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Suppression of *Bipolaris* Leaf Blotch and Improvement of Wheat Growth by Plant Growth Promoting Rhizobacteria Bacilli

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Abstract: *Bipolaris* leaf blotch, induced by *Bipolaris sorokiniana*, poses a notable risk to wheat cultivation, resulting in considerable annual declines in yield. Plant Growth-Promoting Rhizobacteria (PGPR) are acknowledged for their colonization of the wheat rhizosphere and provision of various advantages to plants, including disease control and improved growth. This investigation seeks to explore the feasibility of utilizing PGPR strains as a viable substitute for harmful chemical inputs to combat *Bipolaris* leaf blotch and foster the growth of three wheat varieties while pinpointing the optimal PGPR-wheat variety combination for future field application. Laboratory and pot experiments were conducted utilizing a Completely Randomized Design (CRD) with three replications for each treatment. Data Analysis Involved Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) to ascertain the statistical significance ($p = 0.05$) of treatment effects. Following an initial screening of seven PGPR isolates for their *in vitro* antifungal activities against *B. sorokiniana*, two promising antagonists, *Bacillus subtilis* PPB9 and *Bacillus amyloliquefaciens* PPB12, were selected for further evaluation due to their multiple plant-growth-promoting and biocontrol characteristics. Subsequent pot experiments in a growth room revealed that seed treatment followed by foliar spraying with these strains effectively suppressed *Bipolaris* leaf blotch infection across all three wheat varieties-Kanchan, Shatabdi and BARI gom-27, compared to the untreated control. Additionally, PGPR treatment led to significantly ($p = 0.05$) higher levels of root and shoot growth compared to untreated plants. The efficacy of the two PGPR strains varied in disease suppression and growth promotion, with *B. amyloliquefaciens* PPB12 outperforming *B. subtilis* PPB9. Similarly, among the three wheat varieties, Shatabdi exhibited a more prominent response to PGPR treatment. These findings emphasize the importance of carefully selecting rhizobacterial strains to effectively manage *Bipolaris* leaf blotch and enhance wheat growth. The study accentuates the significant potential of *Bacillus* strains and their compatibility with a preferred wheat variety, offering a novel and sustainable alternative for *Bipolaris* leaf blotch management and wheat cultivation improvement.

Keywords: *Bacillus, Bipolaris sorokiniana*, Disease Incidence, Disease Severity, Colonization

Introduction

Wheat (*Triticum aestivum*), originally originating from the Levant region, is now a global staple crop with vast cultivation. Wheat plays a pivotal role in providing nourishment to nearly 35% of the world's population, supplying nearly 20% of the world's protein and calorie intake (Farooq, 2009). Today, wheat is the most cultivated commercial crop and remains the primary source of sustenance for humanity. As the global population continues to grow, projections suggest that by 2025, a 60% rise in wheat output will be essential to fulfill the food requirements of developing nations (FAO, 2020). This increased demand makes enhancing wheat

productivity a critical concern, particularly for countries with high poverty rates that heavily rely on wheat for food security (Lidwell-Durnin and Lapthorn, 2020).

In Bangladesh, wheat initially emerged as an alternative food crop but has now become a second staple, driven by shifts in consumer dietary preferences and a thriving baked goods market (Hossain *et al*., 2017a). Wheat production in Bangladesh stands at 1.084 million tons annually, with a per-acre yield of 7.32 metric tons (BBS, 2022). Nevertheless, the cultivation of wheat within the country has been insufficient to keep pace with rising demand. To fill the gap between domestic needs and production, Bangladesh has to import wheat from the global market. With approximately 5.5 million tons of wheat imports, Bangladesh ranks ninth in wheat imports in the world, making it one of the leading global wheat importers (FAO, 2020). Addressing this reliance on imports necessitates a considerable increase in wheat yield, which can be attained through improved crop management, especially in combating various diseases.

Diseases caused by pathogenic microorganisms are persistent and significant risks to wheat cultivation (Hossain *et al*., 2017b). Spot Bloch or blight disease, associated with *Bipolaris sorokiniana*, is a primary plant pathogen of wheat (Hossain *et al*., 2014). *B. sorokiniana* is known to infect a comprehensive spectrum of hosts including wheat, rye and barley (Hossain *et al*., 2014). This pathogen is accountable for various diseases, including seedling blight, common root rot, leaf spot blotch, leaf blight and black point of the grain. Spot blotch, a prevalent wheat ailment, is observed across all continents (Al-Sadi, 2021). Its symptoms manifest as brown lesions encircled by yellow halos, progressively expanding to cover larger areas of the leaves. Under humid conditions, these lesions turn olive-brown and exhibit fungal sporulation (Gupta *et al*., 2018). Managing *B. sorokiniana* is challenging due to its high variability in morphology, physiology and genetics, resulting in yield losses ranging from 10-21% (Malaker *et al*., 2004). Notably, regions with warmer climates experience particularly elevated losses, reaching as high as 16-43% (Ayana *et al*., 2018). South Asia, in particular, serves as a hotspot for *B. sorokiniana* diseases (Devi *et al*., 2018; Sultana *et al*., 2018).

While chemical interventions are effective against the disease, their continued use is constrained by various factors. Researchers are actively exploring alternative methods of controlling plant diseases, focusing on beneficial microbes. Currently, substantial research efforts are dedicated to identifying and exploiting beneficial microbes for stimulating plant growth and controlling diseases. One group of rhizospheric bacteria, known for their dual function in boosting growth and controlling diseases, are prevalently designated as Plant Growth-Promoting Rhizobacteria (PGPR) (Islam *et al*., 2016). *Azotobacter, Pseudomonas, Azospirillum*, *Bacillus, Stenotrophomonas, Enterobacter, Klebsiella, Variovorax and Serratia* are widely recognized as PGPR (Glick, 2012; Islam *et al*., 2016). They provide various benefits to plants, including nutrient acquisition, improved soil properties and secretion of secondary metabolites, hormones, antibiotics and signal compounds, all contributing to enhanced plant growth (Masum *et al*., 2018; Backer *et al*., 2018). PGPR has also been extensively studied as a biocontrol agents against numerous plant diseases (Islam and Hossain, 2012a; Islam *et al*., 2016). Particularly, *Bacillus* strains have shown effectiveness in controlling diverse pathogens and boosting plant growth (Islam *et al*., 2016). However, the usefulness of PGPR in combating *B. sorokiniana* in wheat has been inadequately investigated. The *B. subtilis* strain TE3 and *B. amyloliquefaciens* DB2 have exhibited highly propitious inhibition against *B. sorokiniana* in durum (Villa-Rodriguez *et al*., 2019) and common wheat (Luan *et al*., 2023). Although a few investigations have been done on the use of *Bacillus* strains in controlling spot blotch, there is scarce information on the variability of various PGPR strains in their capacity to confer disease-controlling and plant growth-promoting effects in different wheat cultivars. Consequently, the compatibility of PGPR strains with specific wheat cultivars and the optimization of their interaction to maximize their effectiveness remain largely unknown. Therefore, this study aims to address these gaps by investigating the potential of two PGPR strains in suppressing *Bipolaris* leaf blotch and promoting growth across three distinct wheat cultivars, while identifying the optimal combination of PGPR and wheat variety for future field application.

Materials and Methods

Planting Materials

Three popular wheat varieties Shatabdi, Kanchan and BARI gom-27 were selected. Kanchan is a semi-dwarf variety (90-100 cm) and it requires 106-112 days from seeding to ripening (Azad *et al*., 2017). Shatabdi (BARI gom-21) is also a semi-dwarf variety with a crop duration of 105 days. BARI gom-31 is high-yielding, early maturity and tolerant to heat stress. The variety Kanchan is susceptible, while Shatabdi and BARI gom-31 are known to be moderately tolerant to *Bipolaris* leaf blight under natural field conditions (Azad *et al*., 2017). Seeds of these varieties were obtained from the Bangladesh Agricultural Research Institute (BARI), located in Gazipur, Bangladesh.

Bacterial Isolates and Fungal Pathogen

Seven PGPR *B. amyloliquefaciens* PPB4, *B. amyloliquefaciens* PPB6, *B. subtilis* PPB8, *B. subtilis* PPB9, *B. amyloliquefaciens* PPB10, *B. subtilis* PPB11 and *B. amyloliquefaciens* PPB12 preserved in the Plant Pathology Laboratory of BSMRAU, Gazipur-1706,

Bangladesh were taken for this study. These strains had originally been isolated from the cucumber rhizosphere and preserved at -80°C in an 80% glycerol solution (Hossain and Sultana, 2018). Additionally, a purified culture of *Bipolaris sorokiniana* BS-1, the fungal pathogen, stored at 4°C in a PDA slant in the Plant Pathology Laboratory was used in the study.

In vitro Antagonistic Activity Against the Pathogen

To assess the *in vitro* antagonism of the bacterial strains against *B. sorokiniana* BS-1, a dual culture technique was employed. PDA plates were prepared in triplicate. A 5 mm diameter fungal plug was cut from the actively growing edge of the pathogen colony and placed at the midpoint of the plate. Separately, a fresh culture of the rhizobacteria was streaked at 5 cm distance from the plug. Control plates containing pathogen culture without bacteria were prepared. Subsequently, incubation of all plates was done at 28°C for a duration of 7 days and the diameter of the fungal colony was measured. The growth inhibition of the fungus by each bacterium was calculated using the following formula (Hossain, 2024):

$$\%\,Inhibition\ of\ growth = \frac{X - Y}{X} \times 100$$

where,
X = Fungal colony growth in dual culture plates
Y = Fungal colony growth in control plates

Antifungal Activity of Culture Filtrates of PGPR

The antifungal activity of bacterial culture filtrates against the *Bipolaris sorokiniana* isolate BS-1 was assessed. The bacteria were cultured in YP broth on a shaker (120 rpm at 28°C) for two days and culture filtrates were collected by centrifuging the broth at 15,000 rpm at 4°C and filtered (0.45 µm). Autoclaved PDA was amended with culture filtrate at concentrations of 10, 25, or 50% (v/v) before plating. Control plates without culture filtrate amendment were prepared. A 5 mm diameter fungal plug was cut from the actively growing edge of the pathogen colony and placed at the midpoint of the plate. Subsequently, incubation of all plates was done at 28°C for a duration of 7 days and the diameter of the fungal colony was measured. The growth inhibition of the fungus by each bacterium was calculated using the formula described above (Hossain, 2024).

Characterization of Selected Bacteria for Plant Growth-Promoting (PGP) Traits

The two selected PGPR strains underwent testing for the following PGP traits as they were deemed important (Sultana and Hossain, 2022).

Phosphate Solubilization

Pikovskaya Agar (PA) plates were prepared following the method of (Hossain and Sultana, 2018). PGPR isolates were grown in Nutrient Broth (NB) overnight and spot inoculated at the midpoint of PA plates. After incubation at 37°C for six days, visual observations were made for the presence of a clear zone around colonies.

Diazotrophic Nitrogen Fixation Ability

Each bacterium was grown in YP broth media under shaking conditions at 120 rpm for 24 h at 28°C. Then, the culture was centrifuged and diluted to 10^7 CFU mL^{-1}. A 2 µL aliquot of the bacterial suspension was spot-inoculated onto a nitrogen-free solid LG medium (Hossain and Sultana, 2018). Following incubation at 28°C for 10 days, bacterial growth in the nitrogen-free medium indicated a positive reaction.

Indole-3-Acetic Acid (IAA) Production

Each bacterium was cultured at 37°C for two days in NB supplemented with 0.1% tryptophan. The culture was centrifuged and supernatants were collected. Two drops of ortho-phosphoric acid were included in 2 mL of the supernatant. Salkowski reagent was prepared and added to the supernatant and ortho-phosphoric acid mixer (Hossain and Sultana, 2018). IAA production was assessed by measuring color strength at 530 nm.

Siderophore Production

Chrome Azurol S (CAS) agar plates were prepared and utilized (Hossain and Sultana, 2018). A 10 µL aliquot culture of the bacterium was inoculated onto the CAS agar plates and incubated at 37°C for 12 days. The production of siderophore by the bacteria was indicated by orange halos around the colonies.

(ACC) 1-Aminocyclopropane-1-Carboxylic Acid Deaminase Assay

The rhizobacteria were cultured overnight in NB as previously described. Subsequently, the DF-ACC medium, comprising of Dworkin and foster minimal salts medium and ACC was inoculated with bacteria. The DF-ACC medium without bacterial inoculation was used as a control. Both the inoculated and control cultures were then nurtured at 30°C on a rotary shaker at 160 rpm for 2 days. The cultures were then centrifuged to collect the supernatant and dilute it by adding DF medium (100:1 ratio). To assess ACC deaminase activity, 2 mL ninhydrin reagent was pipetted to the diluted supernatant. The mixture was then placed in a hot water bath and allowed to incubate for 30 min. During this time, the solution developed a purple color. Following the incubation in the hot water bath, the samples were left to cool at room temperature (30°C) for 10 min. Then, absorbance was taken at 570 nm. The

control medium, which lacked the bacterial culture, served as a blank in this analysis.

Characterization of Selected Bacteria for Biocontrol Traits

The production of acetoin, indole, ammonia, HCN, Hydrolytic enzymes and biofilm formation are important biocontrol characteristics (Niazi *et al*., 2014; Sultana and Hossain, 2022; Haque *et al*., 2023). Therefore, the PGPR strains were evaluated for the possession of these traits.

Production of Acetoin

Production of acetoin by rhizobacteria was qualitatively assessed. Yeast extract salt broth was prepared in 20 mL glass vials (5 mL/vial) triplicates for each bacterium and autoclaved. Then, 50 µL of 1-day culture of the bacterium was pipetted into the broth and incubated at 28±1°C for 4 days. A 1 mL aliquot of the culture was taken to a new test tube and added with 5% (w/v) alpha-naphthol in absolute alcohol (600 µL). Developing a crimson to ruby color at the top or all over the mixtures within 4 h implied an acetoin production.

Indole Production

The capability of the rhizobacteria to produce indole was qualitatively assessed. A broth containing 1.0% tryptone and 0.1 L-tryptophan was prepared in 20 mL glass vials (5 mL/vial) triplicates for each bacterium and autoclaved. Next, 10 µL of an overnight culture was inoculated into the broth and then it was placed in a shaker at 28±1°C, with agitation at 160 revolutions per minute (rpm). Following 4 days, 0.5 mL of Kovac's reagent was added to the culture and mixed. The appearance of a red hue on the upper part of the culture indicated a positive presence of indole production.

Ammonia Production

The ability of the rhizobacteria to produce ammonia was qualitatively assessed. Each rhizobacterial strain was cultured overnight in NB as described above. 5 mL of peptone water (Sigma-Aldrich, Germany) was taken in 20 mL glass vial triplicates for each bacterial strain. Then, 100 µL bacterial culture was added to the glass test tubes and incubated at 28±1°C. Following three days, Nessler's reagent (1 mL) (Sigma-Aldrich, Germany) was supplemented. The formation of a yellow-brown color implied positive results for ammonia production.

Hydrogen Cyanide (HCN) Production

Each rhizobacterial strain was streak inoculated onto Nutrient Agar (NA) plates containing 0.44 % (w/v) of glycine. Whatman filter papers (no. 1) soaked in a sterile filter containing a solution of sodium carbonate (2% w/v) and picric acid (0.5% v/v), were placed on the bottom side of Petri plate lids. Upon sealing with parafilm, the plates were subjected to an incubation period of 48 h at 28±2°C. Production of HCN by the bacteria was indicated by alterations in the color of the filter paper, transitioning from yellow to orange.

Production of Hydrolytic Enzymes

Chitinase Activity

The rhizobacteria were grown in NB overnight as discussed above. M9 medium supplemented with colloidal chitin (1% w/v) was prepared and spread onto 9 cm Petri dishes. Subsequently, 2 µL of the culture (with a concentration of 10^7 CFU/mL) were inoculated onto the plates, which were then placed in an incubator set at 37°C for 72 h. The formation of a translucent clear zone around bacterial colonies indicated chitinase production (Hossain and Sultana, 2018).

Production of Amylase

Each rhizobacterial strain was spot inoculated onto starch agar plates and incubated at 28±2°C in darkness for 96 h. The Petri dish surface was overflowed with a solution containing iodine (1%) and potassium iodide (2%). A clear zone is formed around the colony due to a positive amylase activity.

Protease Assay

Each rhizobacterial strain was spot inoculated onto casein agar petri plates for incubation at 28±2°C in darkness for 96 h. After washing with bromo cresol green dye, the appearance of a clear zone around the colony indicated a positive proteolytic activity.

Lipase Assay

A medium containing peptone with pH 6.10 was prepared. After autoclaving, filter-sterilized tween 20 was added to the medium. The medium was plated and inoculated with bacterial strain, followed by incubation at 28°C for 3 days. The formation of a clear zone indicated a positive lipase activity.

Catalase Test

The catalase test was done following the method of (Hossain and Sultana, 2015) with slight modifications. Each bacterial strain was inoculated on NA plates and incubated at 28±2°C for 2 days. The bacterial colony was transferred with a needle to a clean slide containing one drop of 3% H_2O_2. The formation of copious bubbles in the hydrogen peroxide indicated the presence of catalase.

Production of Biofilm by Rhizobacteria

The rhizobacteria were grown in NB as described in the above section and continued until OD_{660} reached 0.6-0.8. 5 mL of SOBG salt-optimized broth plus glycerol) were inoculated in a glass test tube with 50 µL of bacterial

cultures and incubated at 28°C under static conditions. After 3 days, the production of Air-Liquid (AL) and/or Solid-Air-Liquid (SAL) biofilm was examined and the photograph was taken. The biomass of biofilms was gently removed and washed two to three times with sterile distilled water. The optical density ($OD_{600\ nm}$) of both biomass biofilm and planktonic culture was recorded (UVS 1800, Shimazu, Japan) (Haque *et al*., 2023).

Effect of Bacteria on Germination of Conidia of Bipolaris sorokiniana

Bacteria were cultured in NB for 5 days (OD_{600} = 1.0). Culture filtrates were collected as described above. *Bipolaris sorokiniana* isolate BS-1 was incubated on PDA for 9 days. Spore suspension was prepared by washing the petri dishes twice with sterile water. Culture filtrate/bacterial culture (10^6 cells/mL) and *Bipolaris* spore suspension were added to a flask at a 1:1 volume ratio. A control culture was maintained without bacteria or culture filtrate. After incubation, for 24 h at 28°C, fungal spore germination, germ tube length and appressorial formation of at least 100 spores per replicate were examined microscopically and percent inhibition was calculated from the test data as follows:

$$\%Inhibition = \frac{A-B}{A} \times 100$$

where,

A = Numbers in the control slides
B = Numbers in the treated sample slides

Preparation of PGPR Inoculants

Bacterial strains were cultured and wheat seeds were treated following the techniques described by Islam *et al.* (2016). Fifteen-gram seeds (30/31 seeds) coated with bacteria were dried overnight at room temperature. To assess the bacterial load on seeds, 1 mL of sterilized water was added to 10 seeds from each sample and vortexed for 1 min, followed by 1 min of sonication (BactoSonic, Bandelin). The resulting suspension was then diluted to a factor of 10^{-5} and plated onto nutrient agar to count the number of Colony Forming Units (CFU/grain).

Preparation of Bipolaris Sorokiniana Spore Suspension for Pathogen Inoculation

B. sorokiniana was grown on PDA for 7 days. By adding 10 mL sterilized water to the colony, conidia along with mycelial mass, were scrapped with a sterilized glass slide. The suspension was sieved through a double-layer cheesecloth to discard mycelial mass and spore suspension with Tween 20 was prepared at 5×10^5 spores/mL.

Effect of PGPR Treatments on the Suppression of Bipolaris Leaf Blotch

A pot culture experiment was designed to assess the impact of two PGPR strains on the bipolar leaf blotch disease and the growth of three varieties of wheat: Kanchan, Shatabdi and BARI gom-27. Three sets of plants for each wheat variety were prepared; two for two bacterial strains and one for untreated control. Ten plastic pots, each measuring 14.5×8.5 cm, were prepared for each treatment within each wheat variety. The soil in these pots underwent two rounds of autoclaving, with each round lasting 20 min at 121°C and 15 psi and the total sterilization process spanned 24 h. Approximately 450 g of sterilized soil was then placed in each of the prepared plastic pots. Seeds were treated with the inocula of two bacteria strains as described above. For both bacteria-treated and untreated categories, 20 seeds were sown in each pot and grown in a growth room under a 16 h light and 8 h dark cycle at 20°C, with a humidity level of 75±5%. After germination, seedlings were thinned to allow only ten seedlings to continue growing in the culture room. When seedlings reached 4 weeks of age, seedlings were again treated with the two bacteria by spraying with bacterial suspension. Untreated control plants were sprayed with sterilized distilled water. Once the seedlings reached four weeks of age, they were subjected to a second round of bacterial treatment through the application of a bacterial suspension via spraying. The untreated control plants, on the other hand, were sprayed with sterilized distilled water. 2 days following the bacterial and water treatment, the seedlings were exposed to *B. sorokiniana* by spraying a spore suspension onto their leaves. Subsequently, the plants were placed in a humid chamber for approximately 48 h before being transferred to a growth room. After 7 days of pathogen inoculation, the severity of the disease in all leaves of the plants within each pot was assessed using a scale ranging from 0-5, with the following criteria: 0 indicating no visible lesions on the leaves, 1 representing necrotic spots without chlorosis, covering up to 5% of the leaf area, 2 denoting necrotic spots with mild chlorosis, 3 signifying necrotic spots with pronounced chlorosis and coverage of 21-40% of the leaf area, 4 indicating enlarging lesions with coverage of 41-60% of the leaf area and 5 representing merged lesions affecting more than 60% of the leaf area (Adlakha *et al*., 1984). Disease incidence and severity index were calculated using the following formula (Monma and Sakata, 1997):

$$Disease\ Incidence\ (\%) = \frac{Number\ of`\ infected\ plants}{Total\ number\ of\ plant\ assessed} \times 100$$

$$\text{Severity Index} = \frac{\text{Summation of all ratings}}{\text{Total number of rating} \times \text{Maximum disease grade}} \quad (5)$$

A mean disease incidence (%) and severity index for each replication were calculated based on the values derived from each pot. From these replication means, the mean disease incidence (%) and severity index for each treatment were then calculated. After estimating the disease severity and incidence, wheat seedlings were harvested and data on root and shoot growth parameters were recorded.

Colonization of Plants by PGPR Strains

The determination of root and shoot colonization by bacterial isolates followed the protocol outlined by Hossain *et al*. (2008b). At the end of the experiment, the roots were harvested from randomly selected plants, meticulously cleaned, washed and gently dried by blotting. After weighing, the roots were homogenized in SDW, serially diluted and plated on PDA. The number of CFU per gram of root was determined after 24 h of incubation at a temperature of 28±2°C. In the case of the above-ground shoots of PGPR-treated plants, a similar procedure was followed to assess colonization by bacteria, as described for the roots.

Design of Experiments and Analysis of Data

All experiments for this study were implemented either *in vitro* or pot-based settings and the experimental units were homogeneous. Therefore, the experiments were conducted following a Completely Randomized Design (CRD), taking three replications for each treatment. All experiments were repeated at least twice. Statistical analysis was performed using the Statistics 10 program package. The data underwent Analysis of Variance (ANOVA) using a single factor test and the means of the treatments were compared using a Duncan Multiple Range Test (DMRT) at a significance level of $p = 0.05$ to determine any significant differences.

Results

In vitro Antagonistic Activities of the Bacterial Isolates

In dual culture assays, all seven rhizobacterial isolates significantly inhibited the growth of *B. sorokiniana* BS-1. The mycelial growth of *B. sorokiniana* BS-1 in control plates measured 88.58 mm, while in bacteria-treated plates, it ranged from 13.86-50.65 mm, resulting in the inhibition of *B. sorokiniana* BS-1 growth, ranging from 58.22-84.35% (Table 1). Of these, isolate *B. subtilis* PPB9 (84.35%) recorded the maximum inhibition, followed by isolate *B. amyloliquefaciens* PPB12 with a mycelial growth of 15.21 mm (82.83%). The culture filtrates from all isolates demonstrated notable antagonism against the target pathogen, significantly suppressing the mycelial growth of *B. sorokiniana* across all three concentrations compared to the control (Table 1). The growth of *B. sorokiniana* in untreated control plates ranged from 87.34 to 89.23 mm. In culture filtrate-treated plates, growth was observed to be between 52.33 and 74.79 mm at 10% concentration, 34.27-67.44 mm at 25% concentration and 9.31-41.82 mm at 50% concentration (Table 1). At a concentration of 50%, the culture filtrates from strain PPB9 exhibited the highest inhibition rate (89.40%) of *B. sorokiniana* mycelial growth, a result statistically comparable to that of PPB12 (85.98%).

Biochemical Characterization of Bacillus subtilis PPB9 and Bacillus amyloliquefaciens PPB12

Both isolates of PGPR were rod-shaped bacteria, forming rapidly growing colonies with round to irregular shapes, raised elevations and smooth surfaces. Neither isolate exhibited pigmentation. They displayed motility and tested positive for gram staining, citrate utilization, catalase activity and oxidase activity, but tested negative for the KOH solubility test (Table 2). Both isolates were positive for phosphate solubilization, N_2 fixation, siderophore, 1-aminocyclopropane-1-carboxylate deaminase, amylase, acetoin, indole, ammonia, HCN, chitinases, protease and lipase tests. The selected elite PGPR strains also produced biofilm and indole acetic acid (Table 2).

Inhibition of Conidial Germination

The inhibitory effects of PPB9 and PPB12 on the germination of *B. sorokiniana* conidia were assessed using conidial suspension. Following 24 h of incubation, both the live inoculum and culture filtrate of each bacterium significantly reduced conidial germination compared to the control (Table 3). The germination rate of conidia in the control suspension (where conidia were dispersed only in water) was 96%, of which 83% produced appressoria. In contrast, germination of PGPR-treated conidia ranged from 11-28%, leading to 88.56-70.83% inhibition. The highest inhibition of conidial germination was recorded by *B. amyloliquefaciens* PPB12 culture filtrate, followed by *B. subtilis* PPB9 culture filtrate. Appressoria formation and germ tube length were also significantly inhibited by both rhizobacteria. In both cases, the highest inhibition (92.77-94.88%) was recorded by *B. amyloliquefaciens* PPB12 culture filtrate, followed (87.95 and 89.14%) by *B. subtilis* PPB9 culture filtrate.

Table 1: Inhibition of mycelial growth of *Bipolaris sorokiniana* BS-1 by bacterial cells and their culture filtrates

Antagonists	Inhibition of *B. sorokiniana* in dual culture (%)*	Inhibition of *B. sorokiniana* at different concentrations of culture filtrate of antagonists (%)		
		10%	25%	50%
Control	0.00±0.00e** (88.58)*	0.00±0.00e (89.23)	0.00±0.00e (87.34)	0.00±0.00e (87.81)
Bacillus amyloliquefaciens PPB4	67.69±3.78c (28.62)	29.24±1.51b (63.14)	52.05±1.32b (41.49)	70.39±4.04bc (26.00)
B. amyloliquefaciens PPB6	65.49±3.18c (30.57)	29.42±1.45b (62.98)	45.18±2.71c (47.88)	63.50±3.52c (32.05)
B. subtilis PPB8	66.74±3.74c (29.45)	33.91±1.12b (58.97)	53.94±1.35b (40.23)	73.90±3.19b (22.92)
B. subtilis PPB9	84.35±2.84a (13.86)	41.35±2.71a (52.33)	60.10±1.85a (34.85)	89.40±3.48a (9.31)
B. amyloliquefaciens PPB10	71.41±3.10b (25.33)	33.29±2.03b (59.53)	49.29±3.26bc (44.29)	68.19±3.77bc (27.93)
B. subtilis PPB11	42.82±1.65d (50.65)	16.18±1.23c (74.79)	22.79±2.02d (67.44)	52.37±3.21d (41.82)
B. amyloliquefaciens PPB12	82.83±4.70a (15.21)	41.70±2.13a (52.02)	60.76±2.37a (34.27)	85.98±3.85a (12.31)

*Values in parenthesis indicate the redial mycelial growth (mm) of *B. sorokiniana* BS-1.**Different letters denote significant differences among treatments for each column according to Fisher's LSD test (p<5%)

Table 2: Characterization of elite rhizobacterial antagonists for plant growth promoting and biocontrol traits of *Bacillus subtilis* PPB9 and *Bacillus amyloliquefaciens* PPB12

Biocontrol traits	*Bacillus subtilis* PPB9*	*Bacillus amyloliquefaciens* PPB12
Phosphate solubilization	++	+++
N_2 fixation	+++	++
Indole acetic acid	47.24±0.32	40.41±0.18
Siderophore	++	+++
1-aminocyclopropane-1-carboxylate deaminase	++	++
Amylase	++	++
Acetoin	+	+
Indole	+	+
Ammonia	+++	+++
HCN	+	++
Chitinases	+	++
Protease	+	+
Lipase	+	+
Catalase	+	+
Biofilm (OD_{600})	0.24±0.01	0.31±0.01

*+, positive; −, negative result for the test

Table 3: Spore germination, appressoria formation and germ tube length of *Bipolaris sorokiniana* BS-1 co-cultured with cells or sterile culture filtrate of *Bacillus subtilis* strain PPB9 and *Bacillus amyloliquefaciens* strain PPB12

Treatment	Conidial germination		Appressoria formation		Germ tube	
	Number	Inhibition (%)	Number	Inhibition (%)	Length (μM)	Inhibition (%)
Bacillus subtilis PPB9 culture	16±1.82d	83.33±3.42	10±1.41d	87.95±2.63	11.76±2.28d	89.14±4.27
B. subtilis PPB9 culture filtrate	28±2.31b	70.83±3.52	19±2.23b	77.11±2.17	36.29±0.99b	66.47±3.46
B. amyloliquefaciens PPB12 culture	11±0.92e	88.56±4.58	6±1.04e	92.77±3.30	5.54±0.86e	94.88±5.67
B. amyloliquefaciens PPB12 culture filtrate	21±1.15c	78.13±5.92	14±2.51c	83.13±3.14	29.27±2.26c	72.96±5.35
Control	96±7.62a	-	83±5.83a	-	108.24±6.11a	-

Suppression of Bipolaris Leaf Blotch

The potential of two PGPR strains on suppression of *Bipolaris* leaf blotch was investigated. The results revealed that regardless of varieties, the highest disease incidence was recorded in untreated control plants compared to rhizobacteria-inoculated plants (Table 4). Among the control plants in all three varieties, the maximum *Bipolaris* leaf blight incidence (80.00%) was recorded in var. Kanchan, which was followed by var. BARI gom-27 (66.67%) and Shatabdi (60.00%). In contrast, the lowest disease incidence among the treated plants was observed in *B. amyloliquefaciens* PPB12-treated plants compared to *B. subtilis* PPB9-treated plants in all three varieties. *B. amyloliquefaciens* PPB12 reduced *Bipolaris* leaf blight incidence by 67.50, 77.78 and 70.00% in var. Kanchan, Shatabdi and BARI gom-27, respectively. Similarly, *B. subtilis* PPB9-treated plants showed a reduction of 58.34%, 66.67-61.00% in disease incidence in var. Kanchan, Shatabdi and BARI gom-27, respectively. Likewise, control plants exhibited the highest disease severity compared to rhizobacteria-inoculated plants. Among the controls, the lowest severity (3.01) was recorded in var. Shatabdi and the highest was in var. Kanchan (3.97) (Table 4). In rhizobacteria-treated plants, treatments with *B. amyloliquefaciens* PPB12 resulted in the lowest disease severity than those with *B. subtilis* PPB9. As in var. Kanchan, the lowest severity index was recorded in plants treated with *B. amyloliquefaciens* PPB12 (2.50) which was followed by *Bacillus subtilis* PPB9 (2.96) inoculated plants. In var. Shatabdi, severity index was estimated to be 1.01 and 2.09 in plants treated with *B. amyloliquefaciens* PPB12 and *B. subtilis* PPB9, respectively. Similarly, in var. BARI gom-27, the lowest disease severity, was recorded with *B. amyloliquefaciens* PPB12 (2.18) treatment, followed by *Bacillus subtilis* PPB9 (2.84). The percent reduction in severity index in *B. amyloliquefaciens* PPB12 treated plants over that in control was recorded to be 37.03, 64.45-32.51% in var. Kanchan, Shatabdi and BARI gom-27, respectively, while the values were 25.44, 30.56 and 12.07%, respectively in plants treated with *B. subtilis* PPB9.

Effect of PGPR on Growth Attributes of Plants after Inoculation with B. sorokiniana

Significantly higher plant growth was observed in PGPR-pretreated plants compared to untreated plants in all three wheat varieties (Fig. 1). In var. Kanchan, Shatabdi and BARI gom-27, higher shoot length was obtained in plants pretreated with *B. amyloliquefaciens* PPB12 compared to those pretreated with *B. subtilis* PPB9 and inoculated with *B. sorokinana*. In untreated control plants of the Kanchan, Shatabdi and BARI gom-27 varieties, the shoot lengths were 16.32, 15.79 and 17.01 cm, respectively. When treated with *B. amyloliquefaciens* PPB12, these varieties had shoot lengths of 26.44, 28.51 and 27.87 cm and when treated with *B. subtilis* PPB9, the shoot lengths were 25.33, 26.11 and 25.88 cm, respectively. The increase in shoot length in three varieties by *B. amyloliquefaciens* PPB12 over control ranged from 60.80-80.55% and that by *B. subtilis* PPB9 ranged from 52.14-65.35%. The root lengths of untreated control plants for Kanchan, Shatabdi and BARI gom-27 were recorded as 6.42, 7.32 and 6.77 cm, respectively. When treated with *B. subtilis* PPB9, these varieties exhibited root lengths of 10.46, 12.25 and 10.93 cm and when treated with *B. amyloliquefaciens* PPB12, the root lengths were found as 11.15, 13.93 and 11.55 cm, respectively. *B. subtilis* PPB9 increased root length in these three varieties by 61.44-67.37%, while *B. amyloliquefaciens* PPB12 increased it from 70.60-90.30%. In both rhizobacterial treatments, var. Shatabdi showed higher enhancement in shoot and root length compared to var. Kanchan and BARI gom-27. The rhizobacterial pretreatment of wheat plants also stimulated significantly higher fresh and dry shoot weights per plant as compared to untreated plants (Fig. 1). Treatments with *B. amyloliquefaciens* PPB12 resulted in the highest fresh and dry shoot weights in all three varieties, measuring 0.28 g and 0.11 g for Kanchan, 0.37 g and 0.15 g for Shatabdi and 0.33 g and 0.13 g for BARI gom-27, respectively. The percent increase in fresh and dry shoot weight by *B. amyloliquefaciens* PPB12 over control ranged from 55.56-94.73% and 160.00-200.00%, respectively. Similarly, the percent increase in fresh and dry shoot weight in *B. subtilis* PPB9-treated plants was recorded to be 36.84-47.36% and 120-140%, respectively (Table 4). Wheat var. Shatabdi showed the highest increase in fresh and dry shoot weight which was followed by var. Kanchan and BARI gom-27. As for root weight, wheat plants pretreated with two rhizobacterial strains showed significantly higher root fresh and dry root weights than those of untreated infected plants (Fig. 1). The highest fresh and dry root weights were observed in plants treated with *B. amyloliquefaciens* PPB12 for all three varieties, recording 0.10-0.04 g in Kanchan, 0.12-0.05 g in Shatabdi and 0.11-0.04 g in BARI gom-27, respectively. When treated with *B. subtilis* PPB9, the fresh and dry root weights for all three varieties were appraised as 0.09-0.03 g in Kanchan, 0.11-0.04 g in Shatabdi and 0.09-0.03 g in BARI gom-27, respectively. The percentage increase in root fresh and dry weight by *B. amyloliquefaciens* PPB12 over the control was 66.66-100.0% for Kanchan, 71.42-150.0% for Shatabdi and 70.6-57.14% for BARI gom-27, respectively. Similarly, the increase in root fresh and dry weight in plants treated with *B. subtilis* PPB9 over the control was as follows for the three varieties: 50.0-50.0% for Kanchan, 57.14 and 100% for Shatabdi and 61.44 and 28.57% for BARI gom-27, respectively. In terms of varietal responses to PGPR treatments for root weight, the maximum increase was observed in var. Shatabdi, followed by var. Kanchan and var. BARI gom-27.

Table 4: Disease incidence and severity of *Bipolaris* leaf blotch in three wheat varieties inoculated with *Bacillus subtilis* PPB9 and *Bacillus amyloliquefaciens* PPB12

	% Disease incidence*			% Disease severity		
PGPRs	Kanchan	Shatabdi	BARI gom-27	Kanchan	Shatabdi	BARI gom-27
Bacillus subtilis PPB9	33.33±1.56^{b} (58.34)	20.00±1.56^{b} (66.67)	26.00±1.56^{b} (61.00)**	2.96±0.02^{b} (25.44)	2.09±0.02^{b} (30.56)	2.84±0.02^{b} (12.07)
Bacillus amyloli quefaciens PPB12	26.00±1.42^{c} (67.5)	13.33±1.42^{c} (77.78)	20.00±1.42^{c} (70.00)	2.50±0.01^{c} (37.03)	1.01±0.01^{c} (64.45)	2.18±0.01^{v} (32.51)
Control	80.00 ± 2.31^{a}	60.00 ± 2.31^{a}	66.67 ± 2.31^{a}	3.97 ± 0.01^{a}	3.01±0.01^{a}	3.23±0.01^{a}

* The disease was measured four weeks after seeding in the pots. Values are means ± SE (n = 3); one replication consists of ten plants. **Values within parenthesis indicate the % reduction in disease incidence over control (*B. sorokiniana*). The data presented were from representative experiments repeated at least twice with similar results

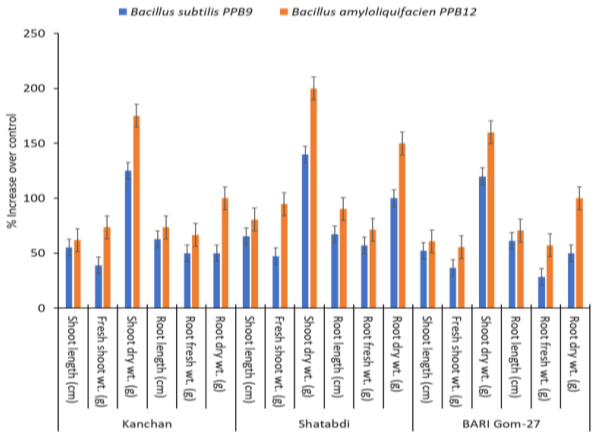

Fig. 1: Effect of treatment with *Bacillus subtilis* PPB9 and *B. amyloliquefaciens* PPB12 on the shoot and root growth of three wheat varieties Kanchan, Shatabdi and BARI gom-27. The percent increase in shoot and root growth in bacteria-treated plants was calculated over untreated control plants. Plant parameters were evaluated four weeks after seeding in the pots. The reported values represent means ± Standard Error (SE) with a sample size (n) of 3; each replication comprised ten plants

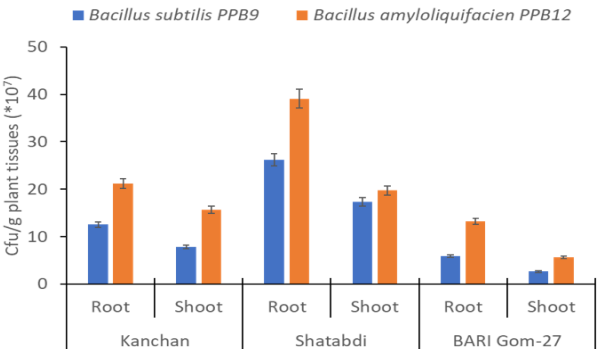

Fig. 2: Colonization of wheat var. Kanchan, Shatabdi and BARI gom-27 roots and shoots by *Bacillus subtilis* PPB9 and *Bacillus amyloliquefaciens* PPB12. Bar values represent colony forming unit (cfu) of PPB9 and PPB12 per gram root and shoot tissues and are means of three replicates, each from three randomly selected plants. Error bars are SE from three replicates that received the same treatment

Root and Shoot Colonization by PGPR

The ability of two rhizobacterial isolates was examined whether they colonized the roots and shoots of three varieties of wheat. Both isolates were isolated at higher frequencies from the roots and shoots of var. Kanchan, Shatabdi and BARI gom-27 (Fig. 2). The number of cfu of *B. amyloliquefaciens* PPB12 per gram root tissues in three varieties ranged from 13.2-39.1×10^{7}, while that of *B. subtilis* PPB9 varied between 5.9 and 26.2×10^{7}. On the other hand, the number of cfu of *B. amyloliquefaciens* PPB12 per gram shoot tissues in three varieties ranged from 5.8-19.5.1×10^{7}, while that of *B. subtilis* PPB9 varied between 2.6-17.3×10^{7}. The bacterium *B. amyloliquefaciens* PPB12 consistently maintained a higher population than *B. subtilis* PPB9 in both roots and shoots. Additionally, the highest number of cfu per gram of root and shoot tissues was observed in Shatabdi, while BARI gom-27 had the lowest number of cfu per gram of tissues. No bacteria were detected in the untreated roots and shoots of the three varieties.

Discussion

This study investigated the antagonistic activities of seven bacterial isolates, including *B. subtilis* PPB9 and *B. amyloliquefaciens* PPB12 strains, against *B. sorokiniana* BS-1 using dual culture assays. The bacterial isolates significantly inhibited the growth of *B. sorokiniana* BS-1, with inhibition ranging from 58.22-84.35%. Moreover, culture filtrates of all isolates effectively suppressed the mycelial growth of *B. sorokiniana* BS-1 at various concentrations. Among the isolates, *B. subtilis* PPB9 exhibited the highest inhibition (84.35%), followed by *B. amyloliquefaciens* PPB12 (82.83%). These results align with previous studies that have demonstrated the significant antagonistic activity of *B. subtilis* and *B. amyloliquefaciens* against plant pathogens including *B. sorokiniana* (Masum *et al*., 2018; Rahman *et al*., 2018; Villa-Rodriguez *et al*., 2019). In a study with antagonistic bacteria, the application of cell suspension or culture filtrate of *B. subtilis* TE3 showed broad-spectrum inhibition of *B. sorokiniana* (Villa-Rodriguez *et al*. 2019).

The cell-free filtrate obtained from *B. amyloliquefaciens* DB2 disrupted the integrity of the mycelial cell membrane, reduced mitochondrial transmembrane potential, triggered the accumulation of reactive oxygen species and induced nuclear damage, ultimately leading to cell death in *B. sorokiniana* (Luan *et al*., 2023). Similarly, *B. amyloliquefaciens* strain XZ34-1 remarkably inhibited the mycelial growth of *B. sorokinian* (Yi *et al*., 2021). These results show that the rhizobacteria *B. subtilis* PPB9 and *B. amyloliquefaciens* PPB12 used in this study acted as strong antagonists, directly inhibiting the mycelial growth of *B. sorokiniana.*

Both *Bacillus* isolates tested positive *in vitro* for the production of hydrolytic enzymes including chitinases, proteases, lipases and catalases. Additionally, they demonstrated the secretion of HCN, a volatile compound known for its high inhibitory effect on pathogens. This indicates that both *B. subtilis* PPB9 and *B. amyloliquefaciens* PPB12 possess multiple biocontrol traits, further enhancing their potential as effective biocontrol agents (El-Rahman *et al*., 2019; Yi *et al*., 2021). The biochemical characterization also revealed that *B. subtilis* PPB9 and *B. amyloliquefaciens* PPB12 exhibited several PGP traits, including IAA production, phosphate solubilization, siderophore production and nitrogen fixation. Previous research findings indicate that the *Bacillus* strains possessing these traits are efficient PGPR (Islam *et al*., 2016; Sultana *et al*., 2020; Sultana and Hossain, 2022; Hossain, 2024). Zakry *et al.* (2012) reported the presence of the *nifH* gene in *Bacillus*, enabling them to produce nitrogenase and fix atmospheric nitrogen. Similarly, phosphate solubilization and siderophore production, have been known as key PGP traits of PGPR, including *Bacillus* (Islam and Hossain, 2012a). Undeniably, the presence of multiple PGP traits in *B. subtilis* PPB9 and *B. amyloliquefaciens* PPB12 emphasizes their potential to be used as PGPR.

Both bacteria in this study acted as strong antagonists, directly inhibiting the germination and formation of appressoria in *Bipolaris* conidia. Several studies have shown that *B. subtilis* and *B. amyloliquefaciens* significantly inhibited the germination and appressoria formation of *Bipolaris* conidia. For example, *B. subtilis* and *B. amyloliquefaciens* significantly inhibited the conidial germination of *Bipolaris cactivora* (Bae *et al*., 2013). Similarly, the culture filtrate of *B. amyloliquefaciens* strain XZ34-1 notably inhibited the spore germination of *B. sorokiniana* (Yi *et al*., 2021). *Bacillus* species have been reported to induce structural abnormalities, disrupt cell membrane integrity, reduce mitochondrial transmembrane potential, trigger an accumulation of reactive oxygen species and cause nuclear damage, ultimately leading to cell death in *B. sorokiniana* (Chaurasia *et al*., 2005; Luan *et al*., 2023). These findings suggest that *Bacillus* has the capacity to produce bioactive substances with antifungal properties.

In the biocontrol experiment, significantly lower incidences and severities of leaf blotch disease were observed in plants treated with *B. subtilis* PPB9 and *B. amyloliquefaciens* PPB12 compared to non-treated control plants across all three wheat varieties. Notably, treatments with *B. amyloliquefaciens* PPB12 resulted in lower disease severity than those with *B. subtilis* PPB9. Furthermore, the percent reduction in disease incidence was highest in var. Kanchan compared to the other two varieties. Previous studies have demonstrated the effectiveness of antagonistic bacteria in reducing wheat spot blotch. For instance, the application of cell suspension or culture filtrate of *B. subtilis* TE3 has been shown to reduce wheat spot blotch under highly conducive conditions (Villa-Rodriguez *et al*., 2019). Similarly, in potted experiments, the culture filtrate of *B. amyloliquefaciens* XZ34-1 exhibited significant control over *B. sorokiniana* in wheat (Yi *et al*., 2021). Moreover, in both detached leaves and potted plants, *B. amyloliquefaciens* DB2 demonstrated remarkable suppression of *B. sorokiniana* in wheat (Luan *et al*., 2023). These findings collectively indicate that *B. subtilis* and *B. amyloliquefaciens* are well-documented PGPR strains capable of safeguarding wheat plants against spot blotch. Multiple evidence suggests that the inhibition of *B. sorokiniana* by *Bacillus* is closely related to its ability to secrete hydrolytic enzymes and HCN (El-Rahman *et al*., 2019; Yi *et al*., 2021). These antifungal compounds have the potential to degrade fungal cell walls, inhibit mycelial growth, impede conidia germination and disrupt the formation of appressoria in *Bipolaris* (Gong *et al*., 2015; Wang *et al*., 2020; Yi *et al*., 2021). Consequently, they serve to prevent successful infection. Additionally, both PGPR strains might induce Induced Systemic Resistance (ISR) against the pathogen. Signal-based ISR is considered an indirect mechanism of plant disease control by PGPR, creating a heightened state of defense effective against a wide range of pathogens and parasites (Chowdhury *et al*., 2015; Hossain *et al*. 2017b). While the elicitation of ISR by PGPR is a well-documented phenomenon, there are limited reports regarding ISR against *B. sorokiniana*, particularly in wheat. Kilic-Ekici and Yuen (2003) observed that treatments with *Lysobacter enzymogenes* strain C3 induced resistance against *B. sorokiniana* in tall fescue foliage and roots. Similarly, Kilic-Ekici and Yuen (2004) found that soil drenches with various PGPR strains resulted in systemic resistance in leaves against *B. sorokiniana* in tall fescue. Additionally, the foliar application of heat-killed C3 cells effectively controlled *B. sorokiniana* in wheat and reduced the severity of brown patches, caused by *Rhizoctonia solani*, in tall fescue (Kilic-Ekici and Yuen, 2003). These

findings suggest that the induction of resistance by PGPR may not solely depend on direct interactions with the pathogen but may also involve microbial elicitors produced by PGPR.

The results show significant enhancement in plant growth observed in three wheat varieties, namely Kanchan, shatabdi and BARI gom-27, pretreatment with *B. subtilis* PPB9 and *B. amyloliquefaciens* PPB12 compared to untreated controls. Specifically, plants treated with *B. amyloliquefaciens* PPB12 showed superior growth compared to those treated with *B. subtilis* PPB9. Among the varieties tested, var. Shatabdi exhibited the highest response to PGPR treatments, followed by var. Kanchan and var. BARI gom-27. Soleimani *et al*. (2005) demonstrated that seed coating and soil drenching with rhizobacteria not only reduced disease caused by *Bipolaris australiensis* and *B. sacchari* but also positively influenced the growth and yield of wheat cultivars. Thus, the plant growth-promoting abilities of *Bacillus* species serve as a complementary means for plant protection. According to current research findings, *B. subtilis* PPB9 and *B. amyloliquefaciens* PPB12 have multiple PGP traits. Application of nitrogen-fixing and phosphate-solubilizing bacteria make available nitrogen and phosphorus to plants, respectively, thus enhancing plant growth and yield of wheat (Islam and Hossain, 2012b; Zakry *et al*. 2012; Tahir *et al*., 2018). Furthermore, plants can utilize Fe-siderophore complexes to increase iron content in plant tissues, promoting overall plant growth (Khedher *et al*., 2021; Yi *et al*., 2021). *Nocardiopsis dassonvillei*, as a biocontrol agent, effectively controlled *B. sorokiniana* and enhanced wheat growth, owing to its capacity to produce siderophores, hydrogen cyanide and IAA (Allali *et al*., 2019). The potential contribution to plant growth promotion by *B. amyloliquefaciens* UCMB5113 is its ability to produce IAA, acetoin and siderophore (Niazi *et al*., 2014). While it's evident that multiple mechanisms contribute to growth promotion, the precise interplay between these mechanisms remains a subject of ongoing research.

The colonization study found that both isolates were present at higher frequencies in roots and shoots of all varieties, with *B. amyloliquefaciens* PPB12 consistently showing higher populations in the variety Shatabdi. These microorganisms, when colonizing host tissues, act as competent PGPR and biocontrol agents against plant diseases (Hossain and Sultana, 2020). Microbial colonization also leads to the formation of microcolonies or biofilms and facilitates cell-to-cell communication via a process known as "Quorum Sensing" (QS) (Velmourougane *et al*., 2017). This triggers various cellular events in the host plant and transmits signals systematically from the colonization zone to other parts of the plant, activating systemic defense mechanisms against plant pathogens (Hossain *et al*., 2008a).

Furthermore, the study unveiled variations in the efficacy of PGPR strains in disease suppression and plant growth promotion. *B. amyloliquefaciens* PPB12 demonstrated superior performance in both disease suppression and growth promotion, while the wheat variety Shatabdi displayed greater responsiveness to PGPR treatment. These observations align with previous studies, where specific Arabidopsis ecotype influenced the capacity of PGPR to express ISR and suppress disease (Ton *et al*., 2001a). Shivanna *et al*. (1994), on the other hand, revealed a variety-specific interaction of fungal isolates in their effect on wheat growth in the field. Harman (2006) reported that maize inbreeds treated with a *Trichoderma* strain showed three different types of growth responses: Strongly positive, little effect and negative. These findings suggest a preferential interaction between microbial strains and host plants at the cultivar level, in which a particular cultivar may either be favored or discouraged by a specific PGPR strain (Hossain and Sultana, 2015). The use of an optimum plant cultivar-PGPR combination may help maximize the efficacy of their interaction for plant growth promotion and disease suppression. Existing data support the heritability of the traits regulating plant differential interaction with microbes for expressing ISR (Ton *et al*., 2001b). Hence, incorporating these traits into commercial cultivars through breeding endeavors could be a viable strategy, potentially yielding substantial impacts on breeding outcomes for high-yielding disease-resistant varieties. Despite the obvious significance for agriculture, there are still few studies on how the plant response to microbial inoculants is influenced by plant genotypes in terms of growth promotion and disease suppression.

Conclusion

In conclusion, this study explores the potential of two PGPR *B. subtilis* PPB9 and *B. amyloliquefaciens* PPB12 as an eco-friendly alternative to chemical inputs for controlling Bipolaris leaf blotch and promoting plant growth in three wheat varieties. *B. amyloliquefaciens* PPB12 showed superior efficacy in suppressing diseases and promoting growth compared to *B. subtilis* PPB9, while cultivar Shatabdi was more significantly responsive to PGPR treatment than the remaining two wheat cultivars. The research findings emphasize the importance of carefully choosing a specific PGPR strain adapted to the respective wheat cultivars for efficient disease management and sustainable crop improvement. Future research could benefit from harnessing the interactions between PGPR and plants for potential field application and developing disease-resistant, high-yielding crop varieties by incorporating preferential interaction traits through breeding.

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Author's Contributions

Farzana Zerin Eivy: Drafted research implementation and data collection.

Md. Tanbir Rubayet: Research planned, data collection, drafted and data analysis.

Umakanta Sarkar: Research planned, data interpreted and reviewed.

Md. Motaher Hossain: Conception, fund acquisition, research designed and drafted reviewed.

Ethics

These are original data that haven't been published previously. The corresponding author relates that no ethical concerns exist on the publication of this study.

Conflict of Interest

The authors have no conflict of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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