Ann. Bangladesh Agric. (2017) 21 (1 & 2) : 61-71 *ISSN 1025-482X (Print)*
2521-5477 (Online)

EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON SEED GERMINATION AND GROWTH OF WHEAT PLANTS

M. F. Rahman, A. M. Akanda, F. Z. Eivy and M. M. Hossain*

Abstract

Plant growth promoting rhizobacteria (PGPR) are recognized as important agents for stimulating growth of various plants. The present study was undertaken to explore the potential of two PGPR strains, namely *Pseudomonas stutzeri* PPB1 and *Bacillus subtilis* PPB2 to promote seed germination, plant growth, and leaf nitrogen content in four wheat varieties, namely Kanchan, Shatabdi, BARI Gom-27, and BARI Gom-29. Both the strains significantly increased (18.20 to 21.28%) seed germination in var. Shatabdi compared to un-inoculated control, while in var. BARI Gom-27 and BARI Gom-29, the significant increase in seed germination was observed in treatment with *B. subtilis*. Treatment with both the strains resulted in significant increase in shoot length (16.54% to 44.11%), shoot fresh weight (21.17% to 85.48%), shoot dry weight (29.54% to 110.22%), root length (16.71 to 58.81%), root fresh weight (17.79% to 93.06%-) and root dry weight (21.42% to 77.78%) of wheat plants compared to un-inoculated control. Both the PGPR significantly enhanced leaf nitrogen content (27.17% to 73.78%) in wheat plants of four varieties. The two tested rhizobacteria colonized the wheat roots successfully accounting a population with a range of 73.50×10^7 to 233.60×10^7 cfu/g root tissue. Two PGPR strains varied in effectiveness in stimulating plant growth traits. Higher plant growth promotion was obtained in plants pretreated with *B. subtilis* than with *P. stutzeri*. Application of selected PGPR inoculants, thus, could be an obvious approach to reduce the use of chemical fertilizers in wheat field.

Keywords: *Pseudomonas, Bacillus,* shoot, root, nitrogen content, colonization.

Introduction

Wheat is the second most important cereal after rice in Bangladesh and plays an important role to ensure national food security. Its consumption is increasing day by day. Currently, Bangladesh produces 12-13 lakh metric tons of wheat against the demand of around 40 lakh tons annually (BBS, 2016). This implies that two-thirds of the wheat demand are imported every year. Therefore, it is important to boost up wheat production for ensuring food security as well as saving the hard earned foreign currencies.

Appropriate supply of fertilizers is prerequisite for boost up agricultural production and growth. The benefits of synthetic fertilizer use in the crop field have been immense as they reduce crop losses due to nutrient deficiencies. However, their continuous use in the field has yielded a range of unintended negative consequences on environment and human health. Overcoming these widespread hazards is a major challenge in contemporary agriculture, and the problem must be seriously addressed before their impacts on environment become irreversible. Therefore,

Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706.
*Corresponding Author: hossainmm@bsmrau.edu.bd

emphasis must be given to green technologies which will preserve and prosper natural ecosystem. Natural ecosystems depend directly on beneficial microorganisms that are present in the rhizosphere soil and helps crops to reach higher productivities. The beneficial microorganisms of the rhizosphere are important determinants of plant health and soil fertility because they participate in several key processes in the soil, such as biological control of plant pathogens, nutrient cycling and seedling establishment (Jeffries *et al.,* 2003). Rhizospheric bacteria that exert beneficial effects on plant growth and development are referred to as plant growth promoting rhizobacteria (PGPR). In the last few decades, the use of PGPR for improving plant growth has been extended. PGPR can influence plant growth by production and release of secondary metabolites (plant growth regulators/phytohormones/biologically active substances), lessening or preventing deleterious effects of phytopathogenic organisms in the rhizosphere and/or facilitating the availability and uptake of certain nutrients from the root environment. Selection of effective PGPR is most critical aspect to have maximum benefits from this technology. The present study was undertaken to find the effect of seed priming with PGPR strains on seed germination and seedling growth of wheat.

Materials and Methods

Plant materials

Four wheat varieties namely Shatabdi, Kanchan, BARI Gom-27 and BARI Gom-29 were used in the experiment. Seeds of these varieties were collected from Plant Genetic Resource Center of Bangladesh Agricultural Research Institute, Gazipur, Bangladesh and stored at 4^0 C until use.

Selection of plant growth promoting rhizobacteria strains (PGPR)

Four PGPR strains *Bacillus subtilis* PPB2, *Stenoprophomonas maltophilia* PPB3 and *Bacillus amyloliquefaciens* PPB10 were collected from a stock culture of the Department of Plant Pathology, BSMRAU, Gazipur.

Preparation of PGPR inocula

Rhizobacterial strains were multiplied in 250 mL conical flasks containing 200 mL yeast peptone dextrose (YPD) broth (Islam *et al*., 2015) on an orbital shaker at 120 rpm for 72 hr at 27^O C. The bacterial cells were separated from the broth by centrifugation at 15000 rpm for 1 min at 4^O C. The bacterial cells were washed twice with sterilized distilled water. The bacterial pellets were suspended in 0.6 mL sterilized distilled water and vortexed for 45 seconds. The final suspensions of the rhizobaterial strains were used as inoculum .

Seed priming with PGPR inocula

For priming, 15 g of apparently healthy wheat seeds of each variety were surface sterilized with 1.0% chlorox for 2 min and rinsed in sterilized distilled water for three times. Surface sterilized seeds were soaked in the PGPR suspension for 10 min and air dried overnight at room temperature to ensure better coating of the seeds with the rhizobacteria. Another set of grains were soaked in only sterilized distilled water, which were used for control treatment. After priming with PGPR strains, the number of bacterial cells per seed was counted following serial dilution

plate technique. Weight of primed seeds was taken and homogenized in sterilized distilled water with sterilized mortar and pestle. Serial dilution of the bacterial suspension was prepared with sterilized distilled water up to 10^{-6}. An aliquot of 100 µL suspension of each dilution was spread evenly on Petri dishes containing King's B agar medium. After 24 hr of incubation at 28 ^{O}C, the number of colony-forming units (cfu) per gram of seed was recorded.

Examination of effect of seed priming with PGPR on germination in wheat

In order to determine the effect of the isolates on germination and seedling vigor, 100 seeds inoculated with each isolate were incubated in ten 9-cm petri dishes on two layers of moistened filter paper. As a control treatment, seeds treated with water instead of bacterial suspensions were also established. In order to maintain sufficient moisture for germination, 5 mL distilled water was added to the petri dishes every other day, and seeds were incubated at 28 ± 2^{0} C in a light incubator. Germination was considered to have occurred when the radicles were half of the seed length. The germination percentage was recorded every 24 hours for 7 days. Root and shoot length were measured after the seventh day. The experiment was planned as a completely randomized design with 10 replications for each isolate.

$$\text{Germination rate (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Growing of seedlings and collection of data on germination and seedling growth

Field soil collected from BSMRAU experimental farm was used as potting medium. The soil contained 0.84% organic carbon, 1.84 % organic matter, 0.10% nitrogen, 0.12% phosphorus, and 0.91% potassium. The pH of the potting soil was 6.41. The soil contained 45.52% sand, 36.00% silt, 18.48% clay, and 21.96% moisture. The soil was autoclaved twice at 24 hours intervals for 20 min at 121^{O} C under the pressure of 1.1kg / cm^{2} before pouring it in pot. About 180 g of autoclaved soil was poured in each plastic pot (9.5 cm x 7.0 cm). Before pouring the soil, the pots were washed with 10.0% chlorox for 10 min and rinsed with tap water for three times. Primed seeds of wheat were planted in each plastic pots (9.5 cm×7.4 cm) filled with potting soil. Water treated seeds were planted in another sets of pots which served as control. Each pot received 10 seeds. After seven days of planting, data on seed germination were recorded. Germinated seedlings were thinned to have five seedlings per pot. The seedlings were allowed to grow for three weeks providing proper soil moisture. After three weeks, wheat seedlings were uprooted carefully from the pots to minimize root damage. The root systems were washed with tap water and soaked with blotting paper to remove excess water. Data on shoot weight, shoot length, root weight and root length were recorded.

Root colonization by PGPR strains

After priming with PGPR strains, the number of bacterial cells per seed was counted following serial dilution plate technique (Mian, 1995). After collecting data on root growth, root systems were thoroughly washed with running tap water to remove adhering soil particles. After washing, the root systems were surface sterilized with 1.0% chlorox for 1 min, rinsed in sterilized distilled water for

three times, soaked with blotting paper to remove excess water and air dried. Weight of each of the air dried root system was taken and homogenized in sterilized distilled water with sterilized mortar and pestle. Serial dilution of the bacterial suspension was prepared with sterilized distilled water up to 10^{-6}. Similarly, serial dilution of bacterial suspension was prepared from primed seeds. A 100 µl aliquot of each dilution was spread evenly on Petri dishes containing King's B agar medium (Mian, 1995) and incubated for 24 hr at 25^{O} C. Subsequently, appropriate dilutions were plated onto King's B medium. After 24 hours of incubation at 28^{O} C, the number of colony-forming units (cfu) per gram of infected root tissue and per gram of seed was recorded. Data were expressed in number of bacterial cells per gram of seed and per gram of root.

Determination of leaf nitrogen content

Total nitrogen contents in leaf of seedling was determined following modified Kjeldahl method (Kjeldahl, 1983) using sulfuric acid and salicylic acid digestion techniques followed by distillation and titration (Bramner and Keeney, 1965).

Experimental Design and Statistical Analysis

Experiments were conducted following completely randomized design with three replications. Collected data were analyzed using MSTAT-C and Statics10 program. The experiments were repeated at least twice and data with similar results were presented. Means were separated following least significant difference (LSD) test at 5% level of probability.

Results and Discussion

Effect of seed priming with PGPR on germination of wheat varieties

Priming of seeds with two PGPR increased germination of wheat seeds over control in four wheat varieties, although significant differences from control were not observed in all the varieties. The highest seed germination (88.93%) was observed in the variety Shatabdi treated with *B. subtilis* and the lowest seed germination (66.39%) was found in control treatment in the variety Kanchan (Table 1). The maximum (21.95%) increase in germination over control was obtained in wheat variety BARI Gom-29 treated with *B. subtilis*, which was followed by the treatment of the variety Shatabdi (21.27%) and BARI Gom-27 (20.68%) with the same bacteria. Treatment with *B. subtilis* in var. Kanchan did not increase germination significantly. Similar result was also observed in seeds of the variety Kanchan, BARI-Gom-27 and BARI Gom-29 treated with *P. stutzeri,* while significant increase in seed germination was found in the variety Shatabdi. The percent increase in germination of seeds in Kanchan, Shatabdi, BA.RI Gom-27 and BARI Gom-29 treated wills *P. stutzeri* were 8.73%, 18.20%, 7.63%, and 12.82%, respectively, over control. This indicates that seed treatment with *B. subtilis* resulted the highest increase in germination in all the wheat varieties except Kanchan.

Effect of seed priming with PGPR on shoot and root growth of wheat varieties

Shoot length

Both the rhizobacteria gave significant increase in shoot length in wheat variety Kanchan, compared to control, where

Table 1. Effect of inoculation of rhizobacteria on germination of wheat varieties

PGPR	Germination percentage of four wheat varieties			
	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29
Pseudomonas stutzeri	72.35 de B* (8.73)	86.67 b B (18.20)	73.33 d AB (7.63)	80.23 c AB (12.82)
Bacillus subtilis	73.30 d B (10.15)	88.93 a A (21.28)	82.22 c A (20.68)	86.72 b A (21.95)
Control	66.54 g B	73.33 d C	68.13 f B	71.11 e B

*Values having same letter (lower case in a row and upper case in a column) are not significantly different ($P \geq 0.05$).

maximum shoot length (28.57 cm) was recorded in plants treated with *B. subtilis* (Table 2). Seed treatment of this variety with *P. stutzeri* and *B. subtilis* increased the seedling shoot length by 38.25% and 44.11% over control, respectively. Seed treatment with *P. stutzeri* and *B. subtilis* significantly increased the shoot length of the seedlings of Shatabdi compared to that in control, which were 23.36% and 25.56% higher than control, respectively. Although treatment receiving both the PGPR strains significantly increased shoot length of BARI Gom-27 significantly, the highest increase (30.43%) was observed with *B. subtilis*. Both *P. stutzeri* and *B. subtilis* significantly stimulated higher shoot length compared to control in var. BARI Gom-29, which were 16.54% and 24.77% higher over control, respectively.

Fresh weight of shoot

Seed priming with two PGPR isolates resulted in significant increase in fresh shoot weight compared to control. In all the varieties, the maximum increase in fresh shoot weight was obtained with *B. subtilis*, which were 85.48%, 43.16%, 54.54% and 57.05% in var. Kanchan, Shatabdi, BARI Gom27 and BARI Gom29, respectively (Table 3). On the other hand, the percent increase in shoot fresh weight by *P. stutzeri* over control was 42.74%, 33.81%, 36.36% and 21.17% in var. Kanchan, Shatabdi, BARI Gom27 and BARI Gom29, respectively.

Table 2. Effect of seed priming with plant growth promoting rhizobacteria on shoot length of seedling of wheat varieties

PGPR	Shoot length (cm) of four wheat varieties			
	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29
Pseudomonas stutzeri	25.26 e* B** (38.25)	26.40 cd A (23.36)	29.33 cd A (28.64)	29.17 b B (16.54)
Bacillus subtilis	26.33 c A (44.11)	26.87 f A (25.56)	29.74 de A (30.43)	31.23 a A (24.77)
Control	18.27 h C	21.40 g B	22.80 e B	25.03 de C

*Values having same letter (lower case in a row and upper case in a column) are not significantly different ($P \geq 0.05$)

Table 3. Effect of pre sowing seed priming with rhizobacteria on the fresh weight of shoot of wheat varieties

PGPR	Fresh shoot weight (mg)			
	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29
Pseudomonas stutzeri	177 c* A** (42.74)	186 c A (33.81)	180 c A (36.36)	206 b B (21.17)
Bacillus subtilis	230 c A (85.48)	199 b A (43.16)	204 c A (54.54)	267 a A (57.05)
Control	124 d B	139 d B	132 c B	170 c C

*Values having same letter (lower case in a row and upper case in a column) are not significantly different ($P \geq 0.05$)

Dry weight of shoot

Irrespective of wheat varieties, seed treatment with PGPR significantly increased dry weight of shoot compared to control. Seed priming with *B. subtilis* yielded significantly higher dry weight of shoot compared to treatment with *P. stutzeri* in all varieties (Table 4). The percent increase in dry weight of shoot over control in treated plants with *B. subtilis* and *P. stutzeri* were increased by 110.22% and 29.54%, respectively in var. Kanchan, 75.58% and 55.81%, respectively in var. Shatabdi, 83.11% and 71.42%, respectively in var. BARI Gom-27 and 106.59% and 71.42%, respectively in var. BARI Gom-29 (Table 4).

Root length

Plants treated with *P. stutzeri* and *B. subtilis* produced significantly longer roots in all the varieties compared to control. The maximum (58.83%) increase in root length over control was achieved in BARI Gom-29 treated with *B. subtilis* followed by same variety treated with *P. stutzeri* (50.00%) (Table 5).

Fresh weight of root

Fresh weight of root of wheat seedlings was significantly increased in PGPR-treated plants of all the varieties compared to control. Seeds treated with *B. subtilis* produced higher fresh weight of root in var. Kanchan, Shatabdi, BARI Gom-27 and BARI Gom-29 *P. stutzeri*

Table 4. Effect of pre-sowing seed priming with rhizobacteria on dry weight of shoot of wheat varieties

Plant growth promoting Rhizobacteria	Dry shoot weight (mg)			
	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29
Pseudomonas stutzeri	114 bc B (29.54)	134 a A (55.81)	132 b A (71.42)	156 a A (71.42)
Bacillus subtilis	185 a A (110.22)	151 bc B (75.58)	141 c A (83.11)	188 b B (106.59)
Control	88 e C	86 de C	77 e B	91 d C

*Values having same letter (lower case in a row and upper case in a column) are not significantly different ($P \geq 0.05$).

Table 5. Effect of seed priming with rhizobacteria on root length of seedling of wheat varieties

PGPR	Root length (cm)			
	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29
Pseudomonas stutzeri	8.22 bc*A** (21.06)	9.25 a A (22.84)	8.03 c B (16.71)	9.00 ab A (50.00)
Bacillus subtilis	8.45 b A (24.46)	9.08 ab A (20.58)	9.10 a A (32.26)	9.53 a A (58.83)
Control	6.79fg B	7.53 d B	6.88 f C	6.00 h B

*Values having same letter (lower case in a row and upper case in a column) are not significantly different ($P \geq 0.05$)

Table 6. Effect of seed priming with rhizobacteria on the fresh weight of root of wheat varieties

PGPR	Fresh root weight (mg)			
	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29
Pseudomonas stutzeri	146 c* AB** (35.18)	133 d AB (17.69)	156.0 c B (54.45)	181 b B (49.58)
Bacillus subtilis	149 c A (37.96)	143 c A (26.54)	195 a A (93.06)	198 a A (63.63)
Control	108 f C	113 ef C	101 g C	121 e C

*Values having same letter (lower case in a row and upper case in a column) are not significantly different ($P \geq 0.05$)

than those treated with *P. stutzeri* (Table 6). With regard to percent increase in fresh weight of root over control, the highest value (93.06%) was achieved in plants of BARI Gom-27 treated with *B. subtilis* which was followed by those (63.63%) of BARI Gom-27 treated with *B. subtilis*.

Dry weight of root

Application of both the rhizobacteria significantly increased dry weight of root compared to control in all the varieties of wheat. In var. Kanchan and Shatabdi, the treatment effect of *P. stutzeri* and *B. subtilis* on dry weight of root was statistically similar (Table 7), while in var. BARI Gom-27 and BARI Gom-29, treatment with *B. subtilis* produced significantly the higher dry weight of root than *P. stutzeri*. The percent increase in root dry weight by *P. stutzeri* over control ranged from 21.42% to 59.25%, where the maximum and the minimum increase was observed in var. BARI Gom-27 and BARI Gom-29. Similarly, the highest percent increase (77.78%) in dry weight by *B. subtilis* over control was accounted in var. BARI Gom-29 and the lowest increase (47.82%) was in var. Shatabdi.

Nitrogen content in leaf

Nitrogen content in leaf of wheat seedlings was enhanced significantly in all the varieties by inoculation with PGPR compared to control. Seed treatment with *B. subtilis* resulted in the highest nitrogen content in leaf in all four varieties, ranging from 3.18 to 2.82%. The percent increase in leaf content

Table 7. Effect of seed priming with rhizobacteria inoculation on dry weight of root wheat varieties

PGPR	Dry weight of root (mg) of wheat varieties			
	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29
Pseudomonas stutzeri	34 c* AB** (41.66)	31 d AB (34.78)	34 cd B (21.42)	43 b B (59.25)
Bacillus subtilis	37 c A (54.16)	34 cd A (47.82)	46 a A (64.28)	48 a A (77.78)
Control	24 f C	23 f C	28 e C	27 e C

*Values having same letter (lower case in a row and upper case in a column) are not significantly different ($P \geq 0.05$).

Table 8. Effect of inoculation of rhizobacteria on nitrogen content in leaves of wheat varieties

PGPR Kanchan	% Nitrogen content in leaves of wheat varieties*			
	Shatabdi	BARI Gom-27		BARI Gom-29
Pseudomonas stutzeri	2.40 d* B ** (35.69)	2.20 f B (23.59)	2.34 de B (27.17)	2.60 c B (42.07)
Bacillus subtilis	3.06 ab A (72.88)	2.82 d A (58.42)	2.96 bc A (60.86)	3.18 a A (73.78)
Control	1.77 h C	1.78 gh C	1.84 g C	1.83 j C

*Values having same letter (lower case in a row and upper case in a column) are not significantly different ($P \geq 0.05$).

by the application with *B. subtilis* were 72.88, 58.42, 60.87 and 73.77% in var. Kanchan, Shatabdi, BARI Gom-27 and BARI Gom-29, respectively (Table 8). On the other hand, nitrogen content in leaf in *P. stutzeri*-inoculated plants ranged from 2.20 to 2.60%, where the lowest and the highest nitrogen content were found in var. Shatabdi and BARI Gom-29, respectively. Treatment with *P. stutzeri* increased leaf nitrogen content by 35.59, 23.60, 27.17 and 42.08%, in var. Kanchan, Shatabdi, BARI Gom-27 and BARI Gom-29, respectively.

Root colonization by PGPRs

Two rhizobacterial isolates were examined for their root colonization ability in four varieties of wheat. The number of *Bacillus subtilis* in roots of four varieties ranged from 131.20×10^7 to 200.20×10^7 cfu/g root tissues, where the maximum number was found in var. Kanchan and the minimum in var. Shatabdi (Fig. 1). Similarities, *P. stutzeri* showed root colonization value of 73.50×10^7 to 233.60×10^7 cfu/g root tissues of these varieties, where the maximum cfu was found in var. BARI Gom-29 and the minimum in var. Kanchan. This indicated that higher cfu was recorded in the roots of *B. subtilis*-treated plants of var. Kanchan, Shatabdi and BARI Gom-27 compared to those of *P. stutzeri*-treated plants, while *P. stutzeri* showed higher cfu than *B. subtilis* in var. BARI Gom-29.

The results of the study revealed that application of PGPR significantly increased seed germination, plant height and biomass production compared to untreated control.

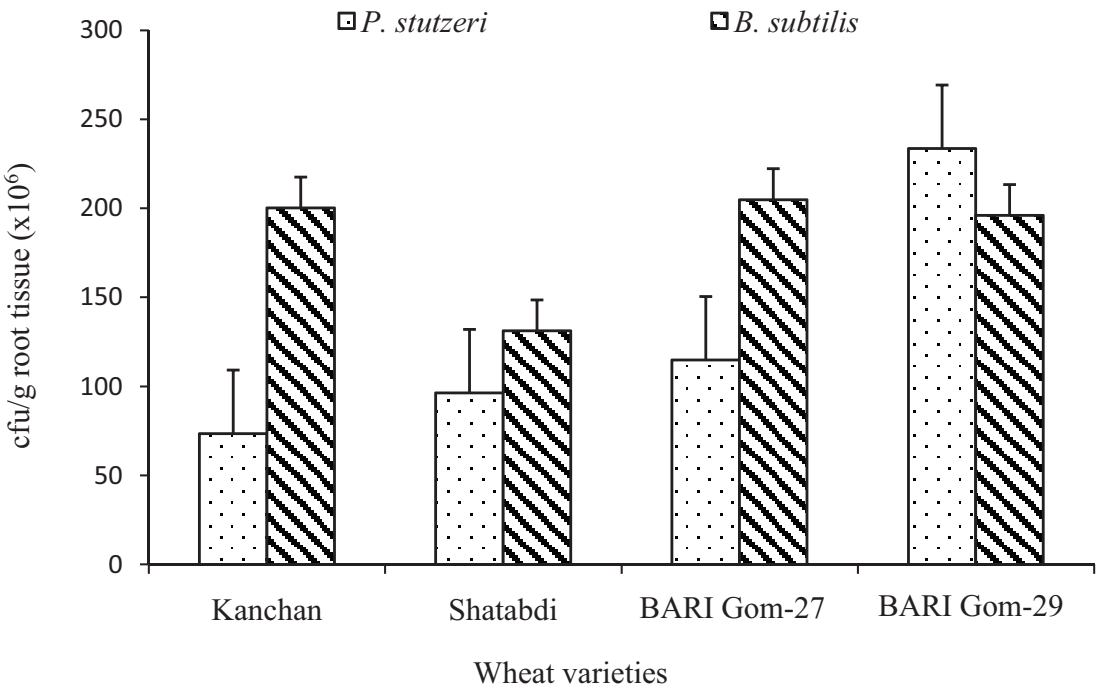

Fig. 1. Growth of rhizobaterial isolates in the roots of four wheat varieties (cfu/g fresh root weight).

Similar increase in plant height and leaf area was observed in different crops inoculated with *Pseudomonas*, *Azospirillum* and *Azotobacter* strains (Saukat *et al.,* 2006a, b). Hamidi *et al.* (2011) reported that seed biofortification by plant growth promoting rhizobacteria significantly enhanced the root length, root fresh and dry weight of maize seedlings. Other studies also showed the positive effect of PGPR on germination and root-shoot growth of plants (Gholami *et al.,* 2009; Mahmood *et al.,* 2010). Such improvement in germination and plant growth might be attributed to N_2-fixing and phosphate solubilizing capacity of bacteria as well as the ability of these microorganisms to produce growth promoting substances (Egamberdiyeva *et al.,* 2002: Awad *et al.,* 2011: Saharan and Nehra, 2011). In the present study, rhizobacterial inoculant increased leaf nitrogen content in wheat plants. Barneix *et al.* (2005) reported that inoculation of plant with rhizobacteria enhanced nitrogen accumulation in the plant, increasing the efficiency of use of the applied fertilizer, with the potential benefit of reducing losses to the environment. Burd *et al.* (2000) reported that plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients as well as facilitating the uptake of nutrients by the plants. Other investigators also reported similar results. However, it is yet to know whether a single or a mixture of different mechanisms are operating in a single plant-microbe interaction.

Rhizobacteria establish root colonization on rhizoplane or in rhizosphere and further

express their activity on host plants (Li and Kremer, 2000). Root colonization takes place at the root surface, inside of the root and/ or the rhizosphere (Weller and Thomashow, 1994). Soil moisture, soil texture, plant species, root exudates composition, and bacterial viability can influence colonization of the rhizosphere and rhizoplane (Howie and Echandi, 1983). In this study, two PGPR strains varied in their effectiveness in stimulating plant growth traits. Higher plant growth promotion was obtained in plants pretreated with *B. subtilis* than with *P. stutzeri*. This might be due to variation in the symbiotic effectiveness between species or within the same species. Till today a little studies have examined the genotypic effects of plants on PGPR-mediated plant growth promotion.

Conclusion

Treatment with two PGPR strains significantly increased seed germination in var. Shatabdi and resulted in significant increase in shoot length, shoot weight, root length and root weight in all four varieties compared to uninoculated control. Both the PGPR colonized the wheat roots and significantly enhanced leaf nitrogen content. Higher plant growth promotion was always obtained in plants pretreated with *B. subtilis* than with *P. stutzeri*. It may be concluded that application of PGPR inoculants for biofertilization could be an attractive option to reduce the use of chemical fertilizers in wheat. Further studies are required to elucidate the exact mechanisms involved in plant growth promotion in wheat by *B. subtilis* and *P. stutzeri*.

Acknowledgments

This study was part of the project, "Microbial priming for plant growth promotion, disease suppression and abiotic stress tolerance in wheat" funded by University Grant Commission through Research Management Committee (RMC) of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.

References

Awad, N. M., A. A. Abd El-Kader, M. Attia and A. K. Alva. 2011. Effects of nitrogen fertilization and soil inoculation of sulfur-oxidizing or nitrogen-fixing bacteria on onion plant growth and yield. *Int. J. Agron.* 2011: 6.

Barneix, A. J., M. I. Saubidet, N. Fatta and M. Kade. 2005. Effect of rhizobacteria on growth and grain protein in wheat. Agronomy for Sustainable Development, Springer Verlag/EDP Sciences/INRA, 2005 25: Pp. 505-511.

BBS. 2016. Yearbook of Agricultural Statistics-2016. Bangladesh Bureau of Statistics (BBS), Ministry of Planning, Government of the People's Republic of Bangladesh.

Bramner, J. M and D. R. Keeney. 1965. Stem distillation methods for determination of ammonium in Kjeldahl analysis of soils. *Commun. Soil. Sci. Plant Anal.* 3:159-165.

Burd, G. I., D. G. Dixon and B. R. Glick. 2000. Plant growth promoting bacteria that decrease heavy metal toxicity in plants. *Can. J. Microbiol*. 46: 237–245.

Egamberdieva, D., D. Juraeva, L. Gafurova and G. Höflich. 2002. Promotion of plant growth of maize by plant growth promoting bacteria in different temperatures and soils. In Proc. of 25th Annual Southern Conservation Tillage Conference for Sustainable Agriculture. Auburn, AL 24-26 June 2002. Special Report no. 1. Alabama Agric. Expt. Stn.RT and Auburn University, AL 36849. USA.

Gholami, A., S. Shahsavani. and S. Nezarat. 2009. The effect of plant growth promoting

rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *World Acad. Sci. Engg. Technol*. 49:19-24.

Hamidi, A., A. Asgharzadeh, R. Chaohan, and M. A. Khalvati. 2011. Maize seed biofortification by plant growth promoting bacteria (PGPR). *Int. J. Agron Plant Produc*. 2: 194-205.

Howie, W. J. and E. Echandi. 1983. Rhizobacteria: influence of cuhivar and soil type on plant growth and yield of potato. *Soil Biol. Biochem*. 15:127-132.

Islam, S., A. M. Akanda, A. Prova, M. T. Islam and M. M. Hossain. 2015. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Front Microbiol*. 6:1360.

Jeffris, P., S. Gianinazzi, S. Perotto, K. Turnau and J. M. Barea. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant and soil fertility. *Biol. Fert. Soils*. 37: 1-16.

Kjeldahl, J.1883. A new method for the estimation of nitrogen in organic compounds. *Z. Anal. Chem*. 22: 366.

Li, J. and R. J. Kremer. 2000. Rhizobacteria associated with weed seedlings in different cropping systems. *Weed Sci*. 48: 734-741.

Mahmood, M., Z. A. Rahman and H. M. Saud. 2010. Influence of rhizobacterial and agrobacterial inoculation on selected physiological and biochemical changes of banana cultivar, Berangan (aaa) plantlets. *J. Agril. Sci.* 2: 115-137.

Mian, I. H. 1995. Methods in Plant Pathology. IPSA-JICA Project. 136 P.

Saharan, B. S. and V. Nehra, 2011. Plant Growth promoting rhizobacteria: a critical review. Life Sciences and Medicine Research, Vol.2011: LSMR-21.

Saukat, K., S. Affrasayab and S. Hasnain. 2006a. Growth responses of *Helianthus annus* to plant growth promoting rhizobacteria used as a biofertilizer. *Intl. J. Agric. Res.* 1: 573-581.

Saukat, K., S. Affrasayab, and S. Hasnain. 2006b. Growth responses of *Triticum aestivum* to plant growth promoting rhizobacteria used as a biofertilizer. *Res. J. Microbiol*. 1: 330-338.

Weller, D. M. and L. S. Thomashow. 1994. Current challenges in introducing beneficial microorganisms into the rhizosphere in molecular ecology of rhizosphere microorganisms, biotechnology and the release of GMOs. F. O'Gara, D.N. Dowling, and B. Boesten, eds. VCH Verlagsgesellschaft, Weinheim, Germany, Pp.1-13.