Growth response of wheat cultivars to bacterial inoculation in calcareous soil

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ABSTRACT

In this study the plant growth-promoting bacteria were analysed for their growth-stimulating effects on two wheat cultivars. The investigations were carried out in pot experiments using calcareous soil. The results showed that bacterial strains *Pseudomonas* spp. NUU1 and *P. fluorescens* NUU2 were able to colonize the rhizosphere of both wheat cultivars. Their plant growth-stimulating abilities were affected by wheat cultivars. The bacterial strains *Pseudomonas* sp. NUU1 and *P. fluorescens* NUU2 significantly stimulated the shoot and root length and dry weight of wheat cv. *Turon*, whereas cv. *Residence* was less affected by bacterial inoculation. The results of our study suggest that inoculation of wheat with *Pseudomonas* strains can improve plant growth in calcareous soil and it depends upon wheat cultivars. Prior to a selection of good bacterial inoculants, it is recommended to select cultivars that benefit from association with these bacteria.

Keywords: wheat cultivars; rhizosphere bacteria; root colonisation

There are many reports on plant growth promotion and yield enhancement by plant growth-promoting rhizobacteria (PGPR) (Lugtenberg et al. 2001). The mechanisms of plant growth promotion by PGPR include: the ability to produce phytohormones, N_2 fixation, antagonism against phytopathogens and solubilisation of insoluble phosphates (Lugtenberg and Kamilova 2009). It is also suggested that PGPR can also prevent the deleterious effects of stresses from the environment (Paul and Nair 2008).

The ecological factors such as soil types, temperature and plant varieties were found to affect rhizosphere microbial communities and PGPR performance (Sørensen et al. 2001). Our previous studies showed that inoculation of wheat with bacterial strains *Pseudomonas fluorescens* PsIA12 and *Pantoea agglomerans* 050309 was found to significantly increase the root and shoot growth in loamy sand at 16°C compared to 26°C (Egamberdieva and Hoflich 2003). Simon et al. (2001) reported that crop varieties affect rhizosphere bacteria community, which can have a profound impact on the plant growth.

Understanding the plant growth-promoting properties of bacterial strains affected by plant cultivar is widely recognized as a key to improving the level and reliability of plant growth stimulation by PGPR. Thus, the objectives of our study were to determine the effect of plant growth-promoting bacteria on the growth of two different wheat varieties in calcareous soil.

MATERIAL AND METHODS

Soil, plant and microorganisms. Soils were sampled from irrigated agricultural site of the Syrdarya province (41°00'N, 64°00'E), north-east of Uzbekistan. The soil is calcareous calcisol with low nutrient content. The main chemical soil properties are: organic matter C_t 2.52%; N_t 0.10%; K^+ 5.7 g/kg; P 1.3 g/kg; pH 7.8.

The bacterial strains *Pseudomonas* spp. NUU1 and *Pseudomonas fluorescens* NUU2 were taken from the culture collection of the National University of Uzbekistan. Wheat (*Triticum aestivum*) cultivars Turon (Uzbekistan) and Residence

Supported by the UNESCO – L'OREAL Fellowship for 'Women in Science'.

(Cebeco Seeds, the Netherlands) were used for pot experiments.

Auxin production. The production of IAA (indole-acetic acid) was determined according to the method of Bano and Musarrat (2003). Tested bacterial strains were grown in the LC medium, a modification of Luria broth base Miller (Difco), with and without tryptophan (100 µg/ml) and incubated at 28°C. After three days of cultivation, aliquots of bacterial cultures were centrifuged at 13 000 × g for 10 min. One ml of supernatant was transferred to a fresh tube to which 100 µl of 10 mmol orthophosphoric acid and 2 ml of reagent (1 ml of 0.5M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added. After 25 min, the absorbance of the developed pink color was read at 530 nm. The IAA concentration in culture was calculated by using a calibration curve of pure IAA as standard.

Colonization of wheat root tip. The colonisation abilities of bacterial strains in the root of wheat were studied under gnotobiotic conditions. The experiments were carried out in test tubes (25 mm in diameter, 200 mm length) described by Simons et al. (1996), containing a 60 g sterilized mixture of washed sand and vermiculite (1:1) soaked with 10% Plant Nutrient Solution (PNS).

The strains were grown overnight in King B (KB) medium; then 1.0 ml of an overnight culture was sedimented by centrifugation (13 000 × g) and the supernatant was discarded. The cells were washed with 1 ml phosphate buffered saline (PBS) and re-suspended in PBS. Cell suspensions were adjusted to OD_{620} = 0.1 that corresponds to cell density of about 10^8 cells/ml. Sterile wheat seeds were obtained by rinsing wheat seeds with household bleach (adjusted to approximately 5% sodium hypochlorite) and stirring in a sterile flask for 3 min. After 3 min, the hypochlorite was removed by washing the seeds five times extensively with 20 ml sterile water, followed with 2 h washing in sterile water during which the water was replaced at least three times. Sterilized seeds were germinated in sterile Petri dishes. Germinated seeds were placed in the bacterial suspension with sterile forceps and shaken gently and after approximately 10 min the inoculated seeds were aseptically planted in the sand column of the gnotobiotic system of glass tubes, 5 mm below the sand surface. The seedlings were grown in a plant growth chamber with 16 h light period at 22°C and 8 h dark period at 16°C and 60% relative humidity for 7 days or until the root tips penetrated the gauze. Then, 1 cm of root tip from plantlets was collected. Bacterial cells were removed from the root tip by vortexing in PBS and plated on KB agar medium. Logarithm of colony-forming units (CFU) was used for statistical analysis.

Plant growth promotion. Seedlings inoculated with bacterial strains were sown in the sterilised gnotobiotic sand system of glass tubes and were grown in a plant growth chamber with 16 h light period at 22°C and 8 h dark period at 16°C and 60% relative humidity. After 7 days the shoot and root length and dry matter of wheat were measured.

Another experiment was conducted in plastic pots (9 cm diameter; 12 cm deep) containing 350 g of soil. The inoculation treatments were set-up in a randomised design with 10 replications. Bacteria were grown in KB medium for 24 h. Wheat seeds were coated with bacteria by dipping the seeds in bacterial suspensions. The pot experiment contained control (no inoculation) and experimental variants (inoculation with bacteria. Plants were grown with a temperature of 18°C to 20°C during the day and 12°C to 14°C at night under open field conditions and after four weeks of growth the shoot and root length and dry matter of wheat were measured.

Statistical analysis. Data were tested for statistical significance using the analysis of variance package included in Microsoft Excel 98 and com-

Table 1. The effect of bacterial strains *Pseudomonas* spp. NUU1 and *P. fluorescens* NUU2 on shoot, root length and dry weight of wheat cultivars (Turon and Residence)

Bacterial strains	Wheat cv. Turon			Wheat cv. Residence		
	shoot[a]	root[a]	dry weight[b]	shoot[a]	root[a]	dry weight[b]
Control	86 ± 0.8	124 ± 1.2	0.019 ± 0.002	95 ± 0.9	136 ± 0.7	0.022 ± 0.002
Pseudomonas spp. NUU1	11.4* ± 1.1	15.4* ± 2.3	0.025* ± 0.002	11.3* ± 0.9	15.2 ± 2.1	0.023 ± 0.001
P. fluorescens NUU2	10.9* ± 0.7	16.1* ± 0.65	0.023* ± 0.002	10.5 ± 1.5	15.3 ± 1.4	0.026* ± 0.001

[a]shoot and root length (mm); [b]dry weight (g/plant). Plants were grown for 7 days in gnotobiotic system of glass tubes. Values represent means for ten plants (n = 10), *significantly different at $P < 0.05$

Table 2. The effect of bacterial strains *Pseudomonas* spp. NUU1 and *P. fluorescens* NUU2 on shoot, root length and dry weight of wheat cultivars (Turon and Residence)

Bacterial strains	Wheat cv. Turon			Wheat cv. Residence		
	shoot[a]	root[a]	dry weight[b]	shoot[a]	root[a]	dry weight[b]
Control	157 ± 0.5	108 ± 1.1	0.49 ± 0.04	171 ± 1.3	125 ± 1.1	0.57 ± 0.066
NUU1	167 ± 0.9	142* ± 1.6	0.52* ± 0.04	170 ± 1.6	123 ± 1.3	0.54 ± 0.029
NUU2	193* ± 1.4	158* ± 1.2	0.62* ± 0.05	182 ± 1.8	152* ± 1.4	0.59 ± 0.039

[a]shoot and root length (mm); [b]dry weight (g/plant). Plants were grown for 30 days in open field condition, values represent means for ten plants (n = 10), *significantly different at $P < 0.05$

parison was done using a Student's t-test. Mean comparisons were conducted using the least significant difference (LSD) test ($P = 0.05$). Standard error and LSD results were calculated.

RESULTS AND DISCUSSION

Prior to testing of bacterial strains for their plant growth-promoting abilities in two wheat cultivars, we investigated their colonisation ability in the rhizosphere of wheat grown in gnotobiotic conditions. The survival of inoculated plant-growth promoting bacteria in the plant rhizosphere is in most cases a precondition for a potential plant stimulation effect during the vegetation time (Höflich et al. 1995). The results showed that *Pseudomonas* spp. NUU1 and *P. fluorescens* NUU2 were able to colonize the rhizosphere of both wheat cultivars. The strains showed (Log CFU/cm of root tip) colonization values in the rhizosphere of wheat cultivar Turon of 4.56 (NUU1), 4.51 (NUU2) and cultivar Residence 4.57 (NUU1), 4.74 (NUU2).

The bacterial inoculation also led to the growth stimulation of wheat in gnotobiotic experiments (Table 1). However their plant growth stimulating abilities were affected by wheat cultivars. The shoot, root length and dry weight of wheat cultivar Turon were significantly stimulated by both bacterial strains, whereas *Pseudomonas* spp. NUU1 significantly increased only shoot growth and *P. fluorescens* NUU2 only dry weight of wheat cultivar Residence (Table 1).

The growth of both wheat cultivars was also influenced by bacterial inoculation in pot experiments (Table 2). The *Pseudomonas* spp. NUU1 and *P. fluorescens* NUU2 stimulated shoot growth of wheat cultivar Turon up to 22%, root growth up to 46% and dry weight up to 25%. There was no significant growth stimulation for wheat cultivar Residence by bacterial inoculants with the exemption of root length (21%) which was stimulated only by *P. fluorescens* NUU2 (Table 2).

Early studies showed that the extent of positive effects of the bacteria on plant growth varied with the species or variety of the host plant (Chanway et

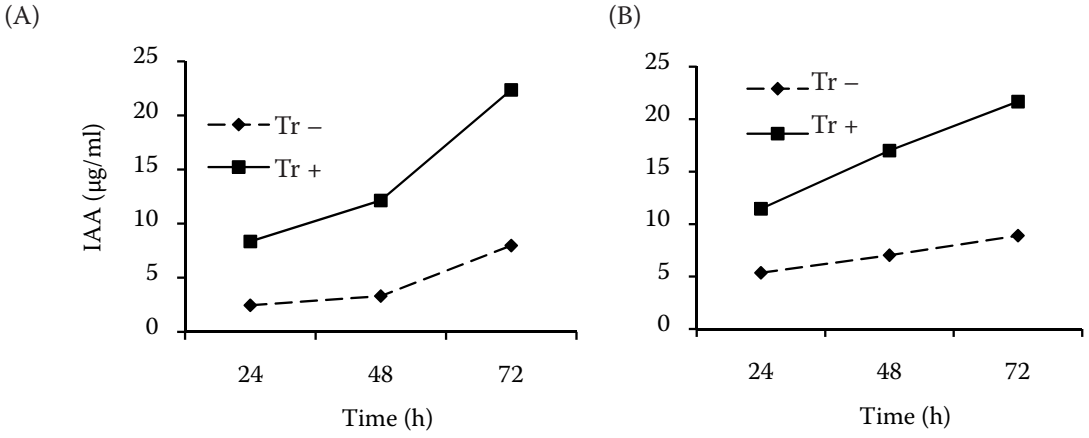

Figure 1. The production of IAA (indole-3-acetic-acid) by *Pseudomonas* spp. NUU1 (A) and *P. fluorescens* NUU2 (B) (bacteria were grown 3 days at 28°C with the absence and presence of 100 ug/ml of the auxin precursor tryptophan)

al. 1988). Alam et al. (2003) also found the cultivar variation in the stimulation of rice growth inoculated with plant growth-promoting bacteria. One of the mechanisms of plant growth stimulation in such environment could be bacterial production of the phytohormone (IAA) (Frankenberger and Arshad 1995). The IAA production by bacterial strains was tested in the absence and presence of 100 µg/ml of the auxin precursor tryptophan. The results obtained from 3-day old cultures showed that bacterial strains appeared to produce IAA and the presence of tryptophan stimulated auxin production (Figure 1).

The results of our study suggest that inoculation of wheat with *Pseudomonas* strains can improve plant growth in calcareous soil and this is dependent upon wheat cultivars. Prior to the selection of good bacterial inoculants, it is recommended to select cultivars that benefit from association with these bacteria.

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Received on March 30, 2010

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