BIOFORTIFICATION OF IRON IN CHICKPEA BY PLANT GROWTH PROMOTING RHIZOBACTERIA

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Abstract
Iron deficiency is a major nutritional disorder being responsible to affect millions of people around the globe. Its malnutrition may be reduced through biofortification: a process to produce micronutrient enriched staple food. Plant growth promoting rhizobacteria (PGPR) can fortify iron content within edible plant tissues by enhancing its availability through various mechanisms. In a pot study, five bacterial isolates (S1, S2, S3, S4 and S5) were tested for improving plant growth and bioavailable iron (Fe) content in chickpea where Fe was applied in the form of iron sulphate solution. Results showed that inoculation with PGPR significantly enhanced the plant height, root length, root fresh and dry weights, shoot fresh and dry weights and Fe content compared to un-inoculated control plants. Application of FeSO4 significantly improved the Fe content up to 100 and 173% in grain and shoot respectively, as compared to control. Application of PGPR along with iron showed 81 and 75% increase in grain and shoot iron contents, respectively, over control. These results suggested that PGPR can help plants to uptake extra Fe from soil, if soil is supplemented with additional Fe. These findings advocate that microbial assisted biofortification in grain can alleviate micronutrient deficiency in humans especially in resource limited countries.

Key words: Micronutrients, Siderophile, Bioavailable, Phytohormones.

Introduction
Micronutrient malnutrition is a serious problem to human health throughout the world, primarily in resource limited countries (Kennedy et al., 2003). According to estimates of World Health Organization (WHO), around two billion people are being suffered with vitamin A, iron and iodine deficiencies. Iron deficiency is a highly prevalent nutritional disorder afflicting 2.5 to 5 billion people around the globe (Yip, 2002) where poor households and pre-school children are severely affected due to high demand for iron (Benoist et al., 2008). Iron acts as co-factor for several enzymes performing basic functions in human body. Inadequate supply of iron contributes to disability, anemia and stunted mental growth (Sheftela et al., 2011). Its malnutrition may be reduced by enhancing the bio-available iron content through iron supplementation and food fortification (Rana et al., 2012). These attempts are usually expensive and difficult to sustain on daily basis particularly in undernourished countries (Best et al., 2011). Therefore, it seems most desirable that crop fortification with iron content would be cost effective approach to overcome the hidden hunger of iron.

Biofortification is a process to produce micronutrient enriched staple food through mineral fertilizer, conventional breeding and transgenic approaches but their success have not always been consistent (Murgia et al., 2012). Plant growth promoting rhizobacteria have been reported to fortify the iron contents in food crops besides improving the soil fertility and crop yield through siderophore production (Rana et al., 2012). Siderophores are low molecular weight organic compound having high affinity for iron. Typically, there are four major groups of siderophore namely catecholates, hydroxamates, hydroxypyridonates, or aminocarboxylates (Neilands, 1977). Siderophores form very stable iron complexes and enhance the dissolution of iron bearing minerals by coordinating with iron atom at mineral surface. Plant growth promoting rhizobacteria synthesize siderophores, release those into surrounding environment, dissolve the iron by making iron chelate complex and translocate towards the plant by growing roots. These iron siderophores complexes are taken up by plant through transporter proteins that are located on plasma membrane of root (Boukhalfa & Crumbliss, 2002). Furthermore, these rhizobacteria enhance plant growth by conferring several beneficial effects such as increased nitrogen fixation, phosphorus solubilization, synthesis of phytohormones, production of organic acid and reduce susceptibility to disease (Ahmed & Kibret, 2014). Sharma et al. (2013) reported that iron content in rice grain was improved by the application of plant growth promoting bacteria (PGPR). Therefore, biofortification of plant through PGPR is considered as safe measure to improve iron content in different edible plant parts and to alleviate its malnutrition.

Chickpea has vital dietary importance in developing countries including Pakistan. Chickpea is ranked 3rd grain legume in world and first in South Asia for its production. It is a rabi pulse crop grown in rain fed areas (Shah et al., 2013). It is cultivated in 1.12 million hectares with 0.98 million tons production in Pakistan (Anon., 2014) Chickpea is cheapest and readily available source of fats (11.40%), protein (19.5%), ash (4.8%) and carbohydrates (57-60%) (Anon., 2014). So, it is imperative to biofortify the chickpea with iron through PGPR. To address this issue, present study was carried out to improve iron uptake and overall growth and yield of chickpea through rhizobacterial inoculation.

References
Benoist et al., 2008. Conference on Nutrition and Health in Asia, Oxford, UK.
Kennedy et al., 2003. Journal of Nutrition, 133(7), 2272S-2278S.
Materials and Methods

Five previously well characterized plant growth promoting bacterial strains (S1, S2, S3, S4 and S5) having ability to produce siderophore were used in this study. 

Inoculum preparation and seed treatment: The inocula of selected strains were prepared by growing them in glucose peptone broth medium. Flasks containing inoculums were incubated at 28± 1°C for 48 hours. Uniform cell density (10^7-10^9 CFU mL^-1) was maintained by measuring the optical density at 535 nm. Inoculum of each culture was injected into sterile peat (100 mLkg^-1) obtained from Chhanga Manga Forest Station and incubated for 24 hours at 28 ± 1°C. For seed inoculation, seeds were dressed with slurry that was prepared by mixing of clay, peat and 10% sugar solution. In case of un-inoculated control, seeds were coated with the same but autoclaved bacterial suspension.

Pot experiment: Pot trial was conducted to study the effect of five bacterial strains (S1, S2, S3, S4 and S5) with and without iron on the growth and iron contents of chickpea. For this purpose, pots were filled with 10 kg soil having sandy clay loam texture, EC _e_1.14 dSm^-1 and saturation percentage (32%). Five inoculated chickpea seeds were sown at 5cm depth in each pot. All the isolates were tested alone and in combination with the iron @ 28 ppm/pot (5.6 kg ha^-1) in the form of iron sulphate solution (soil application). A control treatment was also maintained where neither iron nor PGPR were applied. Another treatment was included where only iron was applied but un-inoculated. All the treatments were arranged according to completely randomized design (CRD) with three replications. Recommended doses of NPK @ (20, 65 and 20 kg ha^-1) were applied in each pot as urea, diammonium phosphate and sulphate of potash, respectively. Pots were irrigated with water, meeting the irrigation quality criteria for crop. Five seeds were sown per pot, after germination, thinning was done to maintain the one plant per pot. Furthermore, plant protection measures were adopted against the attack of pests. Plants were harvested and the data related to yield contributing parameters were recorded at maturity. Root and shoot of plants were analyzed for Fe contents. Wolf (1982) method was followed for the digestion of plant samples. For this purpose, 0.1 g oven dried and ground plant samples were taken in a digestion tube and 2 mL of concentrated sulfuric acid was poured into it. The samples were left over night at room temperature. Then 1 mL 35% extra pure H_2O_2 was poured into the digestion tube along the sides and the tube was rotated. The tube was heated up to 350°C after mounting it in a digestion block for 20 minutes. Then the tube was removed, cooled and added with 1 mL of H_2O_2 and again heated for 20 minutes. Same procedure was continued until the material became colorless. Fifty mL volume of the colorless extract was made by using distilled water and stored for the determination of Fe contents. Samples from different plant parts (grain, shoot and roots) were analyzed for iron accumulation by atomic absorption spectrophotometer. Iron content in soil was determined by ammonium bicarbonate-DTPA (AB-DTPA) extractable method (Soltanpour et al., 1976). The data were analyzed by using statistical procedures (Steel et al., 1997) and means were compared according to Duncan’s Multiple Range Test (DMRT) (Duncan, 1955).

Results

Effect on plant growth and yield: Result regarding the plant growth and yield (Table1) revealed that selected bacterial isolates significantly improved the plant height compared to the un-inoculated control plant, whereas the performance of isolates was better in the presence of iron. Plant height was found to be enhanced with isolate S1 and S4 but maximum plant height was observed by combined application of isolate S2+Fe which was 97% more compared to untreated and un inoculated control plants. A significant increase in shoot fresh weight was also observed with PGPR inoculation compared to control and iron treated plants whereas maximum increase (94%) in fresh weight of shoots was recorded by the combined application of Fe and S1. Similarly, all the PGPR isolates significantly improved shoot dry weight. The highest increase in shoot dry weight (78%) was observed with combined application of Fe and S2 over un inoculated control which was statistically different from other isolates. Results regarding root length of chickpea revealed that root length was significantly improved by all the isolates. The isolate S2 showed the highest improvement in root length (97%) when used in combination with iron and gave significant results compared to other isolates. Moreover, isolate S2 significantly enhanced the root fresh weight but highest root dry weight was noted when isolate S1 was used in combination with iron. Similarly, the highest root dry weight (84% more) was noted with combined inoculation of isolate S1 and iron. It was also observed that application of PGPR alone and in combination also improved the grain yield over control and Fe treated plants. Inoculation with S1 improved the grain yield up to 53% as compared to the treatment where only iron was applied. However, the maximum increase in grain yield (94%) was obtained by the combined application of S1 and Fe compared to control.

Effect on iron concentration in different plant parts: Data regarding the total iron concentration in grains (Table2) showed that PGPR inoculation significantly increased grain iron content compared to uninoculated iron treated plant. However, the maximum increase (81%) in Fe content of grains was observed with the combined application of Fe and isolate S2 as compared to uninoculated iron treated plant which was statistically different from other isolates. Similarly, inoculation had positive effect on shoot Fe content. It was observed that shoot Fe content enhanced up to 75% by combined application of Fe and isolate S2 over uninoculated iron treated plants. Iron content of root was increased up to 62% with isolate S1 as compared to uninoculated Fe treated plants whereas results were more pronounced (97%) when isolate S1 was used in combination with Fe compared to sole application of inoculation and Fe.
Table 1. Application of PGPR alone and in combination with Fe to improve growth and yield of chickpea.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Shoot fresh weight (g)</th>
<th>Shoot dry weight (g)</th>
<th>Root length (cm)</th>
<th>Root fresh weight (g)</th>
<th>Root dry weight (g)</th>
<th>Grain yield (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither iron nor inoculation</td>
<td>12.66f</td>
<td>4.79e</td>
<td>2.40e</td>
<td>13.33g</td>
<td>2.83g</td>
<td>1.03f</td>
<td>1.67g</td>
</tr>
<tr>
<td>Fe alone</td>
<td>16.66e</td>
<td>5.59d</td>
<td>2.83d</td>
<td>18.00f</td>
<td>3.23f</td>
<td>1.2ef</td>
<td>2.12f</td>
</tr>
<tr>
<td>S₁ alone</td>
<td>21.00cd</td>
<td>7.34e</td>
<td>3.60c</td>
<td>22.00de</td>
<td>4.8cd</td>
<td>1.23e</td>
<td>2.50de</td>
</tr>
<tr>
<td>S₂ alone</td>
<td>19.66de</td>
<td>7.5c</td>
<td>3.66e</td>
<td>21.33e</td>
<td>4.8cd</td>
<td>1.26e</td>
<td>2.46de</td>
</tr>
<tr>
<td>S₃ alone</td>
<td>21.66b-d</td>
<td>7.63e</td>
<td>3.80c</td>
<td>21.66de</td>
<td>4.46e</td>
<td>1.3de</td>
<td>2.43e</td>
</tr>
<tr>
<td>S₄ alone</td>
<td>21.66b-d</td>
<td>7.8c</td>
<td>3.70c</td>
<td>21.33e</td>
<td>4.56de</td>
<td>1.33c-e</td>
<td>2.38e</td>
</tr>
<tr>
<td>S₅ alone</td>
<td>21.00 cd</td>
<td>7.36c</td>
<td>3.73c</td>
<td>21.66de</td>
<td>4.63de</td>
<td>1.23e</td>
<td>2.57d</td>
</tr>
<tr>
<td>S₁+Fe</td>
<td>24.33 ab</td>
<td>9.33a</td>
<td>4.53b</td>
<td>22.66c-e</td>
<td>5.03bc</td>
<td>1.90a</td>
<td>3.24a</td>
</tr>
<tr>
<td>S₂+Fe</td>
<td>23.33 a-c</td>
<td>8.66ab</td>
<td>5.06a</td>
<td>26.333a</td>
<td>5.56a</td>
<td>1.60b</td>
<td>3.03b</td>
</tr>
<tr>
<td>S₃+Fe</td>
<td>23.00 a-c</td>
<td>8.86ab</td>
<td>4.63b</td>
<td>24.66b</td>
<td>5.13b</td>
<td>1.46b-d</td>
<td>2.86c</td>
</tr>
<tr>
<td>S₄+Fe</td>
<td>25.00a</td>
<td>8.76b</td>
<td>4.66b</td>
<td>23.00cd</td>
<td>5.1b</td>
<td>1.53b</td>
<td>2.91bc</td>
</tr>
<tr>
<td>S₅+Fe</td>
<td>22.00ad</td>
<td>8.83ab</td>
<td>4.70b</td>
<td>24.00bc</td>
<td>5.03bc</td>
<td>1.50bc</td>
<td>3.00bc</td>
</tr>
</tbody>
</table>

*Means sharing the same letter do not differ significantly at p≤0.05

Table 2. Application of PGPR alone and in combination with iron (Fe) on iron uptake indifferent plants parts of chickpea.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fe Concentration (mg 100 g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grains</td>
</tr>
<tr>
<td>Neither iron nor inoculation</td>
<td>1.20g</td>
</tr>
<tr>
<td>Fe alone</td>
<td>2.40f</td>
</tr>
<tr>
<td>S₁ alone</td>
<td>3.26de</td>
</tr>
<tr>
<td>S₂ alone</td>
<td>3.30c-e</td>
</tr>
<tr>
<td>S₃ alone</td>
<td>3.36b-e</td>
</tr>
<tr>
<td>S₄ alone</td>
<td>3.20e</td>
</tr>
<tr>
<td>S₅ alone</td>
<td>3.40b-e</td>
</tr>
<tr>
<td>S₁+Fe</td>
<td>3.60bc</td>
</tr>
<tr>
<td>S₂+Fe</td>
<td>4.36a</td>
</tr>
<tr>
<td>S₃+Fe</td>
<td>3.50b-e</td>
</tr>
<tr>
<td>S₄+Fe</td>
<td>3.53b-d</td>
</tr>
<tr>
<td>S₅+Fe</td>
<td>3.63b</td>
</tr>
</tbody>
</table>

*Means sharing the same letter do not differ significantly at p≤0.05

Discussion

Iron deficiency is a major nutritional disorder throughout the world. Biofortification is a technique to produce the micronutrient-dense staple crops. Plant growth promoting rhizobacteria have potential to fortify the iron content within edible plant parts through various mechanisms. In the present study, five bacterial isolates were tested alone and in combination with iron to increase iron concentration in edible parts of chickpea. The results of our study revealed that inoculation with PGPR isolates improved the plant growth, yield and Fe uptake. However, combined application of Fe and PGPR showed more pronounced results. Growth promotion by PGPR may be attributed to increased nitrogen fixation, P-solubilization, production of phytohormones and synthesis of organic acids (Remans et al., 2008). It has been reported that PGPR inoculation enhanced the root and shoot growth due to nitrogen fixation, P-solubilization as well as their ability to produce certain compounds (Salantur et al., 2006). PGPR improved the growth of chickpea plant by enhancing P-solubilization and indole acetic acid production (Yadav et al., 2010).

Moreover, siderophore production by rhizobacteria play important role in Fe nutrition of plant and thereby improve the plant growth. Husen (2003) found that siderophore produced by the bacterial isolates Bacillus cereus UW 85 and Azotobacter vinelandii MAC 259 increased the plant growth. Inoculation of soybean and chickpea seed with siderophore producing fluorescent Pseudomonas improved the plant growth and yield (Kumar & Dube, 1992). Similarly, growth factors such as shoot and root dry weight were improved by siderophore producing strains in bean (Omidvari, 2010). Growth promotion and reduction in pathogenicity was observed due to siderophore production by Bacillus megaterium isolated from tea rhizosphere (Chakraborty et al., 2006). Gangwar & Kaur (2009) isolated the E. coli from rye grass and sugarcane which had ability to produce siderophore and ultimately improved the plant growth and yield. Moreover, it has been reported that soybean growth under non-sterilized soil condition improved due to siderophore production by PGPR (Cattelan et al., 1999).

In our study, results regarding Fe concentration in different plant parts (grain, shoot and root) showed that Fe contents were enhanced by PGPR application. It was
observed that combined application of Fe and PGPR inoculation gave best results compared to all other treatments. This enhancement in iron content might be due to siderophore production by PGPR which made this combination more competitive than control and Fe application alone. Many bacteria release ferric iron-specific ligands, known as siderophores, under conditions of Fe scarcity help in binding and transport of iron (Abd-Alla, 1998). Sharma et al. (2013) found that application of PGPR improved iron translocation from root to grain through siderophore production. Similarly, inoculation of wheat seed with three cyanobacterial strains (CW1, CW2 and CW3) and one bacterial strain (PW3) improved the protein content of grain and micronutrients (Fe, Zn and Cu) concentration (Rana et al., 2012). Furthermore, increase in iron content might be due to over expression of ferritin genes. Previous reports showed that increasing capacity for iron storage in plants by over expressing ferritin genes that induced iron uptake mechanism, resulted in enhanced iron content (Goto & Yoshihara, 2001; Curie & Briat, 2003).

Conclusion

Inoculation with PGPR along with Fe increased the iron uptake and overall growth and yield of chickpea. Therefore, it can be concluded that application of Fe with rhizobacteria could be the most promising and cost effective strategy for improving the iron contents of chickpea. However, more work is still needed to reveal the exact mechanism of biofortification of micronutrients by rhizobacteria.

References


Plant growth promotion by plant growth promoting rhizobacteria is a well-known phenomenon and this growth enhancement is due to the fungus Thielaviopsis basicola [55], Pseudomonas putida against Macrophomina phaseolina in chickpea and Azotobacter chroococcum against Fusarium oxysporum in Sesamum indicum respectively in field condition [9]. In recent years, Pseudomonas fluorescens has been suggested as potential biological control agent due to its ability to colonize rhizosphere and protect plants against a wide range of important agronomic fungal diseases such as black. Plant growth-promoting rhizobacteria (PGPR) are bacteria capable of promoting plant growth by colonizing the plant root and they contain plant growth-promoting properties, such as production of ACC deaminase and IAA; nitrogen (N₂ gas) fixation; phosphate solubilization; and siderophore secretion (Zhuang et al., 2007). However, Zn plus Cd treatment affected to increase IAA production and to decrease the protein and lipid degradation and the P solubilization (Munjit et al., 2015). Pseudomonas aeruginosa PDMZnCd2003 is a plant growth-promoting bacteria (PGPB) that was isolated from the rhizosphere of Gynura pseudochina (L.) DC., which are a Zn/Cd hyperaccumulator in Thailand (Mongkhonsin et al., 2016; Panitlertumpai et al., 2013). Plant growth promoting rhizobacteria (PGPR) shows an important role in the sustainable agriculture industry. The increasing demand for crop production with a significant reduction of synthetic chemical fertilizers and pesticides use is a big challenge nowadays. The use of PGPR has been proven to be an environmentally sound way of increasing crop yields by facilitating plant growth through either a direct or indirect mechanism. The mechanisms of PGPR include regulating hormonal and nutritional balance, inducing resistance against plant pathogens, and solubilizing nutrients for easy uptake by plants. Plant growth promoting rhizobacteria (PGPR) can fortify iron content within edible plant tissues by enhancing its availability through various mechanisms. In a pot study, five bacterial isolates (S1, S2, S3, S4 and S5) were tested for improving plant growth and bioavailable iron (Fe) content in chickpea where Fe was applied in the form of iron sulphate solution. Results showed that inoculation with PGPR significantly enhanced the plant height, root length, root fresh and dry weights, shoot fresh and dry weights and Fe content compared to un-inoculated control plants. Iron chelating rhizobacteria. Plant growth promoting rhizobacteria. Siderophore. HPLC. Rana A, Joshi M, Prasanna R, Shivay YS, Nain L (2012) Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. Eur J Soil Biol 50:118â€“126. Article CAS Google Scholar.