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The Effect of PGPR Inoculation on the Growth of Wheat

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ABSTRACT

Many agricultural soils of Iran have high pH, resulting in low availability of Fe and Zn. The potentials of non-symbiotic plant growth-promoting rhizbacteria (PGPR) for stimulating plant growth have been extensively used during recent decades. This experiment was carried out in growth chamber to evaluate the effects of siderophore-producing Pseudomonads on the growth as well as Fe and Zn uptake of wheat. A randomized complete block design experiments was conducted using with Alborz genotype (an efficient phytosiderophore-producing bread wheat) treated with either 7NSK2 strain as a siderophore positive (*sid*$^+$) or with MPFM1 mutant strain of the same isolate as a siderophore negative (*sid*$^-$) treatments with three replications. The potentials of these strains for auxin production and phosphate solubilizing activity were evaluated by standard methods. The results showed that inoculation with *sid*$^+$ strain increased dry matter production in shoots as compared with the control (sterile condition) or with s*id* $^-$ strain. Likewise, the concentration of chlorophyll *a* in leaves of *sid*$^+$ and *sid* $^-$ treatments were 1.27 and 0.41µg mg^{-1} of fresh weight, respectively, and the concentration of chlorophyll *b* were measured to be 1.09 and 0.35 µg mg^{-1} of fresh weight, respectively, indicating significantly more chlorophyll formation due to inoculation with *sid*$^+$ as compared with s*id* $^-$. The uptake of Fe by roots and its rate of translocation to the shoots were greater for the *sid*$^+$ treated plants as compared with the *sid* $^-$ treated ones, indicating that siderophores increased the rate of Fe uptake by wheat. The effect of microbial inoculation on shoot Zn was not significant, but increased the concentration of Zn on roots compared with control. The results suggested that the siderophores of Pseudomonads may involve on increasing bioavailability of iron.

Keywords: Plant Growth Promoting Rhizobacteria (PGPR); Siderophore; Fluorescent Pseudomonads; Iron; Zinc

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are of agronomic importance. Indeed, they produce metabolites such as plant growth regulators that directly promote growth and facilitate nutrient uptake by plants. There is widespread distribution of PGPR that flourish in different geographical habitats (Hafeez et al., 2005). These rhizobacteria significantly affect plant growth not only by increasing nutrient cycling, also by suppressing pathogens by producing antibiotics and siderophores or by bacterial and fungal antagonistic substances and / or by other plant hormones. A divers array of bacteria including *Pseudomonas, Azospirillum, Azotobacter, Bacillus, Klebsilla, Entrobacter* and *Serratia* have been shown to promote plant growth (Khalid et al., 2004). Among these bacteria, fluorescent pseudomonads have high efficiency in host root colonization and plant growth metabolites production as well (Rasouli Sadaghiani, 2005). These bacteria are an important component of the rhizosphere of many plants, and are known to colonize the rhizosphere of wheat,

potato, maize, grasses, pea and cucumber (Cakmakci et al., 2006; Khalid et al., 2004; Howie and Echandi, 1983; Brown and Rovira, 1976). *Pseudomanas* inoculants significantly increased root dry weight in spring wheat (Walley and Germida, 1997) and colonized winter wheat roots (De Freitas and Germida, 1992). Rhizosphere fluorescent pseudomonads are known to be antagonists to plant pathogens via siderophore production (Kloepper et al., 1980). Khalid et al. (2004) screened PGPR for improving growth and yield of wheat and concluded that the strains which produced the highest amount of auxins caused maximum increase in growth as well as yield of wheat.

PGPR improve plants efficiency in iron acquisition. In calcareous soils the availability of Fe is very low due to the high pH of the soil solution and its buffering capacity that may impede Fe uptake mechanisms of many plants. Plants grown in such soils may suffer from sever Fe deficiency (Marschner et al., 1986). In order to avoid Fe deficiency, various graminaceous plants seem to rely on excretion of phytosiderophores by the roots and their uptake as a Fe complex by a highly specific uptake system that is enhanced by Fe deficiency (Marschner et al., 1986).

Microbial siderophores form Fe complexes with high stability constants and therefore play a role in the Fe uptake by microorganisms (Neilands, 1995). Siderophores were found in soil solutions at concentrations that may influence the Fe nutrition of plants. Soil microbial activity is essential for Fe acquisition by soil-grown rape. Similarly, sorghum which is able to release phytosiderophores from the roots requires soil microbial activity to ensure satisfactory Fe supply (Rroco et al., 2003). Furthermore, some strains of *Pseudomonas* and *Bacillus* could be able to solubilize sparingly Zn compounds. These bacteria have been introduced as ZSB (Zinc Solubilizing Bacteria) (Saravanan et al., 2003). Disimine et al. (1998) isolated a strain of *Pseudomonas fluorescens* from forest soil which showed high efficacy in solubilizing insoluble Zn compounds.

Pesudomonas aeruginosa strain 7NSK2, isolated from barley roots (Iswandi et al., 1987), has been genetically marked with a lac element (MPB1) for ecological studies (Hoft et al., 1991). MPFM1 is a pyoverdin-negative mutant of 7NSK2 obtained by Tn5 mutagenesis. In the case of PGPR strain 7NSK2, the fluorescent siderophore pyoverdin plays an important role in plant growth stimulation (Hoft et al., 1991).

The aim of this study was to evaluate the efficiency of siderophore producing PGPR and its siderophore negative mutant. We, therefore, investigated the effectiveness of previously identified strains (7NSK2 and MPFM1) on growth, Fe and Zn uptake in bread wheat.

METHODS and MATERIAL

Microbial Inoculation and Properties

Bacterial strains used were *P. aeruginosa* 7NSK2 as siderophore positive (sid^+) and its siderophore negative mutant *P. aeruginosa* MPFM1 (sid^-) and were prepared by Monika Hoft, Gent University, Belgium. Bacterial inoculations were produced from 48 h culture grown in King's B broth

medium on a rotary shaker at 28°C. Seeds were bacterized by the method of seed inoculation on bacterial culture (ca. 10^8 cfu ml^{-1}) for 15 min. Un-inoculated jars considered as control treatments.

Plant growth-promoting properties of the strains were confirmed with their ability to produce siderophore, indole acetic acid and phosphate solubilization. The potentials of these strains for siderophore production were evaluated by chrome azorel-S assay (CAS blue agar) through color change (Schwyn and Neilands, 1987). Auxin production by the strains was determined by colorimetry. For this purpose sterilized broth of glucose peptone agar medium was put in glass tubes and inoculated at 28 °C for 24 h with occasional shaking. The contents of the tubes were filtered before measuring auxin production as Indole acetic acid (IAA) equivalents. In measuring the IAA equivalents, 3 ml of the filtrate were pipetted into test tubes and 2 ml Salkowski reagent (2 ml 0.5 M $FeCl_3$ + 98 ml 35% $HClO_4$) were added to it. The tubes containing the mixture were left for 30 min for color development. Intensity of the color was measured spectrophotometrically at 535 nm. Similarly, color was also developed in standard solutions of IAA and standard curve was established by measuring the intensity of this color (Asghar et al., 2002). Phosphate solubilization ability was evaluated according to the method of Sperber (1958).

Pltan Culture

Seeds of wheat (*Triticum aestivum* L. cv. Alborz) were surface sterilized for 1 min in 70 % ethanol and then treated in 5 % Na hypochloride solution for 40 min, followed by rinsing the seeds six times with autoclaved distilled water. Wheat genotype was Fe-efficient in terms of phytosiderophore producing (Rasouli Sadaghiani et al., 2007). Bacterized seeds then planted and were grown on sterilized fine sand-derived from Caspian Sea beaches in sand culture method. Pots (Leonard jars) consisted two part, upper part filled with sand and a cotton-based strip in center and lower part included nutrition solution sink with corresponding strip. Nutrient solution was sterilized and containing: 2 mM $Ca(NO_3)_2$, 0.25 mM KH_2PO_4, 0.1 mM KCl, 0.88 mM K_2SO_4, 1 mM $MgSO_4$ 7H2O, 1 µM H_3BO_3, 0.2 µM $CuSO_4$ $5H_2O$ and 0.2 µM $(NH_4)_6MoO_{24}$. Fe and Zn were supplied in sparingly soluble forms as Fe_2O_3 (5 mg kg^{-1} soil) and ZnO (1 mg kg^{-1} soil), respectively. Solution pH was adjusted at 7.1 by adding HCl or NaOH. Plants were cultivated in growth chamber and at aseptic condition. The growing period lasted 32 days and growing condition included 26 °C day (16 h) and 20 °C night temperature (8 h).

At the end of experiment plants were harvested, shoot and root dry weights were determined and their Fe concentration was recorded. Also the concentration of chlorophyll a and b were measured in leaf samples according to the method of Sharma et al. (2003).

RESULTS and DISCUSSION

To evaluate the effects of strains 7NSK2 and MPFM1 inoculation on wheat growth in presence of sparingly soluble compounds of Fe and Zn, an experiment was designed using F_2O_3 and

ZnO. *Pseudomonas* strain 7NSK2 despite from MPFM1 produced high amount of siderophore which determined through CAS blue agar assay. Neither 7NSK2 nor MPFM1 did solubilize insoluble phosphate compounds. Both strains produced auxines (Table 1). The results showed that inoculation with *sid*$^{+}$ strain increased dry matter production in shoots as compared with the control (sterile condition) or with s*id* $^{-}$ strain. Shoot dry weight in 7NSK2 inoculated plants (2.13 g pot^{-1}) were approximately 18% higher than the MPFM1 and were statistically in the same group as the control (1.97 g pot^{-1}). The 7NSK2 treatment (0.47 g pot^{-1}) gave a 46% and 30% increase in root biomass compared to MPFM1 and control treatments, respectively (Fig. 1).

Table 1. Production of plant growth regulators by *Pseudomonas* strains

Isolates	IAA equivalents mg l^{-1}	Phosphate solubilizing ability	Siderophore production
7NSK2	2.27	-	+
MPFM1	2.18	-	-

The control (sterile) plants were comparable to 7NSK2 (sid^{+}) inoculated plants and it was as effective as 7NSK2 in terms of shoot dry weight (Fig. 1). Uninoculated control plants showed higher whole plant dry weight compared to MPFM1 treatments (data not shown). Tabasi genotype has shown to produce large amount of phytosiderophores on Fe deficient condition and classified as efficient wheat genotype (Rasouli Sadaghiani et al. 2007). It seems this genotype could better uptake and utilize nutrients in such condition.

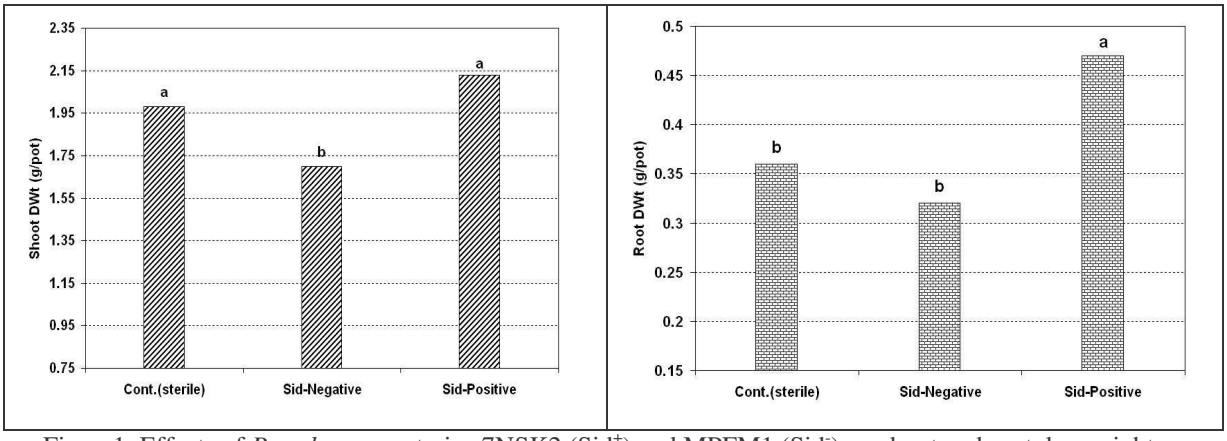

Figure1. Effects of *Pseudomonas* strains 7NSK2 (Sid^{+}) and MPFM1 (Sid^{-}) on shoot and root dry weights

Plant inoculated with MPFM1 showed lower degree of iron chlorosis, which was visualized as interveinal yellowing of wheat leaves. In 7NSK2 treatment along with control plant, a significant (at $P \leq 0.05$) increase in chlorophyll a chlorophyll b and total chlorophyll content was observed as compared to MPFM1 inoculated plants (Table 2). Treatment with 7NSK2 was effective in reducing chlorosis as evident in increased chlorophyll components. Seong et al. (1992) reported that

bacterization of soil with the siderophore producing strain 7NSK2 resulted in a significant dry weight increase in maize compared to MPFM1 strain. In this study, the siderophore-deficient mutant MPFM1 did not affect shoot dry weight as well as chlorophyll content, although its root Zn uptake amount was not impaired (Fig. 1; Table 2). Chlorophyll a, chlorophyll b and total chlorophyll content was correlated with higher iron acquisition (Katyal and Sharma, 1980). The concentration of chlorophyll *a* in leaves of *sid*$^+$ and *sid*$^-$ treatments were 1.27 and 0.41µg mg^{-1} of fresh weight, respectively, and the concentration of chlorophyll *b* were measured to be 1.09 and 0.35 µg mg^{-1} of fresh weight, respectively, indicating significantly more chlorophyll formation due to inoculation with *sid*$^+$ as compared with s*id*$^-$. These results may also indicate that siderophores of strain 7NSK2 involved in Fe uptake.

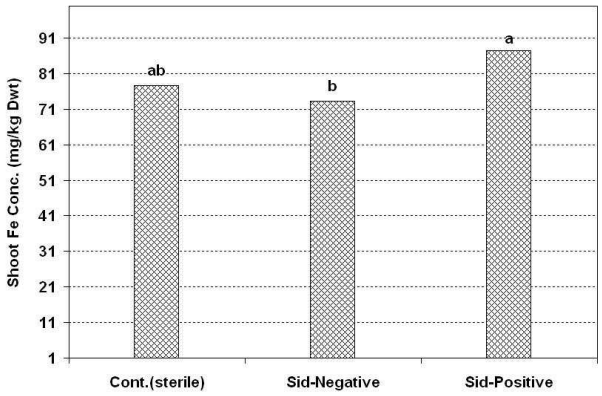

Figure 2. Effects of *Pseudomonas* strains 7NSK2 (Sid$^+$) and MPFM1 (Sid$^+$) on shoot Fe concentration

Table 2. Effects of *Pseudomonas* strains 7NSK2 and MPFM1 on Fe, Zn and chlorophyll concentration

	Fe conc. (mg kg^{-1})		Zn conc. (mg kg^{-1})		Chlorophyll cont. (mg g^{-1} Fresh wt)		
Inoculation conditions	Shoot	Root	Shoot	Root	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Control (no inoculation)	77.75 ab	3606 b	48.50 a	151 b	1.40 a	0.83 a	2.23 a
7NSK2 (Sid$^+$)	87.50 a	5586 a	51.87 a	185 a	1.27 a	1.08 a	2.35 a
MPFM1 (Sid$^-$)	73.25 b	3871 b	47.75 a	180 ab	0.41 b	0.35 b	0.76 b

Data presented in Table 2 shows that in 7NSK2 inoculated plants Fe uptake enhanced (approx. 20%) compared to negative siderophore strain (MPFM1). However, plant with no inoculation (sterile) was also showed relatively high amount of Fe in shoots. It is surprising that although control plant did received pgpr inoculants, its effect on shoot dry weight and chlorophyll content was prominent. This increased Fe uptake resulted from high efficiency of wheat genotype in phytosiderophore release in Fe

deficient condition. Rasouli Sadaghiani et al. (2007) showed that Tabasi genotype as bread wheat released high amount of root exudates mainly phytosiderophores in Fe and Zn deficiency conditions. Therefore, the uptake of Fe by roots and its rate of translocation to the shoots were greater for the *sid*$^{+}$ treated plants as compared with the *sid* $^{-}$ treated ones, indicating that siderophores increased the rate of Fe uptake by wheat.

Microbial siderophores are used as Fe chelating agents that can regulate the availability of iron in the plant rhizosphere. It has been assumed that competition for iron in the rhizosphere is controlled by the affinity of the siderophore for iron (Loper and Henkels, 1999). Interestingly, the binding affinity of phytosiderophores for iron is less than the affinity of microbial siderophores, but plants require a lower iron concentration for normal growth than do microbes (Meyer, 2000). In this study, the effect of microbial inoculation on shoot Zn was not significant, but increased the concentration of Zn on roots compared with control. Disimine et al. (1998) reported that a strain of *Pseudomonas fluorescens* from forest soil showed high efficacy in solubilizing insoluble Zn compounds.

The data presented in this study explores microbe-plant interaction in terms of iron uptake from particularly the insoluble oxide form of iron and supports the mechanisms of heterologous iron uptake in wheat system via microbial siderophores. Chlorophyll content may be used as marker of iron availability to the plant system. For calcareous soils which prevalent in Iran, strains like 7NSK2 will be of great interest to combat iron chlorosis and additionally improve strategic crop yield.

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