± 12.58^{a}	70.67 ± 8.77^{a}	30.85 ± 4.51^{a}	256.67 ± 17.56^{a}	153.17 ± 10.07^{a}	63.57 ± 5.03^{a}

DS-178, *Bacillus subtilis*, DS-179*, Arthrobacter* sp.

accumulating genotypes, soil types (deficient or sufficient for Zn) and also among RDF, RDF+ $ZnSO_4$ and endophyte inoculated treatments (Table 2). Among the genotypes, high Zn accumulating genotypes, irrespective of soil or treatment, gave higher values for all the root parameters recorded. Application of $ZnSO_4$ in Zn deficient or sufficient soils led to a significant increase in all the root parameters with respect to uninoculated control. The root length, surface area, volume and diameter were further improved significantly through the inoculation of endophytes (Fig. 1). Both the endophytes used for Zn fortification (*Bacillus subtilis* DS-178 and *Arthrobacter* sp. DS-179) resulted in statistically similar response with regard to all the root parameters, except root volume, irrespective of the genotypes or soil types.

Biofortification of Zn

The data on acquisition of Zn in root, shoot and grain of wheat genotypes are presented in Table 3 and Figs. 2 and 3. A perusal of the data indicates that irrespective of the treatment, the amount of Zn accumulated was maximum in roots, followed by shoots and grains. In Zn sufficient and deficient soils, on an average, the amount of Zn present in shoots as compared to roots was lower by about 65% and 43% respectively. Likewise, the average amount of Zn present in grains as compared to that in shoots in sufficient and deficient soils was about 34% and 38% respectively. Inoculation of endophytes significantly improved fortification of Zn in grains of both low and high accumulating genotypes and the Zn content in grains due to inoculation was 2 fold higher as compared to uninoculated control. In general, endophyte inoculation fortified wheat grains with Zn which was better or equivalent to application of 40 kg $ZnSO_4$ ha^{-1} both in sufficient and deficient soils. Among the endophytes with respect to accumulation of Zn in grains, no statistically significant differences were observed for all treatments, except for the HASS genotype (K-65). In CIM412, *Bacillus subtilis* DS-178 inoculation resulted in equivalent amount of translocation from root to shoot and from shoot to grains (Fig. 3d).

Yield and yield attributes

The yield and yield parameters were also significantly influenced by application of $ZnSO_4$ and also by inoculation of endophytes both in Zn sufficient and deficient

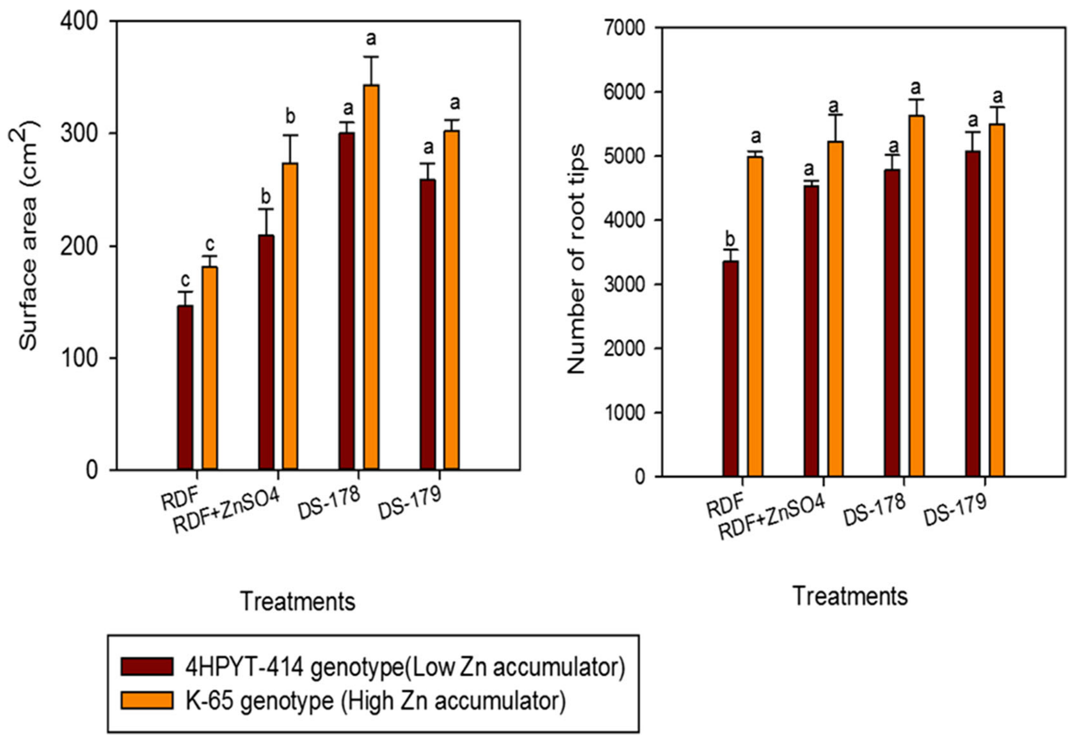

Fig. 1 Response of low and high Zn accumulating wheat genotypes to zinc fertilization and bacterial endophytes inoculation, in terms of root surface area and number of root tips in Zn sufficient soil

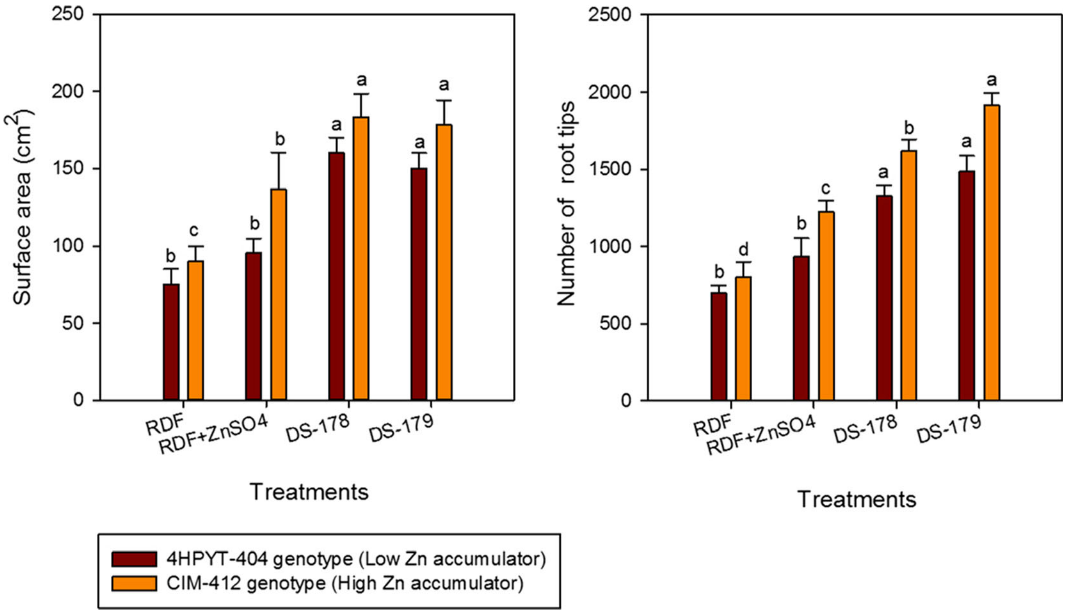

Fig. 2 Response of low and high Zn accumulating wheat genotypes to zinc fertilization and bacterial endophytes inoculation, in terms of root surface area and number of root tips in Zn deficient soil

soils (Fig. 4a–d). In Zn deficient soils, inoculation of *Bacillus subtilis* DS-178 resulted in significantly higher grain yield in both genotypes CIM-412 (HADS) and 4HPYT-404(LADS), as compared to *Arthrobacter* sp. DS-179. In Zn sufficient soil also, both the endophytes were at par with regards to yield and other yield parameters studied. Grain yield responded significantly to amendment of $ZnSO_4$ in Zn deficient soils as compared to uninoculated control, but not in sufficient soil.

Discussion

Microbe- based technologies are gaining importance in recent years for improving soil fertility, enhancing yield and for fortification of plant parts with various micronutrients. In the past few decades, the focus was on the use of rhizospheric microorganisms to enhance accumulation of micronutrients, Zn in particular, in grains of staple crops like wheat, maize and rice (Abaid-Ullah et al. 2015; Prasanna et al. 2015; Rana et al. 2012). However in recent years, the focus has shifted to the internal microbiome that is the endophytes. Endophytes have been reported to control several fungal pathogens (James and Mathew 2015), alleviate drought or salt stress (Naveed et al. 2014) and shown promise in the fortification of micronutrients (Zhang et al. 2012).

The present study was carried out to understand the role of endophytes in fortification of Zn in wheat genotypes with different nutrient use efficiency and in soils deficient and sufficient for Zn. In our earlier study, 13 wheat genotypes were screened for accumulation of Zn in soil deficient or sufficient for zinc and based on the uptake of zinc in grains, the genotypes were classified as low and high accumulators (Singh 2016). Initial studies also revealed that this classification of genotypes was dissimilar when Zn sufficient or deficient soils were used. In Zn deficient soils, genotype 4HPYT-404 was identified as a low accumulator for Zn, while genotype CIM-412 was identified as a high accumulator for Zn. In Zn sufficient soils, genotype 4HPYT-414 was identified as a low accumulator for Zn while genotype K-65 was identified as a high accumulator for Zn.

The high accumulating genotypes of Zn showed significantly higher values of all root parameters analysed as compared to the low accumulating genotypes. The inoculation of endophytes further influenced the root parameters, emphasizing their significance as a contributing factor for the increased uptake of Zn. Root morphological data can be used as an important indicator of efficiency of uptake of nutrients (Singh et al. 2005), which supported our observations. Wang et al. (2014) also reported increase in root surface area,

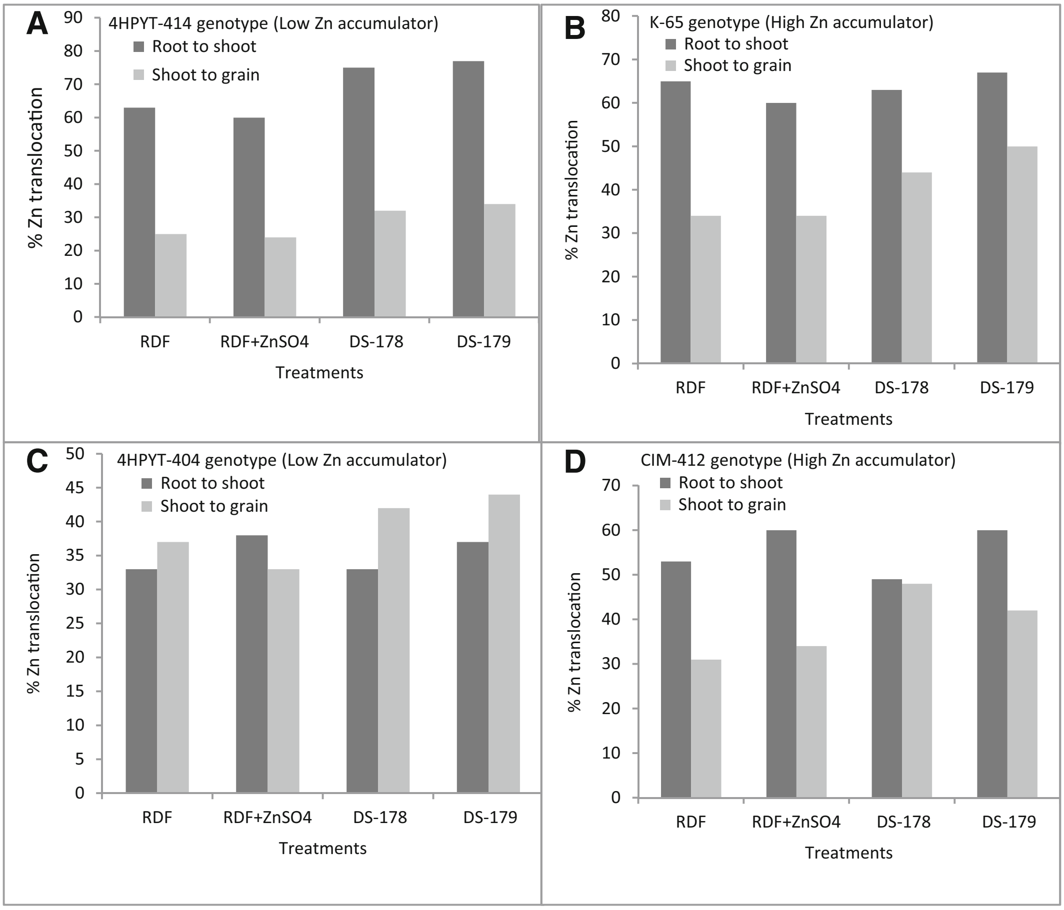

Fig. 3 Elucidation of translocation of Zn from root to shoot and shoot to grain in Zn sufficient and deficient soil represented as percent translocation between different plant tissues in **a** Low Zn accumulator in Zn sufficient soil; **b** High Zn accumulator in Zn sufficient soil; **c** Low Zn accumulator in Zn deficient soil; **d** High Zn accumulator in Zn deficient soil

number of root hairs and root tips due to inoculation of endophytes to wheat crop. The uptake of Zn by genotypes is in proportion to their root volume or density developed by a cultivar (Lynch 2007), and similar observations were reported by researchers in terms of Zn uptake in dry land cereal crops such as wheat and barley (Dong et al. 1995; Genc et al. 2007). The enhancement in root parameters and Zn uptake by plants due to endophytic inoculation could be the result of production of IAA like substances (Vessey 2003). There are many reports on enhancement of plant growth and root parameters due to the inoculation of IAA producing bacteria (Abbamondi et al. 2016; De La Torre-Ruiz et al. 2016). Tariq et al. (2007) reported enhanced Zn mobilization in rice plants and significant increase in root parameters due to inoculation of Zn mobilizing PGPR. Both the endophytic bacteria used in this investigation produced 10 and 16 μg/ ml of IAA (Unpublished data). In treatment with $ZnSO_4$, there was an increase in the values of root parameters over un-amended and un-inoculated control but these values were significantly lower than treatments with endophyte inoculation. This could be due to the rapid fixation of Zn in soil and poor mobility of Zn in soil (Xiaohong et al. 2008). In strawberry plants, application of bio preparation like Biofeed Quality, Biofeed Amin, Vinassa and Florovit Eko increased the root growth parameters in comparison with the plants fertilized with NPK (Derkowska et al. 2015).

The extent of enhancement in the root parameters due to inoculation of endophytes was also determined by the genotype and soil type. If we compare the genotypes grown in deficient soils with regards to root surface area, the high accumulating genotypes showed 107% and 100% higher values over the un-inoculated control due to endophyte inoculation (Suppl. Table 1). Likewise in

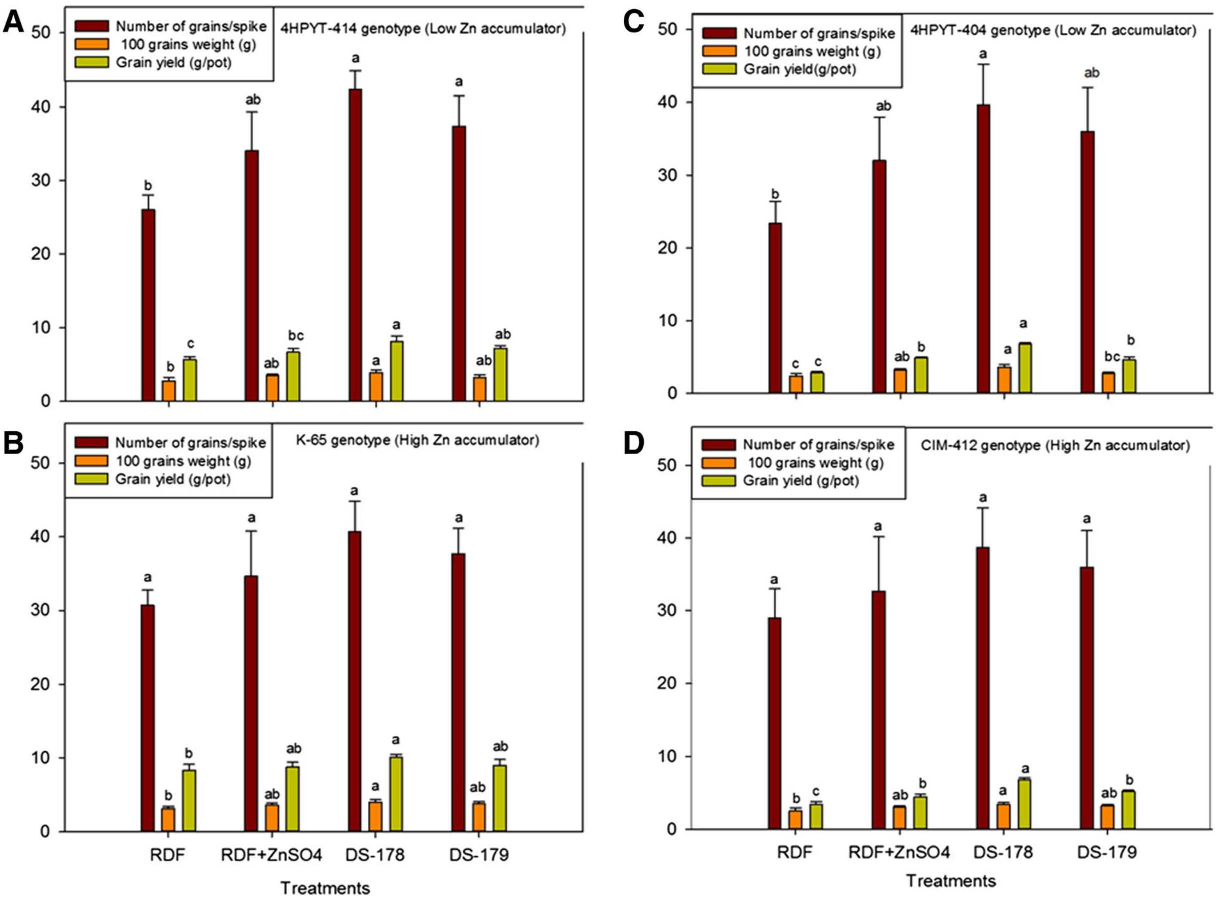

Fig. 4 Yield and yield parameters of low and high Zn accumulating wheat genotypes in Zn sufficient and deficient soils: Low Zn accumulator in Zn sufficient soil (**a**); High Zn accumulator in Zn sufficient soil (**b**); Low Zn accumulator in Zn deficient soil; (**c**); High Zn accumulator in Zn deficient soil (**d**)

sufficient soils, the root surface area of low and high accumulating genotypes was higher by 91% and 78%. However, if the root surface area is compared in sufficient and deficient soils irrespective of the genotypes used, enhancement of 84% and 103% respectively over the control was recorded as a result of the inoculation of endophytes (Supplementary Table 1). The higher percent increase in root parameters either in low accumulating genotypes or in Zn deficient soils due to endophyte inoculation indicates its significance particularly in soils deficient in micronutrients.

The data on accumulation of Zn confirmed earlier reports that micronutrients, such as Zn accumulate in higher concentrations in roots followed by shoots and grains (Chatzistathis et al. 2009). This investigation reveals that the low and high Zn accumulating genotypes respond in an almost identical manner to endophyte inoculation, irrespective of the soil type, however endophyte inoculation definitely enhanced Zn enrichment in grains. An interesting observation was that inoculation of endophyte Bacillus subtilis DS-178 resulted in equivalent amount of translocation from root to shoot and from shoot to grains in Zn deficient soil. These results illustrate that microbe mediated biofortification can be a reality and represent a complementary approach to precision breeding or agronomic fortification. Also, our results clearly show that the genetic potential of any genotype for biofortification of grains with Zn is not realized even when growing in soils sufficient in Zn. This potential can be enhanced by inoculating with zinc solubilizing endophytes.

The second question we posed was whether the endophytes perform the same way in enhancing the uptake of Zn in soils deficient or sufficient for Zn. On comparison of the percent increase of Zn in grains due to inoculation of endophytes irrespective to the soil type, enhancement due to inoculation was 100% and 83% in sufficient and deficient soil respectively (Suppl. Table 2). The results strongly illustrate that the inoculation of endophytes can help in improving the nutrient use efficiency, irrespective of the available levels of Zn in the soil.

Conclusion

This study is the first comprehensive evidence of significance of inoculation of endophytes to crop plants irrespective of their genetic potential to accumulate Zn; and also irrespective of the available Zn present in soil. Therefore, we recommend the use of endophytes for biofortification of micronutrients to help different genotypes realize their genetic potential for accumulation of micronutrients.

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