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PGPR's mix treatment to *Moringa* improved plant growth and iron content in foliage as substantiated by biochemical and molecular methods

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ABSTRACT

In this study, *Bacillus pumilus* SE34 and *B. pumilus* T4 were combined with *Bacillus subtilis* GBO3, *B. pumilus* INR7 and *Pseudomonas fluorescens* UOM14 to form COM1, COM2 and COM3, respectively. All combinations were used to find their synergistic effect on *Moringa oleifera* for growth promotion and Fe accumulation efficiency in foliage. The results indicate a significant increase in Fe content in foliage using COM3 (405.70%) followed by COM2 (105.83%) in comparison to the control with a simultaneous decrease in the soil Fe content. Increased expression of iron-transport-related genes like iron-phytosiderophore oligopeptide transporter and natural-resistance-associated macrophage protein, in foliage of *Moringa* using real-time PCR correlates with the enriched iron content in foliage of treated plants. Increase in citric acid was in direct relation to the Fe accumulation in foliage. Growth promotion and Fe enhancement using plant-growth-promoting rhizobacteria's combination was significantly higher, proving its synergistic effect as a great source for sustainable development in agriculture and nutrition.

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Iron; iron-phytosiderophore oligopeptide transporter; natural resistance associated macrophage protein; organic acids; plant-growth-promoting rhizobacteria

1. Introduction

Human body requires a vast variety of nutrients to function properly and Fe plays a major role. Fe is a vital structural part of hemoglobin and necessary for the normal cell functioning and its development (Erdman et al. 2012). Since, Fe perform imperative roles in the human biology, its deficiency can lead to various diseases. Fe deficiency is the leading cause for anemia worldwide (Lopez et al. 2016), mainly because of its very low quantity in diet consumed by humans, insufficient intake, mal absorption and ineffective utilization (Roohani et al. 2013).

The easiest method to provide the system with commensurate measures of the nutrients is to take a diet rich in Fe. Bioavailability of Fe through plant sources is comparatively less than of animal sources due to the presence of phytate as in case of lentils and beans, whereas the presence of organic acids such as malic and citric acids made a plant source capable of Fe availability to the human body (Gillooly et al. 1982). Other than Fe bioavailability problem, the plants are themselves deficient in this element as they themselves do not receive Fe in sufficient amounts. One of the methods to increase the dietary intake of Fe from plant source is to make sure that the plants receive more than the sufficient amount of Fe from the soil and make them bioavailable to the human body on consumption (Saini, Nile et al. 2016; Pataco et al. 2017).

An approach to overcome poor Fe bioavailability would be to employ genetic engineering strategies by increasing the levels of siderophores, chelating agents, reducing agents, enzymes and transporter proteins in roots that help in increasing Fe uptake. Another method to increase the Fe bioavailability is to decrease the phytate-to-mineral ratio by combining cross-breeding techniques for developing low phytic acid plants with selection of highly nutrient-dense seeds (Zimmermann and Hurrell 2002). However, the introduction of genetically modified crops into the field has various ethical issues; therefore the use of plant-growth-promoting rhizobacteria (PGPR) is a simpler approach to this issue.

Growth promotional activities of PGPR such as superior germination, high vigor, enhanced yield along with endeavoring disease resistance against a wide variety of pathogens by triggering induced systemic resistance in plants are well studied (Zhang et al. 2002; Vessey 2003; Kloepper et al. 2004; Freitas et al. 2015; Rahmoune et al. 2017). PGPR enhances the levels of micronutrients in plants using various mechanisms (Loper and Henkels 1999). In many studies, PGPRs were found to enhance the Fe availability to the plants, making it easier for uptake. *Pseudomonas* species are known to produce siderophores that have high specificity to Fe III, and solubilize it, making it available for plant uptake (Wandersman and Delepelaire 2004). *Bacillus pumilus* SE34, *B. pumilus* T4, *B. pumilus* INR7, *Bacillus subtilis* GB03 are commercially available PGPRs that can be directly used to infect plants via seed coating, seed infection and soil drenching methods. Unlike other PGPR's these are also known for their growth-promotion-producing plant hormones and inducing systemic resistance promoting plant protection. *B. pumilus* SE34, *B. pumilus* T4, *B. pumilus* INR7 and *B. subtilis* GB03 have also been extensively studied individually or in combination for their plant growth promoting effects (Murphy et al. 2003; Kloepper et al. 2004; Kumar et al. 2011). They have also been widely employed on different plant systems such as cucumber, tomato, tobacco, pepper, millets and Arabidopsis against various fungal and viral infections. In recent years, *B. subtilis* GB03 has

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been extensively studied for its Fe accumulation ability in different plants such as Arabidopsis and cassava. *B. subtilis* GB03 was found to release an array of volatiles that were found to indulge in plant growth promotion, plant defense along with iron homeostasis. *B.-subtilis*-GB03-mediated enhancement of plant iron was found to occur via activation of the plant's own iron acquisition machinery (Zhang et al. 2009; Freitas et al. 2015). In the presence of PGPRs, plants are reported to exudate flavonoids and organic acids which perform specific functions such as biofilm formation and chemotaxis of organisms towards plant roots (Tyler and Strom 1995; Zhang et al. 2009; Ahmed et al. 2014). The role of various flavonoids and organic acids in solubilization of iron, phosphorus and other divalent such as Zn was reported from soil (Bais et al. 2006). Furthermore, these acids form complexes with Fe and allow easier uptake, wherein the role of divalent cation transporter (*IRT1*) gene for the transport of Fe from soil to the plant was elucidated (Vert et al. 2002). To boost the uptake of Fe from the soil, the Fe must be present in soluble form and in this regard two mechanisms were demonstrated. In strategy 1 pH of soil is dropped, leading to increase in solubility and uptake of iron via Protons' extrusion from roots of plants in the rhizosphere. In strategy 2 low molecular weight compounds; phytosiderophores (PS) are released in response to iron deficiency, and PS-Fe^{+3} complexes is then transported from soil to root using *IRT1* and other parts of the plants through iron-phytosiderophore oligopeptide transporter (*OPT*) (Buckhout et al. 2009; Yang et al. 2010; Kobayashi and Nishizawa 2012). *OPT* is generally involved in intracellular and long distance Fe transport in plants. *OPT* was also found to transport other divalent metals such as Zn and Mn. *OPT3* (a plasma membrane transporter) was also found to load Fe into phloem, indicating its role in Fe distribution from shoot to root and from mature organs to the developing one (Zhai et al. 2014). Advocating foliar spray and soil loading with PGPRs leads to enhanced production of bioactive metabolites in plants along with nutritional enrichment of plants (Giridhar and Parimalan 2010; Gururaj et al. 2012; Matam and Giridhar 2017).

Moringa, popularly known as drumstick tree, is a much sought-after food plant for addressing the malnutrition problems of the undernourished population of the developing world in view of its optimal levels of major nutrients' profile and also vital health-promoting oils (Anwar et al. 2003). Its foliage was found to be rich in carotenoids, tocopherols, iron, folate, fatty acids, etc. (Saini, Shetty, et al. 2014a; Saini, Shetty, et al. 2014b; Gupta et al. 2017). Use of an abiotic elicitor on plants enhanced accumulation of carotenoids and tocopherols in leaves (Saini, Prashanth, et al. 2014). The leaves were also reported for their superior bioavailability of Fe and folate in rat models (Saini, Manoj, et al. 2014; Saini, Manoj et al. 2016). Being an effective nutritional security provider, commercial cultivation of this high-value energy crop and its further improvement through biotechnological and other traditional methods to overcome cultivar and agroclimatic variation and enhancement of its nutrient profile would facilitate farmers to prefer this for better economical prospective. In view of this, *Moringa oleifera* was chosen as model plant for our study.

Hence in this study we have examined the effects of PGPR application on plant foliage Fe content and other growth promotional activities such as shoot length, primary root length, shoot weight, root weight and lateral root numbers. Levels of organic acids in root exudates were also analyzed as it helps in increasing the bioavailability of Fe. All the studies were restricted to foliage as it is important from food point of view.

2. Materials and methods

2.1. Bacterial growth and infection to seeds and soil

An array of potential PGPR which have been widely studied were selected for the study; these include *B. subtilis* GB03, *B. pumilus* INR7, *B. pumilus* SE34, *B. pumilus* T_4 and *Pseudomonas fluorescens* UOM14 obtained from University of Mysore. Three combinations of these PGPRs were made, based on initial characteristic studies and their competence with each other. The three combinations are COM1 (*B. pumilus*SE34 + *B. pumilus*T4 + *B. subtilis* GBO3), COM2 (*B. pumilus* SE34 + *B. pumilus*T4 + *B. pumilus* INR7) and COM3 (*B. pumilus* SE34 + *B. pumilus* T4 + *P. fluorescens* UOM14).

Two different media were used for the growth of these microorganisms. Tryptic Soya Broth (HiMedia, Mumbai) was used for *B. subtilis* GB03, *B. pumilus* INR7, *B. pumilus* SE34 and *B. pumilus* T_4 and *P. fluorescens* UOM14 was grown on King's B medium (Himedia, Mumbai). Upon inoculation of the microorganisms in their respective medium, the media were kept in a shaking incubator (Orbitek, India) at 120 rpm, with growth conditions of 12 h, 27°C. *M. oleifera*, PKM-1 variety seeds were used for the study. The seeds were washed using Tween 20 and kept under running water for 45 min, following which the seeds were transferred to aseptic conditions and sterilized using sodium hypochlorite (NaOCl) 5% (w/v) for 5 min; these were then rinsed thrice using sterile distilled water to free the seeds of residual NaOCl and infection step was carried (Saini et al. 2012). The broth culture of bacteria obtained by growing them for 12 h was centrifuged and the pellet obtained was washed using 1/8th Hoagland solution to remove residual growth media. The pellet obtained was mixed with (1/4th) strength of Hoagland solution to achieve a culture concentration of 1×10^8 cfu mL^{-1}. To make different combinations, cultures were mixed in equal amount and were used for further study. Surface sterilized *M. oleifera* seeds were transferred to the respective combinations and were kept for 6 h for infection. Subsequently the infected seeds and control seeds were sowed in pots to allow germination. The pots contained soil mix consisting of 2 kg sterile soil present in the ratio of 2:1:1 of soil:vermi compost:coir dust. The soil was also drenched with respective combinations.

Experiment was planned by a randomized block design. All the experiments to study the effect of PGPR on the levels of iron in *M. oleifera* foliage and soil were performed in polyhouse (12 h photoperiod, 25 ± 2°C and 70% relative humidity). Sampling was done at the sixth week after seed germination.

2.2. Fe analysis by atomic absorption spectrophotometry

Acid washed glasswares were used for the study. Ten grams leaf samples from the plants were collected and desiccated in a hot air oven for 3 h at 105°C and charred on an electrical stove until it ceased to smoke. One gram of this sample was transferred into muffle furnace and heated at 550°C to acquire white or grayish ash. Aquaregia (1 mL) was added

to the ash and diluted using 1 N HNO_3 prepared in Millipore water. Total Fe content in sample was estimated using direct aspiration into the Atomic Absorption Flame Emission Spectrophotometer (AA-6710F, Shimadzu). Wavelength was set to 248.3 nm with different Fe concentration ranging from 0.5 to 2 ppm. A calibration curve using four concentration of minimum range in analytical range was prepared.

Wet soil from rhizosphere (2.0 g) on the day of leaf sampling for Fe analysis was collected and extracted in low molecular weight organic acid solution (20 mL) containing acetic, lactic, citric, malic and formic acids in 0.01 M in the ratio of 4:2:1:1:1 so as to evaluate the extractability of Fe in soil treated by PGPR's combinations compared to control. Sample was extracted for 16 h, centrifuged and clear solution was taken for atomic absorption spectrophotometry after diluting to 1:1 with 1 N HNO_3 (Feng et al. 2005).

2.3. Root exudates composition

For the analysis of organic acids, 2 g rhizospheric soil was collected and extraction was carried out in 0.1% acidified methanol followed by centrifugation at 10,000 rpm for 15 min at room temperature. The supernatant collected was concentrated in the fume hood and suspended in 1 mL methanol, which was centrifuged again at 12,000 rpm for 10 min allowing the residual particles to settle. The consequential supernatant was used to carry out HPLC. C_{18} reversed phase SunFire column (250 mm × 4.6 mm × 5 µM) was used for the HPLC analysis of both the compounds. Isocratic elution was performed for the detection of organic acid using 5 mM H_2SO_4 with photodiode array (PDA) detector (Shimadzu SPD-M20A 230 V, Japan) (λ_{max} at 210 nm) with a flow rate of 1 mL per min.

2.4. Gene expression profile

2.4.1. RNA extraction and cDNA synthesis

Fresh leaves from the treatment and control plants were collected in the sixth week and frozen in liquid nitrogen. Spectrum Plant Total RNA Kit (Sigma, Bangalore) was used for the total RNA extraction from the samples, using the protocol provided by the manufacturer. The quality, quantity and integrity of extracted RNA were assessed by Nanodrop (Thermo Scientific, USA) and agarose gel electrophoresis. The RNA was treated with DNase I (Thermo Scientific) and reverse transcription was performed using Revert Aid First Strand cDNA Synthesis Kit (Thermo Fischer Scientific, USA). Gene expression analysis was then carried out using the resulting cDNA obtained from the previous step using RT-qPCR.

2.4.2. Quantitative real-time PCR (RT-qPCR)

Real-time studies were performed to analyze the expression pattern and to correlate it with biochemical analysis. Real-time primers were designed (Table 1) and analyzed to validate the accuracy and specificity using conventional PCR (Biorad). RT-qPCR was performed using the Applied Biosystems QuantStudio 5 Real-Time PCR System with the Maxima SYBR Green/ROX Master Mix (Thermo Fischer Scientific). To normalize sample variance, the *GAPDH* gene was used as reference. The data obtained were analyzed using the mean of the cycle threshold (CT) values of three biological replicates that were normalized to the mean CT values of the endogenous gene. The expression ratios were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). Relative transcription levels are presented graphically.

2.5. Plant growth measurements

Immediately after collecting foliage for non-destructive measurements, plants were harvested for shoot height, shoot dry weight, root dry weight; primary root length and lateral root numbers were measured manually.

2.6. Statistical analysis

The data were expressed as mean ± SD of triplicates and the experiments were repeated twice. Data were subjected to ANOVA (Sigma plot 12.5) analysis with post hoc using Tukey's test (SPSS 17) at the significance level of $P < .05$.

3. Results

All the PGPRs that were used for mix formulation were tested individually for their Fe enhancement ability in *Moringa* foliage. *B. pumilus* SE34, *B. subtilis* GBO3 and *B. pumilus* T4 were found to enhance Fe accumulation by 80–85%. *B. pumilus* INR7 and *P. fluorescens* UOM14 showed only 30–40% Fe accumulation in *Moringa* foliage. *B. pumilus* SE34 and *B. pumilus* T4 were kept constant in all the combinations to study their synergistic effect on *Moringa* foliage with PGPR related to same or other species or other genus. In COM1 *B. pumilus* SE34, *B. pumilus* T4 were combined with *B. subtilis* GBO3. In COM2 *B. pumilus* SE34, *B. pumilus* T4 were combined with *B. pumilus* INR7. In COM2 *B. pumilus* SE34, *B. pumilus* T4 were combined with *P. fluorescens* UOM14.

3.1. Fe analysis by atomic absorption spectrophotometry

The PGPR combination COM3 showed a significant increase of Fe (405 ± 15.34%), followed by COM2 (105.83 ± 4.65%) and COM1 (62.84 ± 2.58%) in foliage of *Moringa* (Figure 1). In soil, the extractability of Fe shown for COM3 (100 ± 4.78%) was significant followed by COM2 (69.11 ± 3.78%) and COM1 (63.13 ± 2.78%) compared to control (Figure 1).

Table 1. List of primers of genes used for real-time studies.

Gene	Accession no	Primers	Function
Glyceraldehyde-3 phosphate dehydrogenase (*GAPDH*)	JQ764560	F- CCTCTTGCAAAGGTCATTCA R- GCTCTTCCACCTCTCCAGTC	Glycolysis pathway
NRAMP	JQ398856	F- AGCTCGAATTCTTGATTGCC R- ACGGGATTTGCATAGGAAAG	Accumulate in response to iron deficiency
OPT	JQ398857	F- AGGCAGGATGAAGAACAACC R- TACCCTATGCATGCTACCCA	Transport small peptides, secondary amino acids that can complex with metals, and the modified tripeptide glutathione

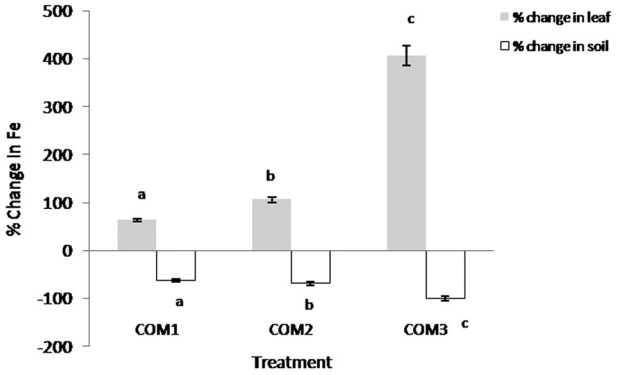

Figure 1. Influence of PGPR's mix treatment on Fe content of *Moringa* foliage and rhizospheric soil. Experiments were repeated three times, each with three replicates. Columns without a common lowercase letter indicate significantly different values among treatments based on Tukey's test ($P < .05$).

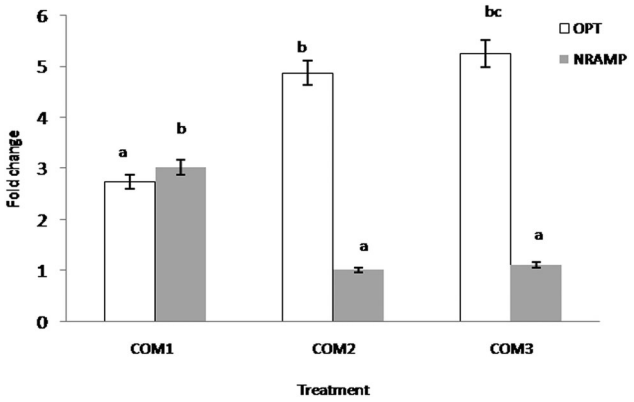

Figure 3. Expression pattern of *OPT* and *NRAMP* in *Moringa* foliage upon treating with various PGPR's combinations. Columns without a common lowercase letter indicate significantly different values among treatments based on Tukey's test ($P < .05$).

3.2. Organic acid composition of root exudates

In root exudates of control, oxalic acid (391.9 ± 11.96 μg kg^{-1}) and ascorbic (131.8 ± 5.28 μg kg^{-1}) acid were detected in higher quantities (Figure 2). However in COM1-, COM2- and COM3-treated plants, root exudates' levels of oxalic acid and ascorbic acid were lower compared to control. Oxalic acid in COM1, COM2 and COM3 was around 318.9 ± 8.97 μg kg^{-1}, 317.2 ± 7.65 μg kg^{-1} and 337.9 ± 9.84 μg kg^{-1}, respectively (Figure 2). Ascorbic acid in COM1, COM2 and COM3 was around 20.8 ± 1.24 μg kg^{-1}, 70.6 ± 3.34 μg kg^{-1} and 42.3 ± 2.27 μg kg^{-1}, respectively (Figure 2). Citric acid was not detected in control, whereas it was present in root exudates of all the treatments. It was found to be maximum in COM3 (1622.5 ± μg kg^{-1}) followed by COM2 (1503.4 ± μg kg^{-1}) and COM1 (1058.8 ± μg kg^{-1}) (Figure 2).

3.3. Gene expression profile

A higher level of expression of *OPT* was seen in COM3 (5.25 ± 0.87 fold) in comparison to COM2 (4.87 ± 0.32 fold) and COM1 (2.73 ± 0.45 fold) (Figure 3). Increase in the expression of natural-resistance-associated macrophage protein (*NRAMP*) was also seen in COM1 (3.02 ± 0.56 fold) followed by COM3 (1.11 ± 0.1 fold) and COM2 (1.01 ± 0.12 fold) (Figure 3).

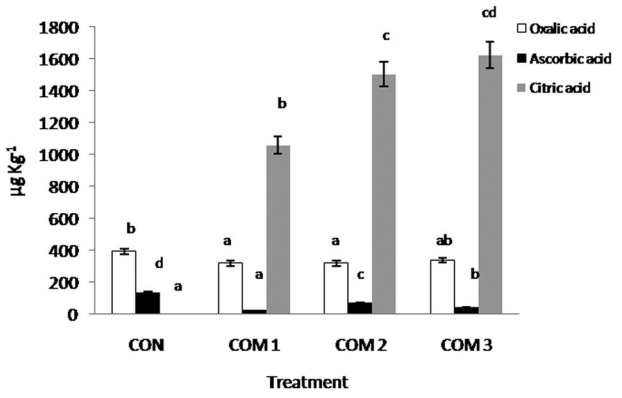

Figure 2. Organic acid composition in root exudates of *M. oleifera* under the influence of PGPR's treatment. Experiments were repeated three times, each with three replicates. Columns without a common lowercase letter indicate significantly different values among treatments based on Tukey's test ($P < .05$).

3.4. Plant growth measurements

All the PGPR mixes have shown a significant effect on shoot length. Maximum enhancement of shoot length was seen in COM2 (50 ± 2.5 cm), followed by COM1 (47.5 ± 1.72 cm) and COM3 (45.5 ± 1.56 cm) (Figures 4 and 5(A)). Number of root hairs was found to be higher in COM3 (55.5 ± 3.67), followed by COM1 (46 ± 2.84) and COM2 (30 ± 1.87) (Figures 4 and 5(B,C)). No significant change in root length was observed (Figure 5(C)). A noteworthy increase in total biomass of *Moringa* (dry weight) was observed in COM2 (0.0031 ± 0.00023 kg), followed by COM3 (0.0024 ± 0.00018 kg) and COM1 (0.002 ± 0.00016 kg) (Figure 4).

4. Discussion

4.1. Fe analysis by atomic absorption spectrophotometry

In the present study, PGPRs' mix showed a profound influence on Fe content of *M. oleifera* foliage and also positively influenced shoot and root growth. Various studies involving the effect of PGPR on Fe have been conducted in economically important plants. The production of siderophores by the PGPR helped to enhance the Fe uptake in cucumber (*Cucumis sativus*) (Wang et al. 1993), maize (*Zea mays* L.) and sunflower (*Helianthus annuus L.*) (Masalha et al. 2000). A study conducted on Raspberry grown in soil supplied with PGPR strains of *Bacillus* (strains OSU-142 and M3) showed significant effects on Fe availability in the soil due to decreased pH and increased mineralization (Orhan et al. 2006). *B. subtilis* GBO3, *B. pumilus* INR7, *B. pumilus* SE34 and *B. pumilus* T4 and *P. fluorescens* UOM14 have been widely used for its disease resistance and growth promotional activities individually or in different combinations (Kokalis et al. 2003; Raj et al. 2003). But their effect in different combinations on Fe accumulation and growth promotional activities directly on food plant such as *Moringa* is scarce. The result obtained in the present study for various growth parameters and Fe accumulation in *Moringa* was further supported by similar studies in another plants. *B. subtilis* GBO3 was found to increase Fe accumulation in cassava leaves by 300% (Freitas et al. 2015). Increased uptake of Fe was attributed to various activities of plants such as volatile, organic acid and flavonoid production, which results in a decrease in soil pH (Zhang et al. 2009).

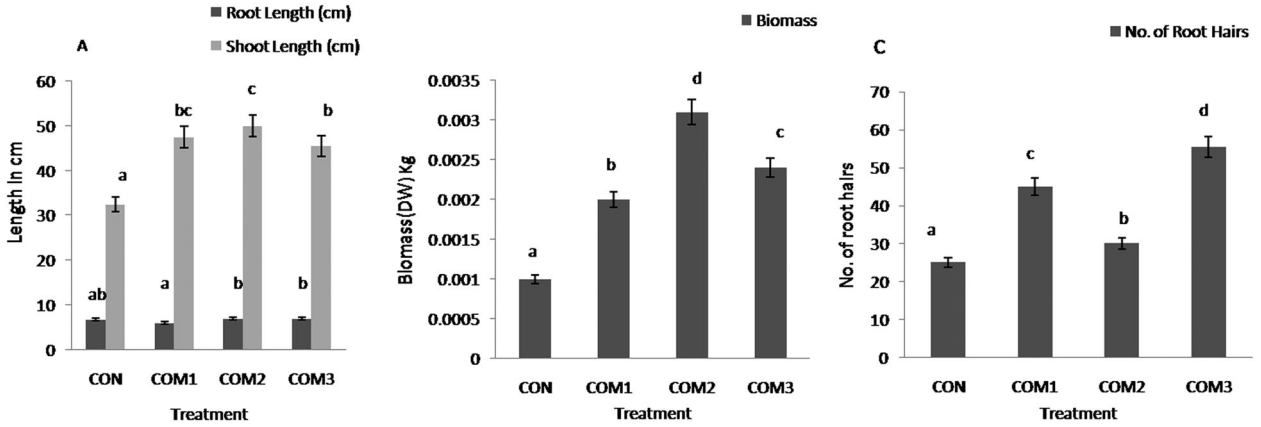

Figure 4. Growth promotion activities of PGPR's mix treatment on (A) shoot length and root length, (B) plant biomass, (C) root hair no. Experiments were repeated three times, each with three replicates. Columns without a common lowercase letter indicate significantly different values among treatments based on Tukey's test ($P < .05$).

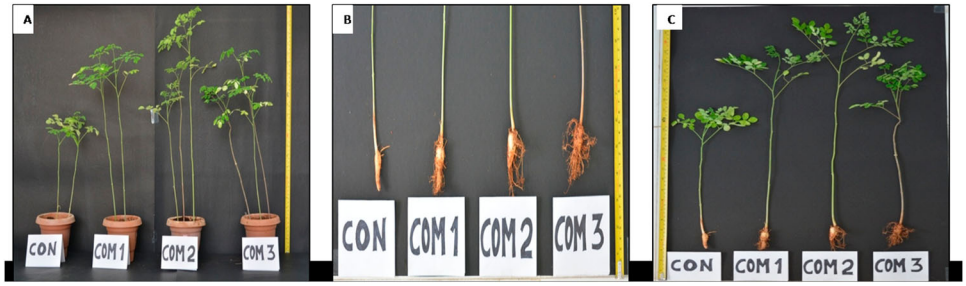

Figure 5. Change in growth characters (A) shoot length, (B) root hairs no. (C) root length of *Moringa* upon PGPR's combinations treatment.

4.2. Organic acid composition of root exudates

Organic acids such as oxalic acid, citric acid, ascorbic acid and malic acid are main components in root exudates (Ling et al. 2011). Similarly, in the present study, oxalic acid and citric acid were detected in all the PGPR's treatments, including control plant root exudates. Ascorbic acid and citric acid were detected in many of the root exudates from various plants. Citric acid was reported to stimulate colonization of PGPR's, such as *P. fluorescens* and *B. subtilis* (Andersen et al. 2007) and also reduce the pH of soil, leading to the formation of citrate. Lower pH leads to increased availability of Fe. Hence, the release of citric acid in root exudates helps plants in Fe accumulation in roots and foliage (Dinkelaker et al. 1989). It is also known to induce biofilm formation within a very short period after incubation (Rudrappa et al. 2008). Reduction of oxalic acid and increase in citric acid shows effective colonization of applied PGPR combination. Malic acid was not detected in any of the samples in our study because its concentration is very low in rhizosphere and its action to biofilm formation and as biocontrol agent may not be significant, but its concentration around roots may be high, which is difficult to estimate (Rudrappa et al. 2008).

4.3. Gene expression profile

Plants treated with *B. subtilis* GBO3 were also found to mimic Fe deficiency condition, leading to overexpression of genes involved in Fe transport like *OPT* and *NRAMP* to facilitate Fe uptake (Vert et al. 2002; Stacey et al. 2008). In our study, significant upregulation of *OPT* and *NRAMP* was seen demonstrating plants response to cope up Fe deficiency symptoms through enhanced Fe accumulation under influence of respective PGPR's. Expression of OPT also proves long-distance transport of Fe from soil and accumulation in foliage (Zhai et al. 2014). *NRAMP* was found to upregulate in the present study, which can be considered as a way for the plant to mimic Fe deficiency response. Thomine et al. (2003) showed *NRAMP*-enhanced expression under Fe-deficient condition. *AtNRAMP* was also found to be upregulated in *Arabidopsis thaliana* in the presence of biotic stress (Zimmermann et al. 2004).

4.4. Plant growth measurements

Increase of Fe content was found to associate with poor growth and low yield in most of the approaches. Use of PGPR was found to enhance Fe accumulation and growth of plant simultaneously. In fact, many endophytes (including arbuscular mycorrhizal fungi) and PGPRs are known for their growth promoting nature in plants, including enrichment of desired metabolite production (Matam and Giridhar 2017). But with reference to enrichment of Fe content in treated plant foliage, this study is the first of its kind in general and on *Moringa* in particular. All the PGPR combination that were used in the present study have shown positive effects on morphological characters such as germination; shoot length, primary root length, lateral root numbers, and shoot biomass, which can mainly be correlated with characters of organisms such as auxin production, phosphate

solubilization, phytohormone production and rhizosphere engineering (Kloepper et al. 2004; Kumar et al. 2011).

5. Conclusion

A combination of PGPRs comprising *B. pumilus* SE34 + *B. pumilus* T4 + *P. fluorescens* UOM14 (COM3) was found to exhibit a significant influence on enhancement of Fe accumulation in *Moringa* foliage. It was also found to be a good Fe extractor from soil, which can be correlated to the citric acid content in the root exudates. They also increased the expression of Fe transporters significantly. PGPR mix also modified root morphology so as to enhance the uptake of Fe. This study concludes that PGPR's performs synergistically and significantly in combinations. It can effectively be used as an alternate for breeding, transgenic, chemical fertilizer, etc. PGPR mix can successfully enhance the Fe content in edible parts of various crops. Enhancing Fe in edible parts of plants can help to restrain the problem of hidden hunger to a greater level. But more research needs to be focused on different formulations using different PGPR and their effect in open field.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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