



Effect of plant growth promoting bacteria on early growth of wheat cultivars

Sang Gyu Lee¹ · Hyeri Lee¹ · Jimin Lee¹ · Byung Cheon Lee¹ · Hojong Lee¹ · Changhyun Choi² · Namhyun Chung¹

Received: 17 July 2019 / Accepted: 26 July 2019 / Published Online: 30 September 2019
© The Korean Society for Applied Biological Chemistry 2019

Abstract Wheat is one of the most important grains. Its consumption is increasing globally. Many countries are making efforts to increase the extent of wheat harvest. It is known that plant growth promoting rhizobacteria (PGPRs) have beneficial effects on various plants. Two PGPRs including *Paenibacillus pabuli* strain P7S (PP7S) and *Pseudomonas nitroreducens* strain IHB (PnIHB) were employed to investigate effects of PGPRs on early growth of three wheat cultivars (Koso, Seakumkang, and Jokyoung). While PP7S had adverse effects on Seakumkang and Jokyoung, PP7S had positive effects on Koso except root length compared to control group having no treatment of PP7S. However, all treatments with PnIHB had adverse effects on germination rate, root/shoot lengths, vigor index, and dry root/shoot weights of all three wheat cultivars. These positive effects with PP7S on Koso might be related to the earlier emergence of wheat seed above soil which is known to be an indicator of increased yield. Results of the present study suggest that if proper PGPR strains are selected, they could have positive effects on early growth rate of a wheat cultivar.

Keywords Germination rate · Koso cultivar · Plant growth promoting rhizobacteria · Seedling emergence · Vigor index

Namhyun Chung (✉)
E-mail: nchung@korea.ac.kr

Changhyun Choi (✉)
E-mail: chchhy@korea.kr

¹Department of Biosystems Engineering, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea

²National Institute of Crop Science, RDA, Wanju-Gun, Jeollabuk-do 55365, Republic of Korea

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Wheat is one of the most grown and consumed grains around the world. Consumption of wheat is increasing continuously. For example, world wheat consumption in 2019 was expected to increase up to 741 million tons, an increase of 0.27% (2 million tons) compared to that in the previous year [1]. However, wheat production continues to decline for various reasons. For example, world wheat production in 2019 was expected to be 733 million tons, a decrease by 3.8% (29 million tons) compared to that in the previous year [1]. This indicates that a serious food security problem might continue if there is no proper measure. In order to prevent the decrease of wheat production, each country has made various efforts in accordance with the environment of its own country.

While wheat consumption in Korea increased every year, wheat self-sufficiency rate of Korea was less than 2% in 2018 [2]. One way to increase the self-sufficiency rate is by increasing yield of wheat which might result in lower wheat price. Many efforts have been made in Korea to increase wheat yield. Although synthetic fertilizer containing nitrogen as a major element has been commonly used, overuse of synthetic fertilizers often contributes to the pollution of soil and water, causing adverse environmental impacts [3]. Therefore, a natural fertilizer that does not induce environmental pollution needs to be developed.

Plant growth promoting rhizobacteria (PGPRs) could be considered as a major component of natural fertilizer. PGPRs are rhizobacteria that can inhabit the rhizosphere of plant roots. They are known to have beneficial effects on plant growth. There are many different kinds of PGPR, among which *Pseudomonas* and *Bacillus* species have often employed as bacterial components of natural fertilizer [4,5]. Although many studies have been performed about beneficial effects of PGPR on various crops, there are not many research results regarding the effect of PGPR on different varieties of wheats. Therefore, the objective of this study was to determine the effect of PGPR on early growth stage (i.e., seedling emergence) of

Korean wheat cultivars.

Materials and Methods

PGPR strains

Bacterial strains used in this study were *Paenibacillus pabuli* strain P7S (PP7S) and *Pseudomonas nitroreducens* IHB B 13561 (PnIHB). Details on the isolation and identification of these strains have been described previously [5,6]. Bacterial cell stock was stored at -70°C in 80% glycerol solution. Three day prior to inoculation with each wheat seed cultivar, PP7S was grown in Luria-Bertani (LB) agar plate at 30°C for 18h in an incubator (BF-150IN, Biofree, Seoul, Korea). The next day, bacteria were grown in 3 mL of LB broth at 30°C for 18h in a shaker chamber (SI-300R, HYSC, Seoul, Korea). One day prior to soaking seeds, 3 mL of culture broth containing bacteria was transferred to 20 mL LB broth for continued growth. For paper towel experiment, bacterial suspension was diluted to aliquot of 10^9 colony forming unit per mL of LB broth in a conical tube.

Wheat seed sterilization

A total of three wheat seed cultivars were used in the present study: Koso cultivar, Seakumkang cultivar, and Jokyoung cultivar. These cultivars were provided by National Institute of Crop Science (Wanju-gun, Korea). Twenty seeds of each cultivar were put into 50 mL conical tube, sterilized with sodium hypochlorite for 20 min with shaking, and then washed three times with 40 mL sterilized distilled water [7]. These seeds were then placed in 50 mL conical tube containing 40 mL sterile distilled water and stored at 23°C for one day.

Germination test

Germination test was performed using published paper towel method [8] with modification. Briefly, paper towels (Geneall, Seoul, Korea) were spread in two layers in a Petri-dish (90 mm \times 15 mm) which received 10 mL of sterilized distilled water. Then 20 seeds were transferred into the 50 mL conical tube containing bacteria for mixing in a shaker chamber for 1.5 h. These infected 20 seeds in a conical tube were transferred to the prepared Petri dish described above. After seedling, Petri-dishes were wrapped around with parafilm to prevent evaporation and placed in a plant growth chamber at 23°C in the dark. Germination rate, root length, shoot length, dry root weight, and dry shoot weight were measured at 5 days after the experiment. When the radicle grew more than 2 mm, the number of seeds germinated was counted. Dried weight was measured after drying at 60°C for 48 h in an incubator (BI-600M, Jeio tech, Seoul, Korea).

Vigor index

Vigor index was calculated based on root length, shoot length, and germination rate using the following formula suggested by Abdul-

Baki and Anderson [10]: vigor index = (mean root length + mean shoot length) \times % germination. To measure root length and shoot length, the beginning part of the root and the beginning part of the leaf were cut from germinated seeds.

Data analysis

Data were statistically treated by analysis of variance (ANOVA) and least significant difference (LSD) test at probability level of 0.05. ANOVA F-test was used to separate means in case of a significant effect of the treatment.

Results

Germination rate was measured after growing the three wheat cultivars in Petri-dishes for five days. Fig. 1 shows germination rate of each cultivar upon treatment with two PGPRs. For Koso, PnIHB-treated group had the lowest germination rate while PP7S-treated group had the highest germination rate. PP7S-treated group had 10.7% higher germination rate than the control group without any treatment. Average germination rate for Koso was in the order of PP7S-treated group > control > PnIHB-treated group (Fig. 1). For Seakumkang, germination rate in PP7S-treated group decreased by 7.86% compared to the control. However, germination rate in PnIHB-treated group decreased more (by 17.97%) compared to the control. Average germination rate for Seakumkang was in the order of control > PP7S-treated group > PnIHB-treated group (Fig. 1). The overall germination rate for Jokyoung was lower than that of Seakumkang. However, the order of average germination rate for Jokyoung tended to be similar to that of Seakumkang. It had the following order: control > PP7S-treated group > PnIHB-treated groups (Fig. 1).

After checking germination rate, five seeds were randomly selected from Petri-dish. Roots and shoots grown from these seeds were then cut to measure lengths of roots and shoots. Vigor index

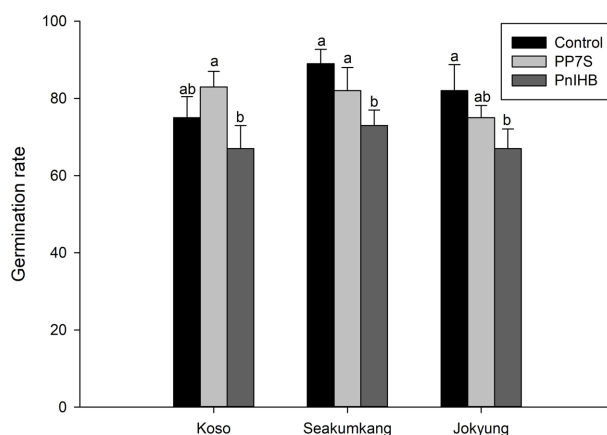


Fig. 1 Germination rates of three wheat cultivars after PGPR treatment. Within each wheat cultivar, values followed by the same capital letter are not significantly different ($p=0.05$)

Table 1 Root/shoot length and vigor index of wheat seedlings after treatment with PGPRs

Cultivar	Treatment	Root length (cm)	Shoot length (cm)	Vigor index
Koso	Control	4.1042 a	3.5951 a	581.95 ab
	PP7S	3.1839 b	3.8937 a	603.62 ab
	PnIHB	2.8068 b	3.1535 a	402.00 a
Seakumkang	Control	5.9312 a	5.2030 a	978.06 cd
	PP7S	4.6951 b	4.6888 ab	802.27 bcd
	PnIHB	3.9163 b	3.9243 b	588.29 ab
Jokyung	Control	5.7251 a	6.5364 a	1016.43 d
	PP7S	4.3839 b	6.4481 a	800.69 bcd
	PnIHB	4.4815 b	6.0556 a	733.09 bc

Within each wheat cultivar and measured item, values followed by the same small letter are not significantly different ($p=0.05$)

was calculated using germination rate together with lengths of roots and shoots (Table 1). Lengths of roots were the longest in control group for all wheat cultivars. Control group of Seakumkang had the longest root length among control and two PGPR groups. The average root length was in the order of control > PP7S-treated group > PnIHB-treated group for Koso and Seakumkang. For Jokyung, the average root length was in the order of control > PnIHB-treated group \geq PP7S-treated group (Table 1). The average shoot length among the three wheat cultivars was in the order of Jokyung > Seakumkang > Koso. The average shoot length was in the order of PP7S-treated group > control > PnIHB-treated group for Koso. On the other hand, the average shoot length was in the order of control > PP7S-treated group > PnIHB-treated group for Seakumkang and Jokyung (Table 1). For vigor index, overall trend was very similar to the order of shoot length among control and 2 PGPR groups. For example, the average vigor index among the three wheat cultivars was in the order of Jokyung > Seakumkang > Koso. The vigor index was the highest for PP7S-treated group of Koso. For Seakumkang and Jokyung, the vigor index was higher for the control group than those of the 2 PGPR groups (Table 1).

Dry root and shoot weights were measured after 2 days of drying process of root and shoot obtained from root and shoot length measurement (Table 2). Dry root weight was lower in the PnIHB-treated group than that of control or PP7S-treated group for all three wheat cultivars. Dry shoot weight was higher in the PP7S-treated group than that of the control or PnIHB-treated group for Koso and Jokyung. For Koso and Seakumkang, shoot/root dry weight ratios tended to be higher in the PP7S-treated group than with those in the control or PnIHB-treated group. However, shoot/root dry weight ratios tended to be higher in the PnIHB-treated group than those in the control or PP7S-treated group for Jokyung. Dry root/shoot weight ratios were between 5.2 and 6.4 (Table 2). They were not significantly different among all calculated values in the previous study [11].

Table 2 Dry root and shoot weights of wheat seedlings after treatment with PGPR

Cultivar	Treatment	Dry root weight (g)	Dry shoot weight (g)	Shoot / Root Dry weight ratio
Koso	Con	0.0007 a	0.0035a	5.380 a
	PP7S	0.0008 a	0.0046a	6.164 a
	PnIHB	0.0006 a	0.0038a	6.068 a
Seakumkang	Con	0.0011 a	0.0058 a	5.483 a
	PP7S	0.0009 ab	0.0050 ab	5.832 a
	PnIHB	0.0008 b	0.0043 b	5.235 a
Jokyung	Con	0.0014 ab	0.0071 a	5.364 a
	PP7S	0.0014 a	0.0075 a	5.647 a
	PnIHB	0.0011 b	0.0067 a	6.395 a

Within each wheat cultivar and measured item, values followed by the same small letter are not significantly different ($p=0.05$)

Discussion

Results of this study showed that the effect of PGPRs was various depending on wheat cultivar. In most cases, PnIHB-treated groups had adverse effects on various parameters including germination rate, root and shoot lengths, vigor index, and dry root and shoot weights of all three wheat cultivars. Although PP7S had adverse effects on Seakumkang and Jokyung, PP7S had positive effects on all parameters of Koso except root length compared to the control group. Higher germination rate and vigor index, which are known to indicate higher early growth, might be related to earlier emergence and higher emergence rate of wheat seed above soil in the same depth. A previous study has shown an increased yield of early-emerging wheat [12]. Thus, a well-selected choice of wheat cultivar and PGPR (such as Koso and PP7S, respectively) could indicate an increased wheat yield. However, wheat yield might decrease upon treatment with PnIHB as PGPRs. Results of the present study also suggests that if proper PGPR strains are selected for Seakumkang and Jokyung, their yields could be increased upon treatment with PGPRs. Detailed interaction mechanism between Koso and PP7S needs to be studied in the future. In addition, we plan to continue pot and field tests for combination of Koso and PP7S.

Acknowledgment This work was supported by a grant (PJ012496022019) from the Rural Development Administration, Republic of Korea.

Authors' contributions SGL performed experiments and wrote the paper. HL and JL helped the preparation of experiments. BCL and HJL revised the manuscript. CHC and NC edited, revised the manuscript, and supervised the work. All authors read and approved the final manuscript.

Competing interests The authors declare that they have no competing interests.

References

1. International Grain Council (2019) Grain market report. https://www.igc.int/en/gmr_summary.aspx#
2. Kim YJ, Na SI, Kim KH, Kim KM, Shin DJ, Cha JK, Lee SK, Ko JM (2018) Agricultural characteristics and grain quality according to sowing times in spring sowing wheat. *Korean J Agric Sci* 45(4): 615–622
3. Savci S. (2012) Investigation of effect of chemical fertilizers on environment. *APCBEE Procedia* 1: 287–292
4. Hong SH, Kim JS, Sim JG, Lee EY (2016) Isolation and characterization of the plant growth promoting rhizobacterium, *Arthrobacter scleromae* SYE-3 on the yam growth. *KSBB Journal* 31(1): 58–65
5. Ibal JC, Jung BK, Park CE, Shin JH (2018). Plant growth-promoting rhizobacteria used in South Korea. *Appl Biol Chem* 61(6): 709–716
6. Trinh CS, Jeong CY, Lee WJ, Truong HA, Chung N, Han J, Hong SW, Lee H (2018). *Paenibacillus pabuli* strain P7S promotes plant growth and induces anthocyanin accumulation in *Arabidopsis thaliana*. *Plant Physiol Bioch* 129: 264–272
7. Trinh CS, Lee H, Lee WJ, Lee SJ, Chung N, Han J, Kim J, Hong SW, Lee H (2018) Evaluation of the plant growth-promoting activity of *Pseudomonas nitroreducens* in *Arabidopsis thaliana* and *Lactuca sativa*. *Plant Cell Rep* 37:873–885 doi:10.1007/s00299-018-2275-8
8. Forghani AH, Almodares A, Ehsanpour AA (2018) Potential objectives for gibberellic acid and paclobutrazol under salt stress in sweet sorghum (*Sorghum bicolor* [L.] Moench cv. Sofra). *Appl Biol Chem* 61(1): 113–124
9. Raj SN, Shetty NP, Shetty HS (2004) Seed bio-priming with *Pseudomonas fluorescens* isolates enhances growth of pearl millet plants and induces resistance against downy mildew. *Int J Pest Manage* 50(1): 41–48
10. Abdul-Baki AA, Anderson JD (1973) Vigor determination in soybean seed by multiple criteria. *Crop Sci* 13: 630–633
11. Harris RW (1992) Root-shoot ratios. *J Arboric* 18(1): 39–42
12. TeKrony DM, Egli DB (1991) Relationship of seed vigor to crop yield: a review. *Crop Sci* 31(3): 816–822