

EFFECT OF *Bacillus amyloliquefaciens* ON PLANT GROWTH AND SUPPRESSION OF *Bipolaris* LEAF BLIGHT IN WHEAT

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Abstract

Effect of seed priming with a plant growth promoting rhizobacteria (PGPR) strain *Bacillus amyloliquefaciens* on root-shoot growth and suppression of *Bipolaris* leaf blight (*Bipolaris sorokiniana*) in four wheat varieties namely Kanchan, Shatabdi, BARI Gom 27 and BARI Gom-29 was studied. In all varieties seed priming with *B. amyloliquefaciens* gave significant increase in shoot and root length compared to control. Similarly, a significant increase in fresh and dry shoot and root weight over control was achieved with pre-sowing seed treatment with *B. amyloliquefaciens* in these wheat varieties. The percent increase in shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight and dry root weight by *B. amyloliquefaciens* over control ranged from 25.26 to 34.18%, 34.68 to 42.86%, 19.73 to 36.12%, 27.98 to 49.86%, 44.25 to 70.08% and 47.14 to 77.65%, respectively. The percent increase in root-shoot growth was the highest in cv. Shatabdi which was followed by cv. Kanchan. Seed treatment with the PGPR strain also caused significant reduction in percent *Bipolaris* leaf blight Disease Index (PDI) compared to control in all wheat varieties. The highest reduction was achieved with *B. amyloliquefaciens* in all four varieties. The percent reduction in PDI by *B. amyloliquefaciens* over control ranged from 31.83 to 47.59%, respectively. The highest reduction in PDI by *B. amyloliquefaciens* was observed in cv. Shatabdi. High level of root colonization (14 to 39.13 cfu/g root tissues) was observed by the bacterium in all four varieties. Findings of the study reveal that root colonizing PGPR used for seed priming can promote plant growth and suppress the *Bipolaris* leaf blight in wheat.

Keywords: Shoot growth, root growth, Percent Disease Index, root colonization.

Introduction

Wheat (*Triticum aestivum*), among the cereals, is considered as the second most important crop after rice in Bangladesh. The consumption of wheat was 54.9 kg yearly per capita in 1961, while it is 65.43 kg in 2013 (Mottaleb *et al.*, 2018). Two decades ago, studies on the economics of wheat in Bangladesh found Boro (winter) rice to be more profitable than wheat in irrigated zones, but wheat often

generated the highest returns in non-irrigated zones and in areas that are unsuitable for Boro rice production (Morris *et al.*, 1996). While Bangladesh has been highly successful in achieving self-sufficiency in rice production, the increasing wheat consumption is met mainly by import. In this critical backdrop, domestic wheat production must be increased to feed Bangladesh's burgeoning population and changing consumption patterns.

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Wheat suffers from many diseases that incur severe yield losses in the field (Murray and Brennan, 2009). *Bipolaris sorokiniana* (Sacc. in Sorok.) (Teleomorph: *Cochliobolus sativus* Dredchs. ex Dastur) is one of the principal pathogens that routinely harms wheat plants. The pathogen is responsible for causing common root rot, spot blotch, seedling blight and black point of the grain. The main source of inoculum is considered the soil-borne conidia, which under favorable conditions, may result in severe leaf blight disease (Duczek *et al.*, 1996). This disease is a major constraint of wheat cultivation in Bangladesh and may cause up to 88% yield reduction (Hossain *et al.*, 1998). The massive yield losses in wheat by *B. sorokiniana* warrant effective strategies for managing the disease. Chemical fungicides have often been suggested to alleviate the negative effect of *B. sorokiniana* on wheat growth and yield. Long term and frequent uses of fungicides have serious adversaries to environment, public health and even to the success of disease management system. Therefore, alternate strategies are needed which could be applied alone or in combination with other strategies such as in Integrated Pest Management technique. Use of plant growth promoting rhizobacteria (PGPR) for improving plant growth and disease resistance is considered as simple, economical, and sustainable strategy (Islam *et al.*, 2015). PGPR are the soil bacteria inhabiting around/on the root surface and are directly or indirectly involved in promoting plant growth and conferring other beneficial effects. Generally, PGPR facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels,

or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents. Therefore, the aim of this work was to explore the potential of PGPR to stimulate plant growth and to suppress *Bipolaris* leaf blight (*B. sorokiniana*) in wheat.

Materials and Methods

Plant material

Seeds of wheat varieties viz. Shatabdi, Kanchan, BARI Gom-27 and BARI Gom-29 were collected from Plant Genetic Resource Center of Bangladesh Agricultural Research Institute, Gazipur, Bangladesh and stored at 4°C until use.

Plant growth promoting rhizobacteria strains (PGPR)

A PGPR strain *Bacillus amyloliquefaciens* was collected from a stock culture of the Department of Plant Pathology, BSMRAU, Gazipur and used in this experiment. The molecular and biochemical characterization of the PGPR was done previously by Islam *et al.* (2015).

Preparation of PGPR inocula

Rhizobacterial strain was 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°C. The bacterial cells were separated from the broth by centrifugation at 15000 rpm for 1 min at 4°C and then 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 suspension of the rhizobacterial strain was used as inocula.

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 surface sterilized grains were soaked in only sterilized distilled water, which were used for control treatment. After priming with PGPR strain, 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 μ L suspension of each dilution was spread evenly on Petri dishes containing King's B agar medium. After 24 hr of incubation at 28 °C, the number of colony-forming units (cfu) per gram of seed was recorded.

Isolation and identification of *B. sorokiniana*

An isolate of *B. sorokiniana* was isolated from *Bipolaris* leaf blight infected wheat leaves collected from the field following tissue planting method on potato dextrose agar (PDA) medium (Adlakha *et al.*, 1984; Asad *et al.*, 2009). Single spore pure culture of the fungus was prepared and it was identified as *B. sorokiniana* based on cultural and morphological characteristics on PDA as described by Acharya *et al.* (2011). For pathogenicity test, wheat seedlings were grown in earthen pot. At the age of 4 weeks,

the seedlings were inoculated by spraying spore suspension 5×10^5 spore/ml. Inoculated seedlings were placed in a humid chamber overnight and then transferred to the net house. After 7 days of inoculation, the seedlings were checked for disease symptoms. The isolate of *B. sorokiniana* developed characteristic leaf blight symptoms on wheat seedling. The pathogen *B. sorokiniana* was then cultured on PDA slant and stored at 10°C for future use.

Growing of seedlings in pots

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, 0.91% potassium, 45.52% sand, 36.00% silt, 18.48% clay and 21.96% moisture, having pH of 6.41. The soil contained. The soil was autoclaved twice at 24 hr intervals for 20 min at 121°C under the pressure of 1.1kg /cm² before pouring it in pot. About 250 g of autoclaved soil was poured in each pot (12.5 cm x 10.0 cm). Before pouring the soil, the pots were washed with 10.0% chlorox for 10 min and rinsed with tap water for three times. Treated seeds (30 seeds/pot) of wheat were planted in each pot filled with potting soil. Water treated seeds were planted in another sets of pots which served as control. After 7 days of germination, seedlings were thinned to have 20 seedlings per pot. The seedlings were allowed to grow for four weeks providing proper soil moisture.

Collection of data on seedling growth

After four weeks of plant growth in PGPR- and non-treated pots, wheat seedlings were separated into shoots and roots and their fresh weight and length were measured. Shoots and roots were then dried at 70°C for at least 72 h, after which their dry weights were recorded.

Preparation of inocula of *B. sorokiniana*

The fungal pathogen *B. sorokiniana* was grown in PDA for 7 days and conidial suspension of *B. sorokiniana* was prepared following method of Mian (1995). Conidia were collected from 7 days old PDA culture by scraping with sterilized glass slides, suspended in sterilized water and passed through two-ply chess cloth to discard large mycelium fragments and mass of PDA. Spore suspension was adjusted to 5×10^5 spore/ml, where Tween 20 (polyoxyethylene sorbitan monolaurate) at 0.04% was to prohibit the spores from clumping.

Inoculation of wheat seedlings with *B. sorokiniana* and disease evaluation

Wheat plants were grown in pots as described above. When the plants were at 4 weeks, they were inoculated with *B. sorokiniana*. For inoculation, spore suspension at 5×10^5 spores/ml was sprayed over the wheat seedlings until run off. Inoculated seedlings were placed in a humid chamber for 48 hr and transferred to the net house. Seven days after inoculation, percent disease index (PDI) was estimated. Disease severity was recorded using a 0-5 scale, where 0 = No visible lesion on leaf, 1 = necrotic spots without chlorosis and up to 5% leaf area covered, 2 = necrotic spots with light chlorosis, 3 = necrotic spots with pronounced chlorosis and 21-40% leaf area covered, 4 = lesions enlarging and 41-60% leaf area covered and 5 = lesions merging and more than 60% leaf area blighted (Adlakha *et al.* 1984). PDI was calculated using following formula:

$$\text{Severity (PDI)} = \frac{\sum(\text{Symptoms index} \times \text{Number of plants with each symptom index})}{\text{Total number of plants}}$$

Root colonization by PGPR strains

After four weeks of plant growth, wheat seedlings were uprooted carefully from the pots and the root systems were thoroughly washed with running tap water to remove adhering soil particles and then surface sterilized with 1.0% chlorox for 1 min, rinsed in sterilized distilled water thrice, placed on 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 10^{-6} was prepared with sterilized distilled water. 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 28°C. The number of colony-forming units (cfu) in each plate was counted and data were expressed in number of cfu per gram of root tissues.

Design of Experiments and Analysis of data

The experimental design was completely randomized, consisting of 6 replications for each treatment, where the experiment was repeated at least twice. The data on disease and growth promotion in the control and treated plants were subjected to independent paired Student's t-test ($p < .05$). All analyses were performed using XLSTAT Pro statistical analysis software (Addinsoft, New York, USA).

Results and Discussion

Effect of PGPR on growth of wheat plant

Shoot and root length

The shoot length in untreated control plants of four wheat varieties ranged from 18.53 to 25.73 cm, while that in PGPR treated plants varied between 27.17 and 30.50 cm (Table 1). The enhancement in shoot length by PGPR treatment was significant in all wheat varieties. The percent increase in shoot length by PGPR over control was 29.95, 34.18, 25.26 and 32.35% in cv. Kanchan, Shatabdi, BARI Gom-27 and BARI Gom-29, respectively. Similarly, treatment with the PGPR isolate gave significant increase in root length of wheat plants compared to control in all varieties. The highest increase in root length by PGPR treatment was observed in cv. Shatabdi followed by BARI Gom-29, Kanchan and BARI Gom-27. The percent increase in root length by PGPR over control was 41.57, 42.86, 34.68 and 32.47% in cv. Kanchan, Shatabdi, BARI Gom-27 and BARI Gom-29, respectively.

Fresh weight of shoot and root

Seed treatment with the PGPR resulted significantly increase in fresh shoot and root weight of four wheat varieties compared to control. The fresh shoot weight in untreated control plants of four wheat varieties ranged from 578 to 679 mg, while the fresh root in untreated control plants of these wheat varieties varied between 612 and 778 mg (Table 2). The fresh shoot and root weight in PGPR treated plants ranged from 707 to 845 mg and 773 to 1190 mg, respectively. The highest fresh shoot and root weight were

Table 1. Effect of rhizobacterial inoculation on the shoot and root length (cm) of four wheat varieties

Treatment	Shoot length (cm) ^a				Root length (cm) ^a			
	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29
Control	21.10±2.03	22.73±2.43	22.49±1.78	20.53±1.24	7.12±0.32	7.98±0.45	7.67±0.56	6.98±0.42
<i>B. amyloliquefaciens</i>	27.42±2.31 [*] (29.95%) ^b	30.50±2.65 [*] (34.18%)	28.17±2.57 [*] (25.26%)	27.17±1.97 [*] (32.35%)	10.08±1.10 [*] (41.57%)	11.40±1.08 [*] (42.86%)	10.33±0.98 [*] (34.68%)	10.57±1.52 [*] (32.47%)

^aThe plant parameter was measured 5 weeks after seeding in the pots. Values represent mean±SE ($n = 6$); one replication consists of 20 plants.

^{*}Indicates significant difference by t-test ($p < .05$).

^bValues within parenthesis indicate the % increase in plant parameter over control.

Table 2. Effect of rhizobacterial inoculation on the fresh shoot and root weight (g) of four wheat varieties

Treatment	Fresh shoot weight (mg) ^a				Fresh root weight (mg) ^a			
	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29
Control	578±20.12	609±16.12	608±23.12	679±12.23	604±15.26	712±21.56	690±17.15	778±26.24
<i>B. amyloliquefaciens</i>	707±24.12* (22.31) ^b	829±29.33* (36.12)	728±31.24* (19.73)	845±27.34* (24.47)	773±18.72* (27.98)	1067±34.21* (49.86)	959±31.85* (38.98)	1090±39.62* (40.10)

^aThe plant parameter was measured 5 weeks after seeding in the pots. Values represent mean±SE ($n = 6$); one replication consists of 20 plants.

*Indicates significant difference by t-test ($p < 0.05$).

^bValues within parenthesis indicate the % increase in plant parameter over control.

obtained in cv. BARI Gom-29 followed by Shatabdi and the lowest was in cv. Kanchan. The enhancement in fresh shoot and dry weight by PGPR treatment was significant in all wheat varieties. The PGPR-treated plants produced 22.31, 36.12, 19.73 and 24.47% higher fresh shoot weight than control treated plants in cv. Kanchan, Shatabdi, BARI Gom-27 and BARI Gom-29, respectively. On the other hand, the percent increase in fresh root by PGPR treatment over control was 27.98, 49.86, 38.98 and 40.10% in cv. Kanchan, Shatabdi, BARI Gom-27 and BARI Gom-29, respectively (Table 4).

Dry shoot and root weight

Wheat plants pretreated with the rhizobacterial strain had shown significantly higher dry shoot and root weight in all wheat varieties than those of untreated control plants (Table 3). The dry shoot weight in untreated control plants of four wheat varieties ranged from 432 to 594 mg, while the dry root in untreated control plants of these wheat varieties varied between 473 and 661 mg (Table 4). The dry shoot and root weight in PGPR treated plants ranged from 658 to 887 mg and 696 to 1056 mg, respectively. The highest fresh shoot and root weight were obtained in cv. BARI Gom-29 followed by Shatabdi and the lowest was in cv. Kanchan. The percent enhancement in dry shoot weight by PGPR treatment was 46.99, 70.08, 44.25 and 49.32% than control in cv. Kanchan, Shatabdi, BARI Gom-27 and BARI Gom-29, respectively. On the other hand, the percent increase in fresh root by PGPR treatment over control was 47.14%, 77.65%, 57.11 and 59.76% in cv. Kanchan, Shatabdi, BARI Gom-27 and BARI Gom-29, respectively (Table 3).

Table 3. Effect of rhizobacterial inoculation on the dry shoot and root weight (g) of four wheat varieties

Treatment	Dry shoot weight (mg) ^a				Dry root weight (mg) ^a			
	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29
Control	432±16.28	498±14.39	479±14.97	594±17.01	473±14.13	528±13.85	506±19.15	661±21.50
<i>B. amyloliquefaciens</i>	635±24.37* (46.99%) ^b	847±22.81* (70.08%)	691±16.31* (44.25%)	887±20.76* (49.32%)	696±18.94* (47.14%)	938±27.19* (77.65%)	795±19.24* (57.11%)	1056±31.48* (59.76%)

^aThe plant parameter was measured 5 weeks after seeding in the pots. Values represent mean±SE ($n = 6$); one replication consists of 20 plants.

*Indicates significant difference by t-test ($p < 0.05$).

^bValues within parenthesis indicate the % increase in plant parameter over control.

Effect of PGPR on percent disease index of *Bipolaris* leaf blight in wheat varieties

Disease severity of individual plant was rated using the 0-5 scale and computed as percent disease index (PDI). The highest PDI of *Bipolaris* leaf blight was observed in untreated control plants of all four wheat varieties. The PDI in these plants ranged from 3.00 to 3.94%, where the highest PDI was observed in cv. Kanchan and the lowest was recorded in BARI Gom-27 (Table 4). Treatment with *B. amyloliquefaciens* resulted in significantly lower PDI in all four wheat varieties compared to control. The PDI in bacterium-treated plants stretched between 1.78 and 2.66, where the highest and the lowest disease incidence were observed in cv. Kanchan and BARI Gom-27, respectively. The percent reduction in PDI by the bacterium over control was 32.48, 46.54, 27.66 and 20.80% in cv. Kanchan, Shatabdi, BARI Gom-27 and BARI Gom-29, respectively (Table 4). This shows that the highest reduction in PDI by the bacterial treatment was found in cv. Shatabdi which was followed by cv. Kanchan.

Root colonization by PGPRs

High level of root colonization was observed by the bacterium in all four varieties. However, the four wheat varieties showed variation in the bacterial population in their roots. The number of *B. amyloliquefaciens* in roots ranged from 14.0×10^7 to 39.13×10^7 to 200.20×10^7 cfu/g root tissues, where the maximum number was found in cv. Kanchan and the minimum in cv. Shatabdi (Fig. 1). Non-treated control plant root did not show any bacterial colonization in all four varieties.

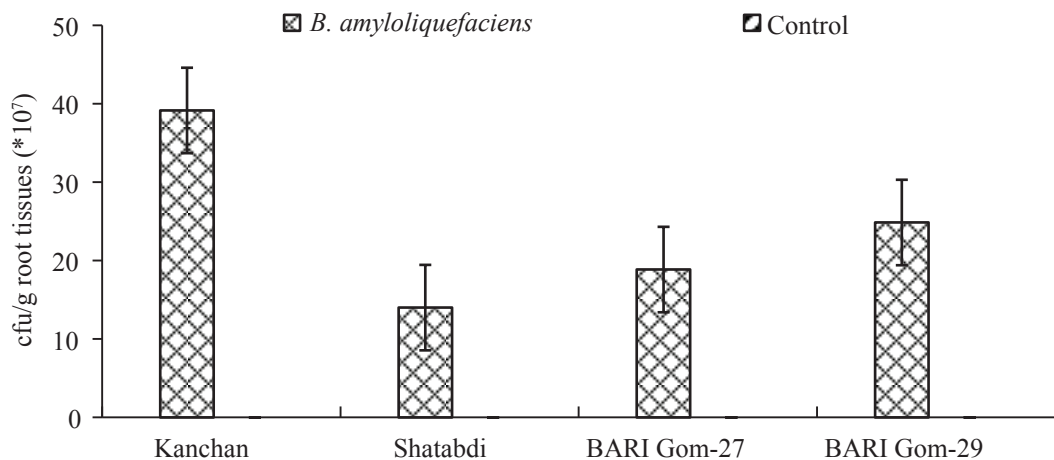
Table 4. Percent Disease Index (PDI) of Bipolaris leaf blight in three wheat varieties inoculated with two PGPR strains

Treatment	Percent Disease Index (PDI) ^a			
	Kanchan	Shatabdi	BARI Gom-27	BARI Gom-29
Control	3.94±0.34	3.33±1.20	3.00±0.19	3.22±0.11
<i>B. amyloliquefaciens</i>	2.66±0.19* (32.48) ^b	1.78±0.40* (46.54)	2.17±0.44* (27.66)	2.55±0.29* (20.80)

^aThe disease parameter was measured 5 weeks after seeding in the pots. Values represent mean±SE ($n = 6$); one replication consists of 20 plants.

*Indicates significant difference by t-test ($p < 0.05$).

^bValues within parenthesis indicate the % reduction in PDI over control.

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

The results of the present study indicate that application of PGPR strain *B. amyloliquefaciens* significantly enhanced root and shoot growth compared to untreated control. Similar plant growth was increased in wheat inoculated with PGPR strains (Rahman *et al.*, 2017). 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 root-shoot growth of plants (Saukat *et al.*,

2006a; Gholami *et al.*, 2009b; Mahmood *et al.*, 2010). Such improvement in plant growth might be attributed to N₂-fixing and phosphate solubilizing capacity of bacteria as well as the ability of these microorganisms to produce growth promoting substances (Awad *et al.*, 2011; Saharan and Nehra, 2011). 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 not clear yet, whether a single or a mixture of different mechanisms are operating in a single plant-microbe interaction.

Findings of this study also revealed that the rhizobacterium *B. amyloliquifaciens* used for seed priming significantly suppressed the incidence and severity of *Bipolaris* leaf blight of wheat. When wheat plants were grown from seed treated with PGPR, leaf blight disease caused by *B. sorokiniana* was suppressed at a distance above the treatment site. Protection of the plant was manifested by a reduction in disease severity in the leaves. PGPR suppressed *B. sorokiniana* infection via induced systemic resistance, since disease reduction took place without direct contact between the two microorganisms. It is believed that signals originating in the roots reached the upper part of plant and activated defense mechanisms (Hossain *et al.*, 2007). Similar results of systemic resistance in leaves of tall fescue against *B. sorokiniana* were reported with PGPR strains (Kilic-Ekici and Yuen, 2004). Soleimani *et al.*, (2005) also reported that seed coating and soil drenching with rhizobacteria not only reduced disease severity and incidence by *Bipolaris australiensis* and *B. sacchari*, but also showed positive influence on growth and yield of wheat cultivars. The main components of systemic-induced resistance are phenolic compounds, genetic and structural modifications, plant resistance activators, and activation of enzymatic weapons (Park *et al.*, 2007). In

this study, wheat varieties varied in their abilities to respond systemic resistance and growth promotion. This implies that genetic variation in host may affect the outcome of the interaction. This observation is in agreement with a finding that capacity to trigger induced resistance and growth promotion is genotype-dependent in plants (Ton *et al.*, 2001; Tucci *et al.*, 2011; Hossain and Sultana, 2015).

Conclusion

This study has shown that PGPR strain *B. amyloliquifaciens* is effective in stimulating root-shoot growth and inducing resistance against *B. sorokiniana* leaf blight in wheat. The ability to express PGPR-mediated disease suppression and growth promotion was found to be dependent on the plant genotypes. Understanding the induction of plant responses by PGPR will lead to develop new strategies for managing plant growth and diseases in wheat plants.

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