



Review Article

Enhancing Iron uptake and Alleviating Iron Toxicity in Wheat by Plant Growth-promoting Bacteria: Theories and Practices

Le He¹, Zonghao Yue¹, Can Chen¹, Chunyan Li¹, Juan Li¹ and Zhongke Sun^{1,2*}

¹College of Life Sciences and Agronomy, Zhoukou Normal University, 466001 Zhoukou, China

²College of Chemistry and Molecular Engineering, Zhengzhou University, 450003 Zhengzhou, China

*For correspondence: sunzh@daad-alumni.de; Phone: +86 (0)394 8178259

Abstract

Though iron is a rich mineral in soil, its bioavailability for many crops is low. Considering the vital role of iron in all organisms, iron deficiency (ID) is one of the most widespread and common nutritional problem in the world. The hidden hunger caused by ID seriously affects the quality of human life and economic development. As one of the major staple food, wheat grain is in short of iron as well. Iron absorbing in wheat is mainly through its root by the strategy II which gets iron from the rhizosphere through the release and uptake of Fe-chelating mugineic acid phytosiderophores. Meanwhile, there are versatile bacteria lived in both the rhizosphere and indigenous region of wheat root. Many of them are commensalistic or termed plant growth-promoting bacteria (PGPB), needing iron for growth and work as well. Contrasting to wheat, bacteria uptakes both dissociative inorganic and associative organic iron combined with siderophores more efficiently, mainly through different ATP binding cassette (ABC) transporters. Laboratory and field experiments showed many PGPB improve wheat iron uptake when iron is deficient by producing different metabolites and regulating transporters, which provided basis for a microbiological strategy to iron biofortification. On the other side, many PGPB reduced iron toxicity to wheat as their strong bioleaching capacity and negative regulation of iron uptake or transport when iron is plenty © 2020 Friends Science Publishers.

Keywords: Iron uptake; Iron deficiency; Iron toxicity; Wheat; PGPB

Iron in Nature and Iron Deficiency in Human

In nature, there are various forms of iron (Fe), e.g. precipitated, dissociative; inorganic, organic; oxidized, reduced. However, the rich mineral of Fe in soils, particular in calcareous soils, is often in oxidized and precipitated forms, which are not readily bioavailable for many crops (Pereira *et al.*, 2014; Cieschi *et al.*, 2019). Meanwhile, versatile microorganisms presented in the rhizosphere and indigenous region of plants influence Fe absorbing in plants. Therefore, plants and microbes have evolved various transporters to get Fe of varying forms from different habitats (Lau *et al.*, 2015; Connorton *et al.*, 2017; Zhang *et al.*, 2017).

As a micronutrient, Fe is almost an essential ingredient in nearly all organisms. In human, plenty iron in diets is necessary as it controls normal red blood cell development and immune function, and involves in key metabolic reactions and multiple physiological functions (<http://www.fao.org/3/y2809e/y2809e0j.htm>, Chapter 13 Iron). However, iron deficiency (ID) is the most widespread and common nutritional problem in the world. Two billion people in the world are in an iron-deficient state and one-fifth of China's population is iron-deficient. ID is a leading cause of anemia and anemia

affects cognitive development, reduces immunity, causes fatigue and particularly in the poor, women, and children (Stevens *et al.*, 2013). The World Health Organization ranks ID as one of the top 10 health problems in modern times (WHO, 2016). It is estimated that about half of pregnant women will have ID-induced anemia, and about 50,000 pregnant women will have ID-induced dystocia. At the same time, ID has caused more than 40% of infants suffering from mental development in developing countries (<http://www.micronutrient.org>).

Iron Deficiency and Iron Toxicity in Wheat

Wheat (*Triticum aestivum* L.) is one of the most important sources of calories and nutrients, including iron and zinc in the human diet, especially in developing countries. However, wheat is typically low in bioavailable Fe, contributing to ID in countries where wheat is consumed as a staple food (Balk *et al.*, 2019). The low bioavailability of Fe is largely because of either the low content of Fe in seeds or the presence of phytates in mineral-rich bran fractions. For example, the mean content of Fe in grains of 198 wheat cultivars grown in France is only 29.1 mg/kg, which is much less than the recommended amount of 59 mg/kg (Baize *et al.*, 2009). The majority wheat cultivars used for

making bread and noodles also have lower Fe than recommended (Zhao *et al.*, 2009). Although a few lines of wheat with high-iron content have been developed by conventional plant breeding (biofortification), this approach is failed in yield and has proved challenging (Beasley *et al.*, 2019). Laboratory studies in transgenic wheat increased Fe contents significantly, but have low acceptability currently (Ludwig and Slamet-Loedin, 2019). Therefore, improving wheat as a source of iron by other approaches is still necessary and important for sustainable agriculture.

On the other hand, less severely than inadequate iron for plant, iron toxicity becomes a threat in some regions. Several crop plants, including wheat, cannot tolerate excess iron, especially in acidic soils. For example, excess iron threatens plant growth in the humid tropical regions of Asia, Africa, and South America, where soils are often flooded or acidified (Guo *et al.*, 2010; Khabaz-Saberi *et al.*, 2010). Iron toxicity in shoot tissues of wheat has been observed and there is a strong negative correlation between iron concentration and dry weight of shoot in wheat (Setter *et al.*, 2008; Khabaz-Saberi *et al.*, 2010). It was also reported that excess iron inhibits root and shoot elongation and there is a linear relationship between iron exposure and wheat shoot elongation (Naveen Kumar *et al.*, 2019). Also, excess iron adversely influences wheat grain yield (Khabaz-Saberi *et al.*, 2012).

Iron uptake and Translocation in Wheat

As found earlier, the monocotyledonous species, like *Triticum aestivum* L, needed higher Fe concentration to reach best growth than the dicotyledonous species did (Christ, 1974). However, iron forms insoluble ferric Fe³⁺ precipitates under aerobic conditions at neutral pH levels that are often unavailable for uptake by living organisms (Guerinot and Yi, 1994). Graminaceous plants get Fe from the rhizosphere mainly through the release and uptake of Fe-chelating mugineic acid phytosiderophores (MAs), a process known as strategy II Fe uptake (Beasley *et al.*, 2017; Li *et al.*, 2016).

It was reported that wheat uses strategy II to absorb iron, and the process is governed by complex mechanisms (Brumbarova *et al.*, 2015). Simply, wheat releases MAs to increase metal solubility greatly under ID stress, and afterwards chelates Fe³⁺. Then, the Fe³⁺-MAs complexes are taken up through a specific transport system in the root plasma membrane (Fig. 1a). Precisely, the Fe³⁺-MA complexes can be taken up into root cells by the YELLOW STRIPE 1 (YS1) and YELLOW STRIPE 1-like (YSL) transporters (Inoue *et al.*, 2009; Lee *et al.*, 2009). Other divalent metal transporters may also contribute to iron uptake in graminaceous wheat. The ZIP (zinc-regulated transporter, iron-regulated transporter-like protein) family discovered as a homolog of *Arabidopsis* IRT1 transports various divalent metals, including Fe²⁺ (Nakanishi *et al.*, 2006; Kobayashi and Nishizawa, 2012). Another family of

transporters, NRAMP (natural resistance-associated macrophage protein), also transports similar divalent metals (Takahashi *et al.*, 2011). However, a bit different from corn and barley, there is neither homologous YS1/YSL nor NRAMP in wheat. As summarized (Table 1), only three un-reviewed ZIP transporters are presented in the annotation dataset of *Triticum aestivum* proteome (<https://www.uniprot.org/uniprot/?query=proteome:UP000019116>). In all, recent progress in genome sequencing has improved our understanding of the genes involved in iron uptake (Zimin *et al.*, 2017).

Fig. 1b schematically illustrated main processes for Fe translocation in wheat. Briefly, iron translocation includes three sequential steps, namely from soil to roots, and then from roots to shoots, last from stems to seeds (Jeong *et al.*, 2017). Many transporters and regulators, including genes nicotianamine synthase (NAS) and nicotianamine aminotransferase (NAAT), responded for Fe translocation from roots to shoots (Connorton and Balk, 2019). This process involves various steps, like passing through the casparian strip, xylem and phloem and moving toward the site of demand (Fe distribution). After transported from stems to grains, Fe was stored. Genes encoding ferritins and vacuolar iron transport (VIT) are responding for Fe accumulation. Frankly speaking, it is difficult to discover the precise contribution of each transporter and the detail process of iron uptake as well as translocation in wheat is still unknown.

Iron uptake in Plant Growth-promoting Bacteria

There are a large number of microorganisms in soil, the rhizosphere and within the tissues of plants. A diverse group of those microorganisms live in association with plants and stimulate the plant growth and/or reduce the incidence of plant disease, thereby termed plant growth-promoting bacteria (PGPB). Some PGPB promote plant nutrition by fixing nitrogen, dissolving phosphorus (Richardson *et al.*, 2009). Some PGPB prevent pathogens and produce versatile metabolites through epiphytic or symbiotic relationships (Lee *et al.*, 2018; Masum *et al.*, 2018).

Owing to the insolubility in nature, bacteria are confronted with a low availability of iron. The PGPB cope with ID using siderophores synthesized by themselves or other neighbor microbes (Braun and Hantke, 2011). Differing from most other nutrients, iron compounds are tightly bound to proteins at the cell surfaces of bacteria. Bound iron is further translocated by specific proteins across the cell wall or the outer membrane. According to the forms of substrate, iron transport in wheat associated PGPB can be divided into two categories: inorganic iron and organic iron: the former includes dissociative Fe³⁺, Fe²⁺ compound, carbonyl iron; the latter includes ferric citrate and siderophores (Fig. 2). Secretion of siderophores and absorption of the combined organic iron is the main way for many bacteria in soils (Fukushima *et al.*, 2013).

Table 1: Potential iron transporters in *Triticum aestivum* based on proteome data*

Entry	Protein names	Gene names	Length	Status
Q5G1L7	Zinc transporter ZIP	?	360	un-reviewed
A0A1D6BHT7	ZIP metal ion transporter	ZTP29	277	un-reviewed
A9NIW8	Putative zinc transporter	ZIP1 TRAES_3BF074300090CFD_c1	355	un-reviewed

*: <https://www.uniprot.org/uniprot/?query=proteome:UP000019116>

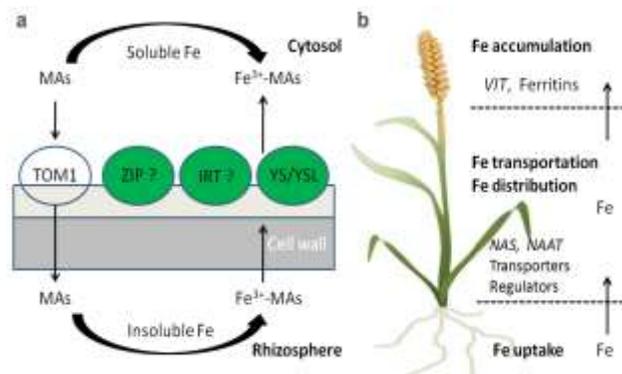


Fig. 1: Schematic representation of iron uptake and translocation in wheat: a, iron uptake by the strategy II mechanism; b, processes during iron translocation. MAs, mugineic acids; ZIP, zinc-regulated transporter; IRT, iron-regulated transporter; YS/YSL, yellow stripe 1 or yellow stripe 1-like; NAS, nicotianamine synthase; NAAT, nicotianamine aminotransferase; VIT, vacuolar iron transport. Note: figure 1b was prepared based on a recent publication with major modification (Connorton and Balk, 2019)

Bacillibactin is a common siderophore of bacilli that are representative wheat associated PGPB, and its synthesis needs adenosine vinyltransferase participation. However, this gene has been mutated in during evolution in many *Bacillus* sp., and only produce a pro-bacteriocin precursor with moderate affinity for Fe^{3+} (Segond *et al.*, 2014). Despite there have been a few reports of siderophore production in *Bacillus* sp. and another PGPB, *Paenibacillus* sp., genomics analysis showed the siderophore synthesis gene cluster exists only on certain species (Raza and Shen, 2010; Hertlein *et al.*, 2014). The results suggest that these genes may be acquired in the late stage of evolution by horizontal gene transfer (Eastman *et al.*, 2014; Xie *et al.*, 2016). Therefore, gaining of a siderophore-dependent organic iron uptake system may provide competitiveness during bacteria-wheat communism and important for its plant growth-promoting properties.

For bacteria that do not produce siderophore, or in soils with high iron solubility in acidic environments, inorganic iron is a preferred source of iron (Cao *et al.*, 2015). In addition, siderophores may be ineffective in anaerobic and/or acidic environments (Mishra *et al.*, 2015). Therefore, getting inorganic iron by PGPB is equally important. However, because of extensive research on absorbing siderophores and heme, and the low abundance of inorganic iron in calcareous soils, little research has been done on the absorption of inorganic iron. So far, four inorganic iron transport systems have been reported:

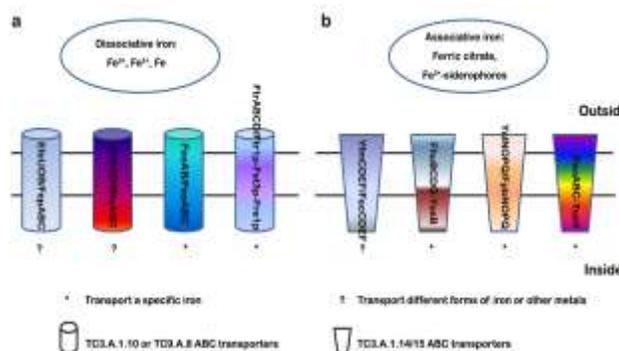


Fig. 2: Schematic representation of iron uptake in plant growth-promoting bacteria by some reported ATP binding cassette (ABC) transporter systems; a, uptake of inorganic iron (dissociative Fe^{3+} , Fe^{2+} , Fe); b, uptake of organic iron (associative ferric citrate and iron-siderophores)

EfeUOB/FepABC, SitABC/MtsABC, FeoAB/FeoABC, Ftr1p-Fet3p-Fre1p/FtrABCD (Sheldon and Heinrichs, 2015; Lau *et al.*, 2015). EfeUOB/FepABC is a widely-existing iron transport system, but because this system is involved in absorbing inorganic Fe^{3+} , Fe^{2+} and heme, its substrate specificity is controversial (Miethke *et al.*, 2013; Temtirath *et al.*, 2017). Similarly, SitABC/MtsABC can transfer of ferrous metals, manganese, and other divalent metals (Sun *et al.*, 2009). FeoAB/FeoABC is a highly conserved Fe^{2+} absorption pump that forms a large complex in the inner membrane and is regulated by iron (Stevenson *et al.*, 2016; O'Connor *et al.*, 2017). Ftr1p-Fet3p-Fre1p/FtrABCD is a reductive, high-affinity Fe^{3+} transporter system found mostly in yeast and gram-negative bacteria (Kwok *et al.*, 2006; Mathew *et al.*, 2014).

Nevertheless, whether it is inorganic iron or organic chelated iron, PGPB mainly use ATP as energy to absorb iron through the ABC transport system (Sheldon and Heinrichs, 2015). According to the classification of ABC transporter systems, inorganic iron is mainly absorbed by transporters in class TC3.A.1.10 and combined organic iron is mainly absorbed by transporters belongs to classes TC3.A.1.14/15 (Saier, 2000). Alternatively, ferrous iron may also pass through cells by transporters of unknown classification (TC9.A.8). In the gram-positive model bacterium *B. subtilis*, these iron transport systems include EfeUOB that absorbs elemental or inorganic iron, YfmCDEF/FecCDEF that absorbs ferric citrate and combined iron-siderophores (ferric amine and iron pigment). While, FhuBCGD-YxeB, YclNOPQ/FpbNOPQ absorb siderophores petrobactin and FeuABC-YusV transport system absorbs siderophores bacillibactin (Pi and Helmann, 2017).

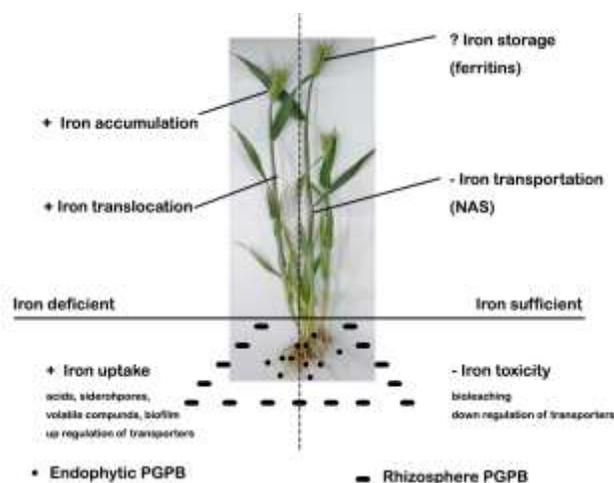


Fig. 3: Potential mechanisms and applications of plant growth-promoting bacteria in wheat: PGPB improve iron uptake when iron deficient (left side); PGPB reduce iron toxicity when iron sufficient (right side). +, improve; -, decrease; ?, unknown effect; NAS, nicotianamine synthase gene

Potential Mechanisms and Application of Plant Growth-promoting Bacteria for Iron Nutrition

Often, most wheat cultivars can neither get enough iron from calcareous soils nor tolerant excess iron in acidic soils. Therefore, there is an urgent need to increase the ability of wheat to uptake or absorb iron and to resist iron toxicity from different soils (Jin *et al.*, 2014). Like a coin has two sides, wheat may suffer from either ID or iron sufficiency (Fig. 3). In brief, when iron is deficient, PGPB either in the rhizosphere or endophytic regions, may improve iron uptake in root, speed iron translocation in stem, and increase iron accumulation in grains. While, when iron is plenty, PGPB may bioleach excess iron in soil, regulate iron uptake and transportation, and up-regulate ferritin expression to improve iron storage. Details mechanisms and examples on how PGPB help plant cope with these two adverse conditions were discussed in following sections.

Enhancing Iron uptake by Plant Growth-promoting Bacteria

Currently, there are many PGPB applied widely for improving nutrition absorption in various plants. PGPB secrete different signals and trigger the secretion of phytosiderophores and hormones in plants, which may regulate iron uptake (Roesti *et al.*, 2006). Bacteria themselves can produce siderophores, organic acids, volatile compounds, and form biofilm (Liu *et al.*, 2017; Sah *et al.*, 2017; Hernández-Calderón *et al.*, 2018). All these molecules solubilize and mobilize iron in soils, improving the bioavailability of iron for plants. It is also possible that bacteria activate genes involved in iron deficiency in plant. For example, *Paenibacillus polymyxa* BFKC01 significant increases plant release of phenolic compounds by activating

membrane-bound iron chelate reductase *fro2* and divalent metal transporter *irt1* and related genes (Zhou *et al.*, 2016). In fact, it has been widely recognized that *Bacillus* sp. and *Paenibacillus* sp. play important roles in aiding plants to gain iron or to cope with iron shortage (Freitas *et al.*, 2015; Verma *et al.*, 2016). Inoculation of PGPB, including *B. altitudinis*, can increase minerals in grains of chickpeas and pigeonpeas, *e.g.* increasing Fe content by 12-18% (Gopalakrishnan *et al.*, 2016). Inoculation with *B. pichinoty* increased the selenium content of the grain by 167% and Fe content by 70% (Yasin *et al.*, 2015). However, the use of genetic engineered PGPB to improve Fe content in wheat grains has not been reported.

Alleviating Iron Toxicity by Plant Growth-promoting Bacteria

Some PGPB can also help plants resist abiotic stresses, including heavy metals and are therefore used to help plants growth in contaminated soils (Jin *et al.*, 2014). Under excess iron, a rhizobacterium *Paenibacillus* sp. FeS53 protected different rice cultivars grow and nutrient uptake to normal levels, showing the bacterium can mitigate iron toxicity in rice (de Souza *et al.*, 2015). Endophytic PGPB are more potent due to less environmental impact and higher colonization capacity in plants. *Bacillus* spp. are either rhizobacteria or endophytes that can be widely isolated from wheat, not only improving iron nutrition but also protecting plants from iron stress and toxicity (Zhou *et al.*, 2018; Zhou *et al.*, 2019). So, to understand how PGPB influence iron mobilization and its uptake by plants in metal-polluted soils is critical.

Siderophores producing microbes in soils are believed to play an important role in heavy metal phytoextraction. Microbial siderophores were used as metal chelating agents that regulate the availability of iron in plant rhizosphere (Sharma *et al.*, 2015). Recent studies also suggested that inoculation with beneficial microbes relieved heavy metal toxicity by increasing the activities of antioxidant enzymes in plants (Pérez-Montañó *et al.*, 2014). We had isolated a series of endophytic bacteria from wheat roots. One of the isolate, *B. altitudinis* WR10 has a strong ability to absorb iron and improves iron tolerance in wheat by up-regulating the expression of ferritin-encoding genes (Sun *et al.*, 2017). The strain produces indole acetic acid (IAA) and siderophores, and has high phytase and 1-aminocyclopropane-1-carboxylate deaminase activities (Yue *et al.*, 2019, unpublished results). Therefore, its potential on alleviating iron toxicity can be expected.

Enhanced Iron Biofortification of Wheat by Plant Growth-promoting Bacteria

There are already several practices on enhancing minerals uptake and yield of wheat through inoculation of PGPB, as summarized in Table 2. A field study examined the effects of several PGPR strains on wheat yield and nutrient contents

Table 2: Examples of plant growth-promoting bacteria enhancing wheat iron uptake and iron fortification

PGPB	Treatment	Tissue	Iron increase	Study conditions	Reference
B. M-3	Information unavailable	leaf, grain, straw	90-105%	Turkey, experiment	field Turan <i>et al.</i> (2010)
<i>P. jessenii</i> , <i>P. synxantha</i> , and AMF	10 ⁵ -10 ⁶ CFU bacteria per seed, 20 infectious propagules per seed, seed encapsulation	grain	30%	India, experiment	field Mäder <i>et al.</i> (2011)
<i>B. sp.</i> AW1, <i>Providencia sp.</i> AW5 and <i>Brevundimonas sp.</i> AW7	10 ⁹ CFU/mL, mixed with charcoal: soil (3:1), prior to sowing of seeds	grain	107-144%	India, experiment	pot Rana <i>et al.</i> (2012)
<i>B. megaterium</i> , <i>Arthrobacter chlorophenolicus</i> , and <i>Enterobacter sp.</i>	10 ⁷ CFU/mL, co-incubation for 30 minutes before seeding	grain	42-49%	India, pot and field experiments	Kumar <i>et al.</i> (2014)
<i>Serratia marcescens</i> , <i>Microbacterium arborescens</i> , and <i>Enterobacter sp.</i>	10 ⁷ -10 ⁸ CFU per seed, co-incubation for 5 days before seeding	grain	16-18%	India, pot and field experiments	Kumar <i>et al.</i> (2017)
<i>P. putida</i>	extracted bacterial siderophores, incubation with seedlings	co- root	51-90%	Iran, hydroponic culture	Rasouli-Sadaghiani <i>et al.</i> (2014)
<i>Arthrobacter sp.</i> and <i>Lysinibacillus fusiformis</i>	iron fortified bacterial siderophore, application during flowering	foliar grain	200%	India, experiment	field Sharma <i>et al.</i> (2019)

Note: AMF, Arbuscular mycorrhizal fungi; *B.*, *Bacillus*; *P.*, *Pseudomonas*

(Turan *et al.*, 2010). All bacterial inoculations, especially *Bacillus* M-3 significantly increased uptake of Fe in grain, leaf, and straw by 90-105%. A two-year field study at seven locations in India revealed that inoculation of arbuscular mycorrhizal fungi (AMF) and PGPR (*P. jessenii* and *P. synxantha*) increased grain yield by 41% and Fe content by 30%, comparing to uninoculated controls (Mäder *et al.*, 2011). Another study with three rhizobacterial strains at fairly high dosage (10⁹ CFU/mL) even showed 107-144% increases in the percentage levels of grain Fe (Rana *et al.*, 2012). Triple combination of strains *B. megaterium*, *Arthrobacter chlorophenolicus* and *Enterobacter* significantly increased Fe content in grain by 49% under pot condition and 42% under field condition (Kumar *et al.*, 2014). However, co-inoculation of *Serratia marcescens*, *Microbacterium arborescens*, and *Enterobacter sp.* increased Fe content in grain by only 16-18% under pot condition and field condition (Kumar *et al.*, 2017). Unlike direct application of PGPB, applying PGPB produced siderophore as chemical fertilizers also showed promising results. Significantly increase of grain yield by 45% and Fe concentration in grains by 200% in wheat was recorded when Fe fortified bacterial siderophore was applied (Sharma *et al.*, 2019).

However, two points need to be noted here. The first is careful selection of bacterial strains. Even closely related bacteria may have different influence on wheat iron uptake. As reported, contrasting to *P. fluorescens* and *P. aeruginosa*, only siderophore produced by *P. putida* improves Fe availability in wheat (Rasouli-Sadaghiani *et al.*, 2014). The study highlighted the importance of bacterial selection. The second is paying more attention to apply of AMF for improving plant nutrient acquisition. A recent study showed that AMF inoculation had no positive effect on concentrating Zn and Fe in durum wheat grain and may even decrease their bioavailability due to increased phytic acid in grains (Tran *et al.*, 2019).

Concluding Remarks

Wheat is one of the staple food but low in iron in grain.

Uptake of iron from soils by wheat root mainly based on the strategy II and is inefficient for human nutritional requirement. However, versatile PGPB evolved different mechanisms for efficient uptake of different forms of iron, mostly by classes of ATP binding cassette transporters. Currently, supplementing soil nutrients by use of chemical fertilizers is the most prevalent approach for getting best yield of crops. However, their use efficiency remains low (Sturz *et al.*, 2000). Extensive application of chemical fertilizers subverts the soil ecology, disrupts the environmental balance, degrades soil fertility, contaminates groundwater and thus leads to sever adverse effects on environment and human health (Joshi *et al.*, 2006).

Therefore, supplementation with biofertilizers, like PGPB, attracted great interest as it can be an environmentally friendly alternative for increasing wheat yields and grain quality, and sustaining the innate fertility of soils (Rana *et al.*, 2012). In fact, inoculation of efficient PGPR not only improved yields, but also influenced minerals concentrations in grains. Therefore, it can be expected that PGPB have a high potential for contributing to food security and for improving nutrition status. Meanwhile, PGPB reduce iron toxicity in wheat when excess iron presented in soils by regulating iron bioavailability in plant rhizosphere and increasing the activities of antioxidant enzymes in plants. However, comprehensive work is still needed to decide the specific strains, bacterial loading dosages, and even delivery approaches during field application.

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