Enhancing grain iron content of rice by the application of plant growth promoting rhizobacteria

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ABSTRACT

Rice is inherently low in micronutrients, especially iron, which leads to severe malnutrition problems in rice-consuming populations. Different plant growth promoting rhizobacterial strains (PGPRs) (viz. *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Azospirillum lipoferum* from a microbial collection and B 15, B 17, B 19, BN 17 and BN 30 isolated from the rhizospheric soils) were applied to field grown rice plants with an aim to increase the iron content of grains. 16S rRNA gene sequence showed that isolates belong to *Enterobacteria* species. Different parameters related to the increase in iron content of plants show an enhancement upon treatment of rice plants with PGPRs. Treatments with *P. putida*, B 17 and B 19 almost doubled the grain iron content. Besides this, the translocation efficiency of the iron from roots to shoots to grains was also enhanced upon treatment with PGPRs. It is therefore concluded that application of PGPR strains is an important strategy to combat the problem of iron deficiency in rice and consecutively in human masses.

Keywords: rice grains; PGPRs; iron uptake; iron translocation

Micronutrients are indispensable for most living organisms, including humans who need a supply of 16 mineral microelements, which can be obtained through a balanced diet (Borg et al. 2009). Current estimate suggests that almost half of the world's population suffers from mineral deficiencies, primarily of iron and zinc. The Copenhagen Consensus (2008) ranks the alleviation of iron and zinc deficiencies as a top priority (Copenhagen Consensus 2008).

Iron is the third among the most limiting nutrients for plant growth primarily due to the low solubility of the oxidized ferric form in aerobic environments (Zuo and Zhang 2011, Samaranayake et al. 2012). Iron deficiency is a common nutritional disorder in many crop plants, resulting in poor yields and reduced nutritional quality. Increasing available Fe levels in staple food crops is an important strategy to reduce Fe deficiency in people (Cakmak 2002). The World Health Organization (WHO) has estimated that nearly 3.7 billion people are iron deficient, with 2 billion of these being anemic (WHO 2007).

A common approach to mitigating Fe deficiency is to promote healthy food, supplementation, and food fortification (Haas et al. 2005), but poor families, especially from developing countries cannot afford these strategies. Biofortification with Fe in staples provides an economical tool to rescue Fe deficiency in target populations globally (Jeong and Guerinot 2008, Nagesh et al. 2012). Biofortification with micronutrients in edible parts of crop can be achieved by utilizing crop and soil management (Zuo and Zhang 2011). Moreover, Fe biofortification is difficult as many beneficial Fe compounds (e.g., $FeSO_4$) are unpalatable, and less soluble Fe compounds are poorly absorbed (Hurrell 2002). Thus, increasing grain Fe content has a great potential in combating Fe deficiency and will have a dramatic impact on human health (Clemens et al. 2002). Increasing Fe content of rice is a difficult task for several reasons. Firstly, although

abundant in soils, plants cannot utilize largely insoluble $Fe^{(III)}$ compounds. Secondly, almost 30% of world's cultivated soils have high pH making situation worse. Finally, Fe can readily accept and donate electrons, making high concentration of Fe toxic as it can lead to production of reactive oxygen species (Bashir et al. 2010).

Rice feeds almost 50–58% of the world's population, hence it can be considered as a global grain (Zeng et al. 2010), but it is considerably deficient in micronutrients especially iron (Bouis and Welch 2010). Therefore, even a small increase in the nutritive value of rice can be highly significant for human nutrition (Zhang et al. 2012). A new and promising technique in this respect is the use of plant growth promoting microorganisms like fungi, bacteria and mycorrhiza. Treatment with *Trichoderma asperellum* resulted in increased grain Fe content in wheat (De Santiago et al. 2011). Also application of a mycorrhizal fungi with *Methylobacterium oryzae* increased Fe content in red pepper (Kim et al. 2010).

With these facts in mind, a field experiment was set up aiming to increase the grain iron content by the application of selected plant growth promoting rhizobacteria (PGPR) strains.

MATERIAL AND METHODS

Bacteria isolates. Three standard PGPR strains viz. *Pseudomonas putida* MTCC 102, *Pseudomonas fluorescens* MTCC 103 and *Azospirillum lipoferum* MTCC 2694 were obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India. Besides these, many bacteria were isolated from rice fields and assayed *in vitro* for Fe solubilisation on chrome azurol-S agar (CAS) media as described by Schwyn and Neilands (1987). Of all the strains, five of the better Fe solubilising strains were selected and tested in the field for Fe solubilising activity *in situ* and they were proved to increase Fe uptake by the plants. Also the sequence analysis and alignment of the 16S rRNA gene of the bacterial isolates show that the isolates belong to the *Enterobacteria* species.

Plant material and treatment. Seeds of three rice genotypes Jaya, PA6444, and Pusa basmati-1, were obtained from the Department of Genetics and Plant Breeding, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, India. The bacterial isolates were grown as liquid cultures; after obtaining sufficient population density they were applied to plants. 21-day old seedlings were uprooted and dipped in the bacterial culture overnight for root inoculation. Then the seedlings were transplanted in the field in a strip plot design for two kharif seasons 2010 and 2011. Fertilizer doses of nitrogen (100 kg/ha), phosphorus (60 kg/ha), potassium (45 kg/ha) and irrigation were applied as per recommendations.

At flowering, 3 plants from each plot were selected and the observations like peroxidase activity and active iron content were determined. Total peroxidase (POD) activity was determined by measurement of the tetraguaiacol produced as described by MacAdam et al. (1992). Active iron content was measured by the method described by Katyal and Sharma (1980).

Three plants from each plot were carefully uprooted. Thereafter root and shoot portions were washed successively with tap water, 0.1 mol/L HCl and then distilled water to remove contamination. Then the samples were oven-dried separately and then 1 g dried samples from different plant parts were taken for iron estimation by atomic absorption spectroscopy, according to the method described by Pirzadeh et al. (2010).

Statistical analysis. The statistical analysis for all the parameters was done by using the analysis of variance for strip-plot design with means being tested at $P > 0.05$ using an STPR software designed at the Department of Mathematics, Statistics and Computer Science, CBSH, G.B. Pant University of Agriculture and Technology, Pantnagar, India.

RESULTS AND DISCUSSION

***In vitro* iron solubilisation**. Of all the cultures tested, three standards viz. *P. fluorescencs*, *P. putida* and *A. lipoferum* were strong iron solubilisers (Table 1). Amongst the isolates, B 15 and B 19 were strong Fe solubilisers; while B 17, BN 17 and BN 30 show a moderate activity to solubilise Fe. This activity of bacteria is due to their siderophore production. *Pseudomonas* and *Azospirillum* produce siderophores and solubilise iron as a part of their life cycle. Thus, Fe solubilising isolates must produce siderophores to account for their Fe solubilising potential. Siderophore production converted Fe into soluble, physiologically available form. Based on the 16S rRNA sequencing, the isolates were identified as *Enterobacteria* species.

Table 1. Iron solubilisation potential of the eight bacterial strains as tested on chrome azurol-S agar medium

Culture	Iron solubilisation
P. putida MTCC 102	+++
P. fluorescens MTCC 103	+++
A. lipoferum MTCC 2694	+++
B 15	++++
B 17	++
B 19	+++
BN 17	+
BN 30	+

++++ very strong solubiliser; +++ strong solubiliser; ++ moderate solubiliser and + weak solubiliser; grading was done on the basis of the area of the siderophore production by the bacteria under study

Similarly, siderophore production by some isolates from a non-rhizospheric soil was demonstrated by Deepa et al. (2010). All five isolates were later applied as treatments in a field experiment to analyze their effect on rice Fe uptake potential and content.

Marker enzyme. Peroxidase uses Fe as a cofactor to form holoenzyme, therefore peroxidase was studied as a marker for Fe uptake. Peroxidase activity (Figure 1) was higher for all the treatments compared with control, especially *P. fluorescens*, *A. lipoferum* and B 15 showed an increase of 2–3 times in activity over control. High Fe solubilisation potential of the treatments B 17 and B 19 was not in accordance with peroxidase activity, because the isolates B 17, B 19, BN 17 and BN 30 have influenced other iron containing proteins like catalase and photosynthetic proteins, as overall increase in the iron content was imminent, similar results were documented by Del Rio et al. (1978) who reported that with an increase in concentration of Fe from 0.6 to 3.0 ppm in the nutrient solution peroxidase activity remained unaltered while catalase increased under similar conditions. The increase in peroxidase activity in rice leaves in response to the treatment with iron and other micronutrients was also reported (Fang and Kao 2000). Almost 1.5 times increase in peroxidase activity was recorded by De Santiago et al. (2011) in wheat in response to *Trichoderma asperellum*. Increased peroxidase activity directly correlates to the Fe content of the plant. Besides the treatment no major difference was recorded in the genotypes.

Active iron content. Iron plays an indispensable role in the plants metabolism participating

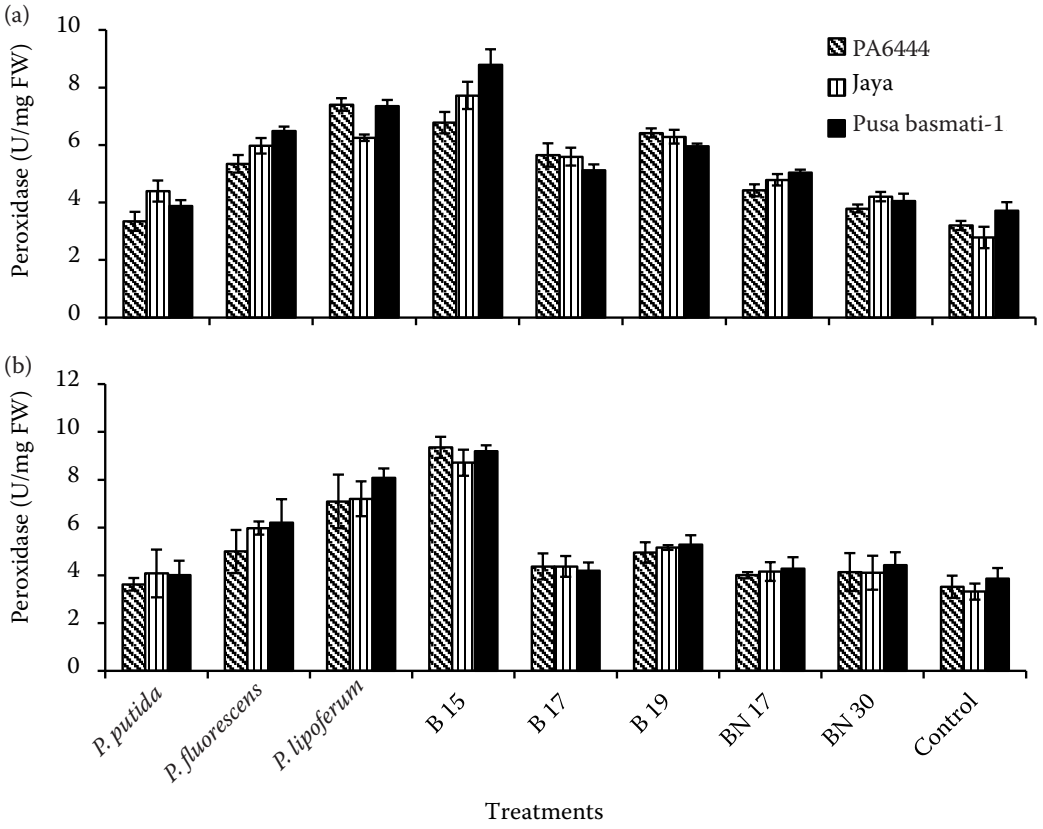

Figure 1. Peroxidase activity of three rice genotypes as affected by the treatment with different strains of plant growth promoting rhizobacteria in the Kharief season (a) 2010; (b) 2011 at the time of flowering

Table 2. Effect of seedling treatment with different plant growth promoting rhizobacteria on active iron content (µg/g DW) in three genotypes of rice at the time of flowering

Treatments	Active iron					
	Jaya		PA6444		Pusa basmati-1	
	2010	2011	2010	2011	2010	2011
P. putida	19.03	19.24	15.40	15.16	19.63	18.33
P. fluorescens	15.54	18.45	16.86	16.14	18.61	18.12
A. lipoferum	22.85	15.75	24.89	15.16	25.30	17.61
B 15	20.21	17.49	17.18	13.81	22.27	13.32
B 17	20.53	15.26	20.10	19.73	22.67	16.33
B 19	19.60	13.21	17.69	13.25	19.03	14.77
BN 17	17.10	13.67	16.53	14.79	19.77	14.60
BN 30	16.54	16.52	16.20	15.54	16.08	16.98
Control	13.52	11.95	14.87	12.06	14.39	12.93
Critical difference $P > 0.05$	2010			2011		
	genotypes (G)	treatments (T)	G × T	genotypes	treatments	G × T
	0.65	0.18	0.67	0.56	0.39	0.85

in various processes right from photosynthetic electron transport in the form of cytochromes and quinones up to its involvement in the functioning of important enzymes like peroxidase and catalase, these all form a pool known as active iron content of the plant. The data on active iron (Table 2) indicated an increase in iron content of plants compared with control leading to an increase of up to 40% in 2010 and 30% in 2011. Treatments with *A. lipoferum* and B 17 during 2010 and *P. putida* and *P. fluorescens* during 2011 were the most responsive. A general increase without a clear cut trend for any treatment or genotype was observed. Active iron content represents a pool of Fe in cell involved in various cellular activities or present as free ion (i.e. outside vacuole), thus an increase in active iron content signified an increased uptake of iron by plants and enhancement of cellular metabolism, as suggested by data on enzyme activity.

Iron content. Iron content in different parts of plant was measured at flowering and harvest. The data on root and shoot Fe content (Table 3) indicate an increased uptake and movement of iron in treated plants. Root iron content increased by 4.7–15.7% during 2010, and 3.1–13.3% during 2011 for different genotypes and treatments. Similarly, shoot iron content increased by 10.7–41.1% during 2010 and by 4.6–42.4% during 2011. Treatments with *P. putida*, *P. fluorescens*, *A. lipoferum* and B 15 were best for all genotypes during both years with B 17 treatment closely trailing behind. Using the same approach, Mishra et al. (2011) reported that iron content doubled in lentil seeds when grown in association with *Pseudomonas* species. This increased Fe content of plants also correlates with increased enzyme activity and active iron content. This increase was also due to the production of siderophores by the bacterial strains that resulted in higher available iron content in the root zone of the plants; increased root activity by the PGPR treatment resulted in higher uptake of Fe by host plants.

Though Fe content in vegetative parts of rice plants increased, we aimed to increase the Fe content of grains. Therefore iron content of grains and husk (Table 3) was determined separately after harvesting. A significant increase in Fe content of grains for all the treatments in both the years was observed. Iron content in dehusked grains was higher – 1.1–2.4 times, and 1.2–2.1 times in 2010 and 2011, respectively than control in all genotypes for different treatments. Similarly upto 1.8 times higher Fe content was observed in husk when compared with control. Translocation efficiency of iron from roots to grains was higher for the *P. putida*, *P. fluorescens*, *A. lipoferum*, B 15, B 17 and B 19 treatments. In different genotypes, grain Fe content was most affected by *P. putida*, B 15 and B 17.

An increase of 1.5 times over control was recorded in Fe content of aerial parts of wheat in re-

Table 3. Total iron content (μg/g DW) in roots and shoots at flowering and in grains and husk after harvesting of the three rice genotypes treated with different strains of plant growth promoting rhizobacteria

Genotypes	Treatments	Root		Shoot		Grain		Husk	
		2010	2011	2010	2011	2010	2011	2010	2011
Jaya	*P. putida*	747.7	742.5	334.7	306.3	141.7	124.0	114.1	112.5
	P. fluorescens	728.3	713.9	327.3	307.3	111.8	105.7	122.5	110.5
	A. lipoferum	731.5	710.3	328.8	339.3	134.2	112.6	134.8	130.9
	B 15	730.0	717.5	345.2	318.0	115.1	98.5	128.8	145.9
	B 17	736.9	722.0	305.0	273.1	120.3	106.2	128.0	130.9
	B 19	732.1	706.7	293.2	301.5	123.5	120.0	112.7	110.5
	BN 17	744.5	736.5	275.0	227.9	90.0	87.2	99.3	96.9
	BN 30	702.4	695.5	271.1	223.4	80.0	87.3	90.0	92.1
	control	658.7	643.7	236.5	213.8	60.7	65.7	83.8	82.9
PA6444	*P. putida*	724.9	725.1	323.4	308.0	123.8	131.8	99.0	100.8
	P. fluorescens	733.7	702.8	346.6	330.7	122.1	106.4	130.4	139.1
	A. lipoferum	699.2	726.8	332.1	346.5	119.4	121.4	139.3	149.3
	B 15	739.2	723.6	348.2	307.2	103.4	107.1	116.4	126.2
	B 17	710.1	715.7	309.4	279.6	131.6	113.9	108.0	115.6
	B 19	718.1	711.8	286.3	296.0	118.5	102.9	141.4	129.0
	BN 17	727.7	712.4	291.7	241.9	82.6	75.8	96.7	108.2
	BN 30	729.0	697.6	250.0	228.7	84.3	73.0	103.9	97.7
	control	666.4	667.8	205.1	211.0	62.7	61.7	83.6	97.5
Pusa basmati-1	*P. putida*	751.0	702.1	301.1	317.8	145.0	111.8	137.8	133.0
	P. fluorescens	742.0	727.2	338.3	308.8	130.9	105.2	103.8	116.6
	A. lipoferum	735.0	725.0	343.3	335.0	126.6	105.7	146.8	126.8
	B 15	739.1	727.3	327.3	340.1	121.4	101.2	142.5	136.6
	B 17	725.4	710.0	303.9	268.6	118.7	96.9	142.5	136.1
	B 19	743.5	718.7	308.3	284.3	110.7	105.7	121.7	111.2
	BN 17	679.4	705.7	256.9	230.3	81.7	77.1	90.0	89.2
	BN 30	695.0	699.2	252.7	222.9	84.9	71.9	104.1	99.8
	control	633.9	677.4	225.5	196.0	71.3	60.3	81.1	80.9
Critical difference $P > 0.05$	genotypes (G)	7.01	9.46	15.08	16.80	8.95	12.85	2.88	11.71
	treatments (T)	4.47	3.95	5.62	4.44	3.09	5.98	1.50	3.96
	G × T	14.09	10.46	20.95	25.02	21.08	21.56	4.67	22.71

sponse to *Trichoderma asperellum* as was reported by De Santiago et al. (2011). Similarly in capsicum, almost a three fold increase in iron content was observed in edible parts in response to treatment with different mycorrhizal and methylobacterium strains (Kim et al. 2010).

In conclusion, although Fe is important for humans, it is lacking in cereals primarily used as staple food crops around the world. Our study aimed at increasing the content of iron in rice grains by using some PGPR strains. The presented results show that the application of several PGPR strains not only led to an increase in iron uptake by plants but also increased the translocation of iron into the grains. This strategy is still under development but it possesses immense potential as an application to increase the micronutrient uptake by the host plants and judicial use of the PGPR strains can also improve the general health of the plant and assist in increasing crop yield.

Acknowledgement

The financial assistance provided by the CSIR, New Delhi, India during the period of this study is duly acknowledged.

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Received on October 12, 2012
Accepted on December 4, 2012

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