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Efficacy of *Bacillus subtilis* MBI 600 Against Sheath Blight Caused by *Rhizoctonia solani* and on Growth and Yield of Rice

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Abstract: Rice sheath blight disease (ShB), caused by *Rhizoctonia solani*, gives rise to significant grain yield losses. The present study evaluated the efficacy of Integral®, the commercial liquid formulation of *Bacillus subtilis* strain MBI 600, against rice ShB and for plant growth promotion. In greenhouse studies, four log concentrations of Integral (from 2.2×10^6 to 2.2×10^9 cfu/mL) were used as seed treatment (ST). After 25 d, seedlings were dipped (SD) into Integral prior to transplanting. At 30 d after transplanting (DAT), leaf sheaths were inoculated with immature sclerotia of the pathogen. At 45 DAT, a foliar spray (FS) with Integral was applied to some treatments. The fungicide control was 50% carbendazim at 1.0 g/L, and a nontreated control was also included. Overall, there were 10 treatments, each with five replications. ShB severity was rated at 52 DAT, and seedling height and number of tillers per plant were rated at 60 DAT. In 2009, two field trials evaluated Integral at 2.2×10^8 and 2.2×10^9 cfu/mL. Integral was applied as ST, and seedlings were produced in a nursery bed. After 32 d, seedlings were treated with Integral as SD and transplanted into 10 m^2 blocks. Foliar sprays were given at 45 and 60 DAT. There were seven treatments, each with eight replications arranged as a factorial randomized complete block design. At 20 DAT, the plots were broadcast inoculated with *R. solani* produced on rice grains. Seedling height before transplanting, ShB severity at 90 DAT, and grain yield at harvest were recorded. Integral at 2.2×10^9 cfu/mL provided significant increase of seedling heights over other treatments under greenhouse conditions. The Integral treatments of ST + SD + FS at 2.2×10^9 cfu/mL significantly suppressed ShB over other treatments. In field studies, Integral provided significant increase of seedling height in nursery, and number of tillers per plant, compared with the control. ShB severity was significantly suppressed with higher concentrations of Integral compared to lower concentrations. Grain yield were the highest at an Integral concentration of 2.2×10^9 cfu/mL. Overall, Integral significantly reduced ShB severity, enhanced seedling growth, number of tillers per plant and grain yield as ST + SD + FS at the concentration of 2.2×10^9 cfu/mL under the conditions evaluated.

Key words: rice; sheath blight; *Rhizoctonia solani*; plant growth-promoting rhizobacterium; *Bacillus subtilis*

Sheath blight (ShB) of rice is an economically important disease in all crop growing areas of the world. Significant grain yield losses were reported due to ShB when susceptible varieties were grown (Prasad and Eizenga, 2008). The disease is caused by a soilborne fungal pathogen, *Rhizoctonia solani* Kuhn. The pathogen survives as sclerotia and mycelia in plant debris and on weeds in rice growing areas (Kobayashi et al, 1997). In temperate regions, the primary source of inoculum is sclerotia produced in previous rice crops (Kozaka, 1961). Germplasm of high genetic resistance for ShB is not available, and the disease is currently managed through use of chemical fungicides (Pal et al, 2005). Fungicidal management of ShB often gives inconsistent results and is not economical. Indiscriminate use of fungicides and chemical fertilizers to increase rice yields has several concerns relating to environmental hazards, pathogen resistance, leaching losses, and destruction of beneficial microflora. Use of plant growth-promoting rhizobacteria (PGPR) as biocontrol agents is gaining popularity in managing rice diseases and in enhancing growth and grain yields (Mew and Rosales, 1992).

Soil bacteria in rice ecosystems have exhibited significant fungistasis on vegetative growth and sclerotia of *R. solani* (Luo et al, 2005). Application of PGPR to control ShB under field conditions was attempted earlier (Mew and Rosales, 1986; Devi et al, 1989; Kanjanamaneesathian et al, 1998). *Bacillus* spp. has been used in biocontrol of ShB. *Bacillus* inoculants

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tolerate desiccation, heat, oxidizing agents, and UV radiations compared to Gram negative bacteria (Jeyarajan and Nakkeeran, 2000). The *Bacillus* spp. causes reduction in pathogen inoculum at infection site due to antibiosis, competition for space and nutrients, inhibition of pathogen related enzymes or toxins, parasitism, or lysis of pathogen hyphae, and through induced systemic resistance (Bacilio-Jiminez et al, 2001; Wang et al, 2009). In addition, plant growth promotion by *Bacillus* spp. is also elicited through increased N uptake, phosphate solubilization, siderophore and phytohormone production. Strains of *B. subtilis* and *B. megaterium* have shown significant inhibition of *R. solani* (Luo et al, 2005). Enhanced plant growth and grain yields in rice with *Bacillus* spp. application have also been well documented (Rabindran and Vidhyasekaran, 1996; Raja et al, 2006; Al-Taweil et al, 2009; Wang et al, 2009).

Several PGPR formulations have been evaluated for management of rice ShB. Most *Bacillus* formulations that were tested included bacterial cell suspensions (Wang et al, 2009), water soluble granules, floating pellets (Kanjanamaneesathian et al, 2007), powder formulations, and empty fruit bunch powders (EFB) (Al-Taweil et al, 2009). The field efficacies of these formulations were not consistent due to varied reasons. The survival rates and application efficiencies of PGPR generally depend on variations in the microclimate of a crop. Furthermore, the field efficacy of a commercial product of PGPR depends on its shelf life, delivery at appropriate dose, type of formulation used, and available concentration of PGPR. The time of application of PGPR can also affect their efficacy in managing ShB (Ren et al, 2006). Since *R. solani* is a soilborne pathogen that will eventually spread to leaf sheath and blades, effective management of the ShB necessitates bacterial application to seeds (Mew and Rosales, 1986), roots (Al-Taweil et al, 2009), or foliage (Kanjanamaneesathian et al, 2007). Synergistic effects in ShB management can be attained by combined applications of PGPR to seeds, roots and foliage (Rabindran and Vidhyasekaran, 1996).

In previous studies, we screened 70 PGPR strains with known efficacies on other crops and pathogens. The majority of the strains showed significant responses against ShB. Specifically, the *B. subtilis* MBI 600 strain significantly suppressed mycelial growth, sclerotial germination, and reduced ShB symptoms caused by *R. solani* under laboratory assays. The strain was found to produce siderophores and enhance rice seed germination and seedling growth under both laboratory and greenhouse conditions. Furthermore, strain MBI 600 was compatible with commonly used fungicides in rice. In the present study, the liquid commercial formulation of the strain MBI 600, available as 'Integral®', was screened against rice ShB, and growth and yield of rice were evaluated. Integral is recommended against root diseases as an in-furrow treatment or incorporation into peat moss or growing media or as seed treatment in crops such as peanuts, soybeans, cotton and other legumes. The objectives of the present study were therefore to screen various concentrations of Integral for suppression of ShB and improve seedling growth under greenhouse conditions, and to test the efficacy of Integral in field trials against ShB and evaluate its effect on grain yield of rice.

MATERIALS AND METHODS

Source of pathogen and production of sclerotia of *R. solani*

A multinucleate and virulent isolate of *R. solani* belonging to anastomosis group AG-1 IA was obtained from the culture collection of Andhra Pradesh Rice Research Institute (APRRI), India. The isolate was originally isolated from ShB infected rice seedlings. The culture was maintained on potato dextrose agar (PDA) for further use. For production of sclerotia, *R. solani* was grown on PDA at (28 ± 1) ºC in the dark. The sclerotia were harvested at different time intervals and categorized according to their ages as follows: immature (< 5-day-old), mature (5–30-day-old), and aged (> 30-day-old). The selected sclerotia were stored at 4 ºC prior to use.

Source of rice variety

Seeds of rice variety Swarna, developed at APRRI, India, were obtained and used. Swarna is a potentially high-yielding, long duration rice variety (150 d) with bold and golden yellow colored grains, and is extremely susceptible to ShB. The seeds were stored at 4 ºC prior to use.

Source and production of *B. subtilis* MBI 600 in liquid formulation

For greenhouse and field studies, the liquid formulation of *B. subtilis* strain MBI 600 was produced by Becker Underwood Inc. at their fermentation facilities located in Ames, Iowa, USA. The formulated product of MBI 600 in liquid was labeled as Integral®. The product contained a minimum of 2.2×10^{10} spores/mL and was packaged in 500 mL bottles and shipped to APRRI, India, to carry out studies described here.

Efficacy of Integral on sheath blight and growth of rice seedlings under greenhouse conditions

The efficacy of Integral on ShB severity and seedling growth of rice was tested under greenhouse conditions (Vidhyasekaran and Muthamilan, 1999; Nandakumar et al, 2001; Kanjanamaneesathian et al, 2007) by adopting the following procedure. Four concentrations of Integral (2.2×10^6, 2.2×10^7, 2.2×10^8 and 2.2×10^9 cfu/mL) were selected for testing. The concentrations of Integral were introduced onto rice seeds as seed treatment (ST), ST + seedling root dip (SD), and foliar sprays (FS). For seed treatment, seeds of rice were surface sterilized with 2% sodium hypochlorite for 5 min, and rinsed with sterile distilled water two times. Surface sterilized seeds were soaked in four concentrations of Integral as described above for 24 h, separately. Seeds were later removed from the bacterial soaked solutions and air dried in a laminar flow hood for 30 min. Seeds were sown into 30-cm-diameter plastic pots containing field soil collected from paddy fields. The soil is typical deltaic alluvial with a pH of 7.2. There were 10 seeds per pot. Carbendazim (0.5 g/L) treated seeds served as a standard chemical control. Seeds soaked in water served as the blank control. There were six treatments, five replications per treatment, with one pot per replication. Replicated pots were arranged on a greenhouse bench in a randomized complete block design (RCBD). The pots were maintained at (26 ± 2) ºC, relative humidity (RH) of 90%, and photoperiod of 16 h (11 h of sunlight and 5 h of artificial illumination using general electrical cool white fluorescent tubes) for 25 d. Germination was observed 7 d post seeding. Seedling growth parameters such as root and shoot lengths were taken at 25 d. Later, 25-day-old seedlings treated with four concentrations of Integral were transplanted into 30-cm-diameter pots containing the same field soil described above, 2 seedlings per pot, after dipping with Integral at appropriate concentrations to boost inoculation. For dipping, roots of seedlings were soaked in Integral for 4 h. Seedlings soaked in water were used as the control.

Immature sclerotia of *R. solani* were used to inoculate the 30-day-old transplanted seedlings. Treated seedlings were artificially inoculated with one immature sclerotium per plant, near the base of leaf sheath above water level to obtain an optimum level of ShB disease to evaluate the efficacy of Integral against ShB. The inoculated portion of the plant was sealed with a cellophane tape and watered immediately. At 15-day post pathogen inoculation, Integral was applied again as a foliar spray onto transplanted seedlings with four concentrations and treated as a separate set of treatments. For foliar sprays, 25 mL of Integral at appropriate concentrations were sprayed on seedlings at 45 days after transplanting (DAT) using a back pack sprayer until run-off. The following treatments were included: 1) ST + SD with Integral at 2.2×10^6 cfu/mL; 2) ST + SD with Integral at 2.2×10^7 cfu/mL; 3) ST + SD with Integral at 2.2×10^8 cfu/mL; 4) ST + SD with Integral at 2.2×10^9 cfu/mL; 5) ST + SD + FS with Integral at 2.2×10^6 cfu/mL; 6) ST + SD + FS with Integral at 2.2×10^7 cfu/mL; 7) ST + SD + FS with Integral at 2.2×10^8 cfu/mL; 8) ST + SD + FS with Integral at 2.2×10^9 cfu/mL; 9) ST + SD + FS with carbendazim at 1.0 g/L; 10) Control.

Each treatment was replicated five times, and replicated pots were arranged on a greenhouse bench in a RCBD and maintained at (26±2) ºC, with an RH of 90%, and photoperiod of 16 h. Pots were fertilized with NPK (1.5-0.5-0.5 g/pot) in the form of urea (46% N), single super phosphate (16% P_2O_5) and muriate of potash (60% K_2O) at the time of pathogen inoculation. Other agronomic practices were followed according to guidelines of APRRI to maintain the seedlings. Seedling height and number of tillers per plant were taken at 60 DAT. ShB disease severity was assessed at 52 DAT according to relative lesion height (RLH) method (Sharma et al, 1990) with the following formula:

RLH (%) = Total lesion height / Total plant height × 100.

Efficacy of Integral on rice ShB, growth of seedlings, and yield under field conditions

Field studies were conducted at APRRI, Maruteru, A. P., India during rainy season (July to November) of 2009. APRRI is a leading center for rice research in India. It is located in the typical deltaic region of Andhra Pradesh at a latitude of 16.38 º N, longitude of 81.44 º E, and an altitude of 5 m above mean sea level. The soils are typical deltaic alluvials with a pH of 7.2. The experimental site is known for its occurrence of ShB disease due to continuous rice cultivation and is designated as an ShB sick field. There were two field trials, 1 km away from each other. Two identical field trials were conducted to minimize the risk of losing a trial in case of flooding due to rains or non-occurrence of disease. The trials were arranged in factorial RCBD. Integral was evaluated at two concentrations (2.2×10^8 and 2.2×10^9 cfu/mL), since these concentrations gave considerable good efficacy results under greenhouse conditions against ShB. Integral was used as ST at time of sowing in the nursery to produce seedlings for field transplanting.

Production of seedlings in nursery

One nursery bed was prepared to produce seedlings for two field trials as follows: The soil was ploughed, puddled with water, and leveled. The puddle mud was later allowed to settle down and the excess water was removed. The nursery area was divided into beds to accommodate various seed treatments. Each bed was 2.5 m in width and 4.0 m in length. NPK was applied at the rate of 5-5-5 g/m^2 to nursery area. Prior to sowing into nursery beds, rice seeds were treated with Integral at two concentrations (2.2×10^8 and 2.2×10^9 cfu/mL) separately. Carbendazim was used as the standard chemical control. Seeds soaked in water served as the control. Treated seeds were sown onto nursery beds by broadcasting at the rate of 50 kg/hm^2. There were four treatments in the nursery and one bed per treatment. The treatments were as follows: 1) ST with Integral at 2.2×10^8 cfu/mL; 2) ST with Integral at 2.2×10^9 cfu/mL; 3) ST with carbendazim at 1.0 g/L; and 4) untreated control. Another dose of 0.5 kg N was applied at 12 d after seeding in the nursery beds. Agronomic practices for rice nursery management developed by APRRI, India were followed. At 30 d after seeding, 20 seedlings from each treatment were collected, washed with water and air dried, after which shoot and root lengths were measured.

Field site preparation and maintenance of transplanted crop

The experimental area intended for transplanting was flooded with water and ploughed until all soil aggregates were broken up. The excess water was drained after 48 h and the site was partitioned manually into eight main blocks. Each main block was divided into seven sub-plots of 10 m^2 and each to accommodate various treatments. Each individual sub-plot was included with earth embankments to prevent water movement among the treatments. Seedlings were pulled from appropriate treatments in nursery beds at 30 d after seeding and were separately grouped into bundles for ease of transplanting. Prior to transplanting, seedling roots were dipped in Integral at concentrations of 2.2×10^8 and 2.2×10^9 cfu/mL, separately, for 6 h. Seedlings dipped in carbendazim at 1.0 g/L served as a standard chemical control, whereas seedlings dipped in water served as the untreated control. Seedlings were then transplanted into sub-plots at a spacing of 15 cm ×15 cm. The transplanted area remained in a submerged condition until harvest. To ensure uniform ShB incidence, *R. solani* multiplied on rice grains was broadcast applied into the field at 20 DAT. NPK was applied at a rate of 80-40-30 kg/hm^2 as follows: Phosphorus and potassium fertilizers were applied as a basal dressing prior to transplanting, whereas nitrogen was equally applied at the basal, active tillering, and panicle initiation stages. Again, two foliar sprays with Integral at 2.2×10^8 and 2.2×10^9 cfu/mL were applied at 45 and 60 DAT onto plants already treated with Integral as ST and SD treatments. Carbendazim (1.0 g/L) was sprayed again on carbendazim treated plants and water was sprayed on the control plants. The following treatments were included: 1) ST + SD with water (control); 2) ST + SD + FS with water (control); 3) ST + SD with Integral at 2.2×10^8 cfu/mL; 4) ST + SD + FS with Integral at 2.2×10^8 cfu/mL; 5) ST + SD with Integral at 2.2×10^9 cfu/mL; 6) ST + SD + FS with Integral at 2.2×10^9 cfu/mL; 7) ST + SD + FS with carbendazim at 1.0 g/L.

There were eight replications for each treatment.

Measurement of seedling growth

Ten seedlings from each replication of transplanted plots in appropriate treatments were carefully harvested at 60 DAT, and plant height and number of tillers per plant were taken. Plant heights were measured from the collar region to the main tip of each seedling. Number of tillers for each plant was counted from the unelongated basal internodes.

Assessment of disease

At 90 DAT, seedlings were rated for ShB severity from appropriatly treated plots. There were 10 seedlings per replication. Percentage of diseased tillers was calculated by comparing the number of diseased tillers to the total tillers in a plant. The height of the ShB lesion from plant base was measured and disease severity was calculated by the RLH method.

Assessment of yield

Seedlings from each treatment were manually harvested for grain yield. Total seedlings from individual replicated plots were collected at 120 DAT, bundled, and dried on site for 2 d. The dried plants were later moved to a threshing floor, and threshed manually for grain separation. Collected grains were stored, dried and weighed.

Statistical analysis

Analysis of variance (ANOVA) was performed using PROC GLM of SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA) to determine the differences in yield attributes such as germination rate, plant height, number of tillers per plant, and for rate of diseased tillers per plant, sheath blight severity and grain yields. Fisher’s unprotected least significant difference (LSD) test was used for comparison of means at $P = 0.05$.

RESULTS

Efficacy of Integral on sheath blight and growth of rice seedlings under greenhouse conditions

Seed treatment with Integral at 2.2×10^8 and 2.2×10^9 cfu/mL significantly increased seedling germination compared to the control at 7 d after seeding (Fig. 1). The highest rate of germination (95.6%) was obtained with a concentration of 2.2×10^9 cfu/mL. Seed treatment with carbendazim gave 90.8% seedling germination. The germination rate in the control was 88.0%. Root lengths of 25-day-old seedlings were significantly greater in seed treatment with Integral at 2.2×10^9 cfu/mL (12.2 cm) and 2.2×10^8 cfu/mL (9.0 cm) over the control (7.9 cm). Shoot length was the highest (40.7 cm) with Integral at 2.2×10^9 cfu/mL. The shoot length in the control was 33.8 cm (Fig. 2).

Plant height and number of tillers per plant at 60 DAT were significantly enhanced in all treatments with Integral compared to the control (Table 1). Plant height was the highest (73.2 cm) with Integral at 2.2×10^9 cfu/mL as ST + SD + FS. Integral gave higher plant height (70.8 cm) as ST + SD + FS at 2.2×10^8 cfu/mL over the untreated control. Plant height with carbendazim as ST + SD + FS was 58.9 cm and was not significant over the control (58.3 cm). Number of tillers per plant was the highest at a concentration of 2.2×10^9 cfu/mL as ST + SD + FS (11.9) and as ST + SD (11.6) with no significant differences between them. At 2.2×10^8 cfu/mL, the number of tillers per plant was 9.6 with Integral as ST + SD + FS, whereas 6.3 in the control. ShB lesions were significantly reduced with all concentrations of Integral (Table 1). ShB severity was the least at a concentration of 2.2×10^9 cfu/mL as ST + SD + FS (9.2%), and with carbendazim (7.8%). ShB severity was up to 24.1%

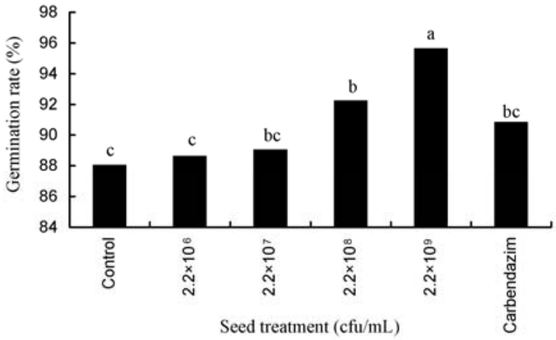

Fig. 1. Influence of various concentrations of *Bacillus subtilis* strain MBI 600 as seed treatment on seed germination of Swarna at 7 d after seeding under greenhouse conditions at Andhra Pradesh Rice Research Institute, India.

Integral applied as seed treatment at 2.2×10^6, 2.2×10^7, 2.2×10^8, and 2.2×10^9 cfu/mL prior to seeding. Values are means of five replications, 10 seeds per replication. Means followed by a common letter are not significantly different according to LSD (at $P \leq 0.05$). The same as following figures.

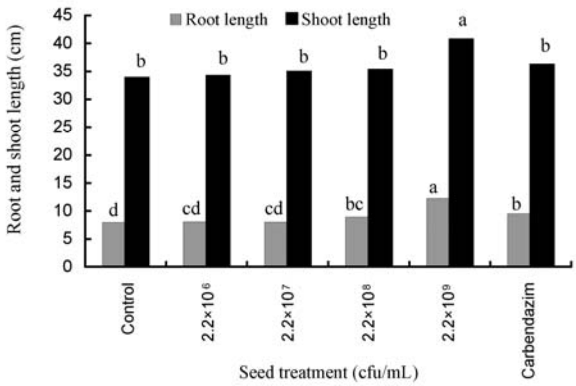

Fig. 2. Influence of various concentrations of *Bacillus subtilis* strain MBI 600 as seed treatment on seedling growth of Swarna at 25 d after sowing under greenhouse conditions during 2009 at Andhra Pradesh Rice Research Institute, India.

Values are means of five replications, 10 seeds per replication.

Table 1. Effect of various concentrations of Integral on growth of rice seedlings and suppression of sheath blight under greenhouse conditions.

Treatment [a]	ShB severity (%) [b]	Plant height (cm)	No. of Tillers per plant [c]
Control	65.5 a	58.3 d	6.3 d
ST + SD (2.2×10^6 cfu/mL)	24.1 b	62.7 c	8.0 c
ST + SD (2.2×10^7 cfu/mL)	20.8 cd	63.1 c	8.0 c
ST + SD (2.2×10^8 cfu/mL)	17.9 d	69.3 b	9.5 b
ST + SD (2.2×10^9 cfu/mL)	14.4 e	72.8 a	11.6 a
ST + SD + FS (2.2×10^6 cfu/mL)	21.5 bc	63.3 c	8.0 c
ST + SD + FS (2.2×10^7 cfu/mL)	18.4 d	63.7 c	8.1 c
ST + SD + FS (2.2×10^8 cfu/mL)	13.5 e	70.8 ab	9.6 b
ST + SD + FS (2.2×10^9 cfu/mL)	9.2 f	73.2 a	11.9 a
50% carbendazim (1.0 g/L)	7.8 f	58.9 d	7.3 cd

Values are means of five replications, two seedlings per replication. Means followed by a common letter in the columns are not significantly different according to LSD ($P \leq 0.05$).

[a] Strain *Bacillus subtilis* MBI 600 was applied as seed treatment (ST) before sowing, as seedling root dip (SD) on 25-day-old seedlings prior to transplanting, and foliar spray (FS) at 45 d after transplanting. The same as following tables; [b] Sheath blight severity was calculated according to relative lesion height method at 52 d after transplanting; [c] Number of tillers per plant was taken at 60 d after transplanting.

with 2.2×10^6 cfu/mL as ST + SD, whereas it was 65.6% in the control.

Efficacy of Integral on rice ShB, growth of seedlings, and yield under field conditions

Seed treatment with Integral significantly improved root and shoot lengths of 30-day-old seedlings compared to the control in nursery (Fig. 3). Root lengths were the highest at concentrations of 2.2×10^9 and 2.2×10^8 cfu/mL (14.0 and 9.3 cm, respectively) with no significant differences between them. Shoot lengths were the highest at 2.2×10^9 cfu/mL (44.9 cm) compared to 2.2×10^8 cfu/mL (37.0 cm). Carbendazim seed treatment significantly improved root length (9.6 cm) over the control (8.4 cm). The shoot length was about 36.0 cm in the control.

On a transplanted crop, application of various concentrations of Integral significantly reduced the rates of diseased tillers per plant and ShB severity compared to the control in the both field trials (Table 2). The mean rate of diseased tillers per plant was the least with carbendazim (29.0%), followed by Integral at a concentration of 2.2×10^9 cfu/mL as ST + SD + FS (31.9%). However, there were no significant differences between carbendazim and Integral at 2.2×10^9 cfu/mL as ST + SD + FS in Trial 1. The mean rate of diseased tillers per plant was 43.0% with a concentration of 2.2×10^8 cfu/mL. In the control, the mean rate of diseased tillers per plant was 97.1%. Mean ShB severity was the lowest in carbendazim treated plots (18.3%), followed by plots applied with Integral at a concentration of 2.2×10^9 cfu/mL as ST + SD + FS (22.9%). However, the efficacy of Integral as ST + SD + FS at 2.2×10^9 cfu/mL was not significantly different from that with carbendazim in Trial 2. The mean ShB severity was 27.0% of Integral as ST + SD + FS at 2.2×10^8 cfu/mL and 65.3% in the control.

Plant height and number of tillers per plant were significantly increased in treatments with both concentrations and methods of application of Integral compared to the control (Table 3). Mean plant heights were the highest at a concentration of 2.2×10^9 cfu/mL

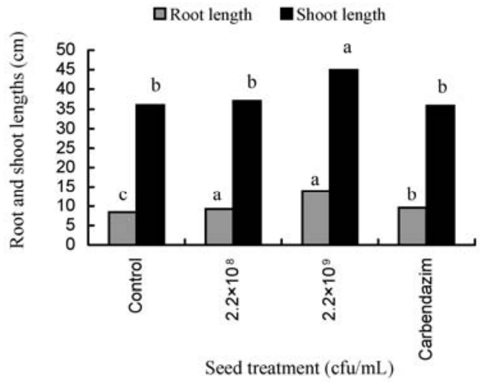

Fig. 3. Influence of various concentrations of *Bacillus subtilis* strain MBI 600 as seed treatment on seedling growth of Swarna at 30 d after sowing under field conditions during 2009 at Andhra Pradesh Rice Research Institute, India.

Values are the means of four replications, 20 seeds per replication.

of Integral as ST + SD + FS (96.9 cm). At the concentration of 2.2×10^8 cfu/mL, plant heights were 93.1 cm and 82.7 cm in the control. Similarly, number of tillers per plant was the highest with Integral at 2.2×10^9 cfu/mL (12.7) followed by Integral at 2.2×10^8 cfu/mL (11.6). The number of tillers per plant in the control was 10.3.

Grain yield was significantly enhanced with different concentrations and methods of Integral application (Table 4). Grain yield was the highest with Integral at 2.2×10^9 cfu/mL as ST + SD + FS (6 065 kg/hm^2), followed by ST + SD of Integral at 2.2×10^9 cfu/mL (5 650 kg/hm^2). Integral at 2.2×10^8 cfu/mL as ST + SD + FS also produced significant yield up to 5 376 kg/hm^2. Mean grain yield in carbendazim treated plots was about 5 507 kg/hm^2. In the control, the grain yield was 4 129 kg/hm^2.

DISCUSSION

Our results showed that Integral, in liquid formulation, was highly effective in suppressing ShB and in promoting rice seedling growth under greenhouse conditions. In field conditions, Integral was also highly effective in reducing ShB severity, promoting

Table 2. Effect of various concentrations of Integral in suppression of rice sheath blight (ShB) under field conditions during 2009 at Andhra Pradesh Rice Research Institute, India.

Treatment	Rate of diseased tillers per plant (%) [a]			ShB severity		
	Trial 1	Trial 2	Mean	Trial 1	Trial 2	Mean
ST + SD (0 cfu/mL)	95.2 a	92.1 b	93.7 b	65.8 b	56.2 b	61.0 b
ST + SD + FS (0 cfu/mL)	94.7 a	99.4 a	97.1 a	69.7 a	60.9 a	65.3 a
ST +SD (2.2×10^8 cfu/mL)	50.3 b	56.1 c	53.2 c	33.7 c	27.5 c	30.6 c
ST +SD+ FS (2.2×10^8 cfu/mL)	46.3 c	39.7 d	43.0 d	29.4 d	24.5 d	27.0 c
ST + SD (2.2×10^9 cfu/mL)	47.8 bc	37.9 d	42.9 d	31.2 cd	22.7 d	27.0 c
ST + SD + FS (2.2×10^9 cfu/mL)	38.6 d	25.1 e	31.9 e	26.5 e	19.2 e	22.9 d
Carbendazim (1.0 g/L)	37.2 d	20.8 f	29.0 e	19.8 f	16.8 e	18.3 e

Values are means of eight replications. [a] No. of diseased tillers per plant was taken at 90 d after transplanting.

Table 3. Effect of various concentrations of Integral on rice growth under field conditions during 2009 at Andhra Pradesh Rice Research Institute, India.

Treatment	Plant height (cm) [a]			No. of tillers per plant [b]		
	Trial 1	Trial 2	Mean	Trial 1	Trial 2	Mean
ST + SD (0 cfu/mL)	84.5 d	87.8 c	86.2 d	10.0 d	10.3 c	10.2 c
ST + SD + FS (0 cfu/mL)	78.5 e	86.9 c	82.7 e	10.5 cd	10.1 c	10.3 c
ST + SD (2.2×10^8 cfu/mL)	90.3 c	94.3 b	92.3 c	11.1 bc	11.6 b	11.4 b
ST + SD+ FS (2.2×10^8 cfu/mL)	91.6 bc	94.6 b	93.1 bc	11.4 b	11.8 b	11.6 b
ST + SD (2.2×10^9 cfu/mL)	94.3 ab	97.8 a	96.1 ab	12.5 a	12.9 a	12.7 a
ST + SD + FS (2.2×10^9 cfu/mL)	95.7 a	98.1 a	96.9 a	12.3 a	12.8 a	12.6 a
Carbendazim (1.0 g/L)	85.5 d	88.2 c	86.9 d	10.8 bcd	10.5 c	10.7 c

Values are means of eight replications. [a] Plant height was taken at 90 d after transplanting; [b] No. of tillers per plant was taken at 60 d after transplanting.

Table 4. Effect of various concentrations of Integral on grain yield of rice under field conditions during 2009 at Andhra Pradesh Rice Research Institute, India.

Treatment	Grain yield (kg/hm^2) [a]		
	Trial 1	Trial 2	Mean
ST + SD (0 cfu/mL)	4 199 d	3 925 e	4 062 d
ST + SD + FS (0 cfu/mL)	4 186 d	4 071 e	4 129 d
ST + SD (2.2×10^8 cfu/mL)	5 227 c	4 882 d	5 055 c
ST + SD + FS (2.2×10^8 cfu/mL)	5 625 b	5 127 c	5 376 b
ST + SD (2.2×10^9 cfu/mL)	5 806 b	5 494 b	5 650 b
ST + SD + FS (2.2×10^9 cfu/mL)	6 207 a	5 922 a	6 065 a
Carbendazim (1.0 g/L)	5 604 b	5 410 b	5 507 b

Values are means of eight replications. [a] Grain yield was taken at 90 d after transplanting.

plant height and increasing number of tillers per plant, and grain yield at a concentration of 2.2×10^9 cfu/mL when seed treatment applications were used in combination with seedling root dips and foliar spraying. These studies have shown that PGPR can be successfully employed in managing soil-borne diseases of crops. However, one of the major hurdles experienced with biocontrol agents is the lack of an appropriate delivery system. Biocontrol of rice ShB using other PGPR strains are successfully demonstrated previously under greenhouse and field conditions (Devi et al, 1989; Rabindran and Vidhyasekaran, 1996; Kanjanamanee-sathian et al, 2007; Wiwattanapatapee et al, 2007). Broadcast application of floating pellet formulation combined with spraying application of water-soluble formulations of *B. megaterium* was found to reduce rice ShB incidence under greenhouse and field conditions (Kanjanamaneesathian et al, 2007). Multiple delivery systems of PGPR strains aimed at protecting spermosphere, rhizosphere, and phyllosphere of crop plants from infection courts of pathogens was a promising means of disease management (Nakkeeran et al, 2005). Application of talc-based formulation or cell suspensions of PGPR to seeds, roots, soils and leaves reduced rice ShB incidence with the added benefit of promoting plant growth and grain yields (Nandakumar et al, 2001). Rabindran and Vidhyasekaran (1996) reported that ShB disease could be effectively suppressed through seed treatment, soil application, and foliar spraying with peat based formulation of PGPR.

Root colonization potential of PGPR also determines its field efficacy in controlling soil-borne diseases. A candidate biocontrol agent should be a potential root colonizer for successfully eliminating the pathogen in the rhizosphere. The exudates of rice roots have a significant effect on motility of PGPR towards roots (Bacilio-Jiminez, 2003). Further, *Bacillus* spp. has excellent root colonization potential. Management of rice ShB disease by Integral in the present investigation could be attributed to its application to seeds and roots, thereby facilitating effective root colonization and subsequent suppression of *R. solani* inoculum in the rhizosphere through competitive saprophytic ability.

Species of *Bacillus* are highly antagonistic to rice ShB pathogen (Luo et al, 2005). The fermented product of *Bacillus* strain Drt-11 reduced hyphal growth, colony diameter, and percentage of sclerotial germination (40%–60%) of *R. solani* (Chen and Kang, 2006). Antibiosis mediated inhibition of ShB pathogen by *B. subtilis* was reported earlier. The *B. subtilis* strain A30 produced a thermostable and proteinase stable antibiotic (P1) that was highly effective against ShB and blast pathogens of rice (He et al, 2002). Production of enzymes such as phenylalanine ammonia-lyase (PAL), peroxidase (PO), and pathogenesis-related (PR) proteins in rice leaves, and accumulation of thaumatin-like proteins, glucanases and chitinases were the mechanisms of *R. solani* inhibition by *B. subtilis* (Jayaraj et al, 2004). Foliar sprays with *B. megaterium* effectively reduced the rate of ShB affected tillers in rice (Kanjanamaneesathian et al, 2007). The efficacy of Integral to reduce ShB in the present study might be due to the production of siderophores, antibiotics and lytic enzymes and induction of defense related enzymes such as PO, PAL, chitinases, β-1-3 glucanases and phenols. Besides, direct antagonistic activity by the production of various bacterial metabolites and induction of systemic resistance by PGPR against diseases have been established as a new mechanism by which plants defend themselves against pathogen attack. Soil inoculum of *Pseudomonas fluorescens* induces disease resistance against foliar pathogens in several crops (Peer et al, 1991; Wei et al, 1991). Any plant has endogenous defense mechanisms that can be induced by insects and pathogens. It is well known that the defense genes are inducible genes and appropriate stimuli or signals are needed for activation. Activation of the plant's own defense mechanisms by prior application of a biological inducer is thought to be a novel plant protection strategy.

Growth promoting abilities of *B. subtilis* in crop plants are well established. Rhizosphere isolates of rice produce indole-3-acetic acid (IAA) and are capable of solubilizing soil organic phosphates. They also promote seed germination, root length, plant height and dry matter production of roots and shoots (Ashrafuzzaman et al, 2009). Inoculation of PGPR to rice fields resulted in enhanced root length (54%), root weight (74%), root volume (62%), root area (75%), shoot weight (23%), panicle emergence index (96%), and zinc mobilization efficiency (Tariq et al, 2007). *Bacillus* spp. have important plant growth promoting traits such as production of IAA, ammonia, hydrogen cyanide, siderophores, and solubilization of phosphorus besides antifungal activity (Ahmad et al, 2008). The culture suspension of *B. licheniformis* CHM-1 was drenched around the roots of rice promoted seedling growth (Wang et al, 2009). Enhanced grain yields in addition to ShB control were reported with PGPR application. Prophylactic sprays with PGPR at 7 d before pathogen inoculation resulted in enhanced grain yield besides reduction in ShB incidence (Singh and Sinha, 2005). Increase in seed germination rate, root and shoot lengths of rice seedlings in nursery, number of tillers per plant, and ultimately grain yield by Integral in the present study might be due to the production of plant growth promoters or indirect stimulation of nutrient uptake, and by producing siderophores or antibiotics to protect the plant from deleterious rhizosphere organisms. Production of siderophores like pseudobactin and pyoverdine, which chelate the available iron in the soil, results in the death of pathogen due to lack of iron for pathogen survival. Iron deficiency in plant pathogens can cause growth inhibition, decrease in nucleic acid synthesis, inhibition of mycelial growth, and sclerotial germinatin of *R. solani*. To conclude, the commercial formulation Integral was highly effective at a concentration of 2.2×10^9 cfu/mL under greenhouse and field conditions as ST + SD + FS in reducing rice ShB and in promoting growth and grain yield.

PGPR are beneficial microbes that colonize rice roots effectively and enhance plant growth through a wide variety of mechanisms. PGPR have the potential to replace chemical fertilizers and pesticides in agriculture (Ashrafuzzaman et al, 2009). However, effective control of rice ShB is feasible only when these biopesticides are used in conjunction with low rates of chemical fungicides (Van et al, 2001). Detailed studies on mechanism of action of commercial PGPR formulations and their population dynamics in soil under submerged crop conditions are essential to formulate effective ShB management strategies at field level.

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