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EVALUATION AND SELECTION OF ELITE PLANT GROWTH-PROMOTING RHIZOBACTERIA FOR SUPPRESSION OF SHEATH BLIGHT OF RICE CAUSED BY *RHIZOCTONIA SOLANI* IN A DETACHED LEAF BIO-ASSAY

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ABSTRACT: Sheath blight (ShB) of rice, caused by *Rhizoctonia solani*, is one of the most important rice diseases worldwide. The objective of this study was to screen selected plant growth-promoting rhizobacteria (PGPR) strains for suppression of ShB under controlled conditions. Sclerotia of *R. solani* were produced on PDA and immature sclerotia (< 5-day-old) were harvested. Leaves of 60-day-old rice plants grown under greenhouse conditions were used to screen PGPR strains by detached leaf assay. Leaf sections of 8 cm in length were cut and placed in Petri dishes, inoculated with immature sclerotia, and incubated in a growth chamber. Approximately 70 PGPR strains were screened. The disease was quantified by the Relative Lesion Height (RLH) method. Among 70 strains, 31 significantly suppressed the RLH of ShB lesions compared to the control. Among these, *Bacillus subtilis* strain MBI 600 resulted in greatest suppression of ShB disease severity under the conditions tested.
Key words : Rice, sheath blight, *Rhizoctonia solani*, Biocontrol, PGPR, *Bacillus subtilis*

INTRODUCTION

Sheath blight (ShB) of rice, caused by the *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk), is a major disease of rice, reducing both grain yield and quality. The pathogen is soil-borne and has a wide host range, often infecting legume crops grown in rotation with rice. Yield losses up to 50% have been reported with rice ShB. The disease is often severe in intense crop production systems especially when susceptible varieties are grown (Lee and Rush, 1983). In midsouth rice-producing areas of the USA, ShB is the most economically important disease (Groth and Lee, 2002; Lee and Rush, 1983; Marchetti, 1983). The pathogen survives in the form of sclerotia and mycelia in plant debris and on weeds in tropics (Kobayashi *et al*., 1997). Strong sources of genetic resistance to ShB are not available. In general, all the rice cultivars are susceptible to ShB; however, the degree of susceptibility varies (Singh *et al*., 2002). In the United States, host plant resistance among cultivable varieties currently ranges from susceptible to moderately susceptible levels (Groth and Bond, 2007). Presently, the disease is being managed through the application of systemic fungicides and antibiotics to seed (IRRI. 1980), soil (Chen and Chu, 1973), and foliage (Dev and Mary, 1986; Lee and Rush, 1983). Use of fungicides in ShB management produces several concerns relating to environmental pollution, pathogen resistance, and escalated costs.

Use of PGPR in ShB management of rice is gaining popularity as an alternative to the chemical fungicides. Although ShB pathogen is soil-borne in nature, disease initiation occurs at the base of seedlings near water line, and the disease subsequently spreads through foliage (Rabindran and Vidhyasekaran, 1996). Therefore, the use of PGPR with good colonization potential in the rhizosphere and or phyllosphere is needed for successful control of ShB disease spread under field conditions. Identification of superior PGPR strains with high antagonistic potential to ShB pathogen and lesion spread on foliage is a vital step for devising effective biological control strategies at field level.

None of the previously published assays has been used for screening of PGPR strains for suppression of ShB lesion disease under controlled conditions. Therefore, the objective of the present study was to screen selective PGPR strains with known activities on an optimized detached leaf assay for selection of best performing strains for control of ShB disease in rice seedlings. The information thus generated will be useful in selecting PGPR strains that potentially reduce spread of the ShB lesions on the rice plant.

MATERIALS AND METHODS

Source of pathogen and production of sclerotia of *Rhizoctonia solani*

A multinucleate and virulent isolate of *R. solani* anastomosis group AG-1 IA was obtained from the culture collection of Dr. D. E. Groth, Rice Research Station, LSU AgCenter, Crowley, Louisiana, USA. The isolate was originally isolated from ShB infected rice seedlings. The culture was maintained on potato dextrose agar (PDA) or on rye kernels for further use. For production of sclerotia, *R. solani* was grown on PDA at 28 ± 1^0C under dark. The sclerotia were harvested at different time intervals and categorized according to their age as follows: immature (<5-day-old), mature (5-30 day-old and aged (>30-days-old). The selected sclerotia were stored at 4^0C prior to use.

Source of PGPR strains

Approximately 70 PGPR strains were obtained from the Phytobacteriology Laboratory strain collection, Department of Entomology and Plant Pathology, Auburn University, AL, USA. The selected strains possessed one or several of the following characteristics: (i) *in vitro* antibiosis against various fungal pathogens, (ii) promotion of root growth on several crops, (iii) enhancement of root and shoot growth of various crops and vegetables, and (iv) capacity to produce plant growth regulators. Purified and identified strains were grown for 48 h at 25^0 C in 20 ml sterile tryptic soy broth (TSB) (Difco, Detroit, Michigan, USA) on a reciprocating shaker (80 rpm). Bacteria were pelletted by centrifugation for 20 min at 10,000 x g. Bacterial cells were then washed (twice) in 0.1 M phosphate buffer (PB) (pH 6.8), resuspended in TSB amended with 20% sterile glycerol, and frozen in vials at -80^0 C for long term storage. In each screening assay a new vial of PGPR was used.

Production of rice seedlings

Seeds of high-yielding, very early maturing long-grain rice, CV. Cocodrie, developed at Rice Research Station, LSU AgCenter, Crowley, Louisiana, USA, were used. Rice seedlings were produced in plastic pots containing field soil amended with Osmocote fertilizer under greenhouse conditions. Pots were initially filled with tap water and the soil was soaked completely for 72 h. Later the soil was agitated manually to break the aggregates, and excess water was drained. Rice seedlings were produced by sowing two seeds per pot and placed on a bench in the greenhouse. Seedlings were under submerged conditions from 4^{th} leaf stage. The pots were maintained at a temperature of 26 ± 2 C, and RH of 90 for 60 days.

Evaluation of select PGPR strains for suppression of ShB in a detached leaf assay

Seventy PGPR strains as described in Tables 1 through 7 were screened for their efficacy in the suppression of ShB symptoms in a detached leaf assay (Guleria *et al*., 2007). In each assay, there were 10 PGPR strains and a control treatment. Each treatment was replicated five times. For testing PGPR strains, strains were retrieved from -80^0 C freezer, thawed, and streaked onto TSA and checked for purity after incubation for 24 h at 30^0 C. Cell suspensions of PGPR were prepared by growing the strains for 48 h at 25^0 C in TSA, harvesting in sterile distilled water, and adjusting the final concentrations at 4×10^8 cfu ml^{-1}. Leaves from 60-day-old seedlings produced as above in the GH were cut and brought to the laboratory in an ice box and surface sterilized as described above. They were then cut into uniform sizes of 8 cm and placed in sterilized glass Petri dishes of 14 CM diameter containing moistened filter paper. There was one leaf piece per Petri dish per replicate of the PGPR strain. In each Petri dish, surface sterilized glass slides were placed on the edges of these leaf pieces to prevent rolling inwards. Each PGPR strain was sprayed onto the surface of leaf pieces in the Petri dish. One immature sclerotium of *R. solani* produced on PDA was placed at the center of the leaf piece. Leaves sprayed with sterile distilled water with inoculated sclerotium served as control treatment in each assay. The Petri dishes with leaves were later placed in plastic trays lined with moistened filter paper. The trays were incubated in a growth chamber at 25 ± 1^0 C and 16 h light. At 7 days after incubation, leaves were rated for ShB disease lesions. The lesion length around the sclerotium was measured and ShB severity was rated by the Relative Lesion Height (RLH) method (Sharma *et al*., 1990) with the following formula:

$$\% \text{ RLH} = 100 \times \text{Total height of lesions} / \text{Total leaf height}$$

Statistical analysis

The data were analyzed using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA) and the treatment means were differentiated by a least significant difference (LSD) at P=0.05 using PROC- GLM.

RESULTS

Evaluation of PGPR for suppression of ShB disease in a detached leaf assay

Of the 70 PGPR tested, only 31 PGPR strains have significantly reduced the ShB lesions when compared to the control (Tables 1 through 7). The disease severity in these significant PGPR strains ranged from 2.9% to 93.3%. Among the PGPR strains tested, maximum inhibition of lesion development was obtained with *B. subtilis* MBI 600 with 2.9% of disease severity (Fig. 1). The next best reduction of ShB was noticed with *B. subtilis subsp. subtilis* strains AP 209 and AP 52 (with 32.1% and 39.58% disease, respectively), and one strain of *B. amyloliquefaciens* AP 219 with 39.2% disease severity.

Table 1. Efficacy of PGPR strains on suppression of rice sheath blight in a detached leaf assay (Assay 1).

Strain	Identification	Sheath blight lesion spread1
AP3	Bacillus safensis	100a
AP7	Bacillus safensis	58.3bc
AP18	Bacillus pumilus	100a
AP40	Bacillus anthracis	87.5a
AP52	Bacillus subtilis subsp. subtilis	39.5d
AP136	Bacillus amyloliquefaciens	65.4b
AP188	Bacillus amyloliquefaciens	45.8cd
AP209	Bacillus subtilis subsp. subtilis	32.0d
AP217	Bacillus macauensis	91.6a
AP219	Bacillus amyloliquefaciens	39.1d
Control	--	100a

Means followed by a common letter within a column are not significantly different at p<0.05
1Sheath blight lesion spread was recorded at 5 days after incubation by Relative Lesion Height method.

Table 2. Efficacy of PGPR strains on suppression of rice sheath blight in a detached leaf assay (Assay 2).

Strain	Identification	Sheath blight lesion spread[1]
AP278	*Bacillus subtilis subsp. subtilis*	64.5^{cd}
AP279	*Bacillus subtilis subsp. Subtilis*	41.6^{e}
AP280	*Bacillus safensis*	100^{a}
AP281	*Bacillus safensis*	90^{a}
AP282	*Lysinibacillus boronitolerans*	87.5^{ab}
AP283	*Bacillus safensis*	100^{a}
AP294	*Paenibacillus peoriae*	70.8^{bc}
AP295	*Bacillus amyloliquefaciens*	48.7^{de}
MBI 600	*Bacillus subtilis*	2.9^{f}
AP302, 299	*B. amyloliquefaciens*	62.5^{cd}
Control	--	100^{a}

Means followed by a common letter within a column are not significantly different at $p \leq 0.05$
[1]Sheath blight lesion spread was recorded at 5 days after incubation by
Relative Lesion Height method.

Table 3. Efficacy of PGPR strains on suppression of rice sheath blight in a detached leaf assay (Assay 3).

Strain	Identification	Sheath blight lesion spread[1]
AP304	*Bacillus amyloliquefaciens*	37.5^{e}
AP305	*Bacillus amyloliquefaciens*	56.2^{cd}
ABU 29	*Bacillus simplex*	100^{a}
ABU 89B	*Bacillus simplex*	95.8^{a}
ABU 161	*Bacillus megaterium*	58.3^{c}
ABU 169	*Bacillus megaterium*	100^{a}
ABU 279	*Bacillus cereus*	100^{a}
ABU 288	*Bacillus megaterium*	93.3^{a}
ABU 334	*Bacillus simplex*	50^{d}
ABU 354	*Bacillus cereus*	78.3^{b}
Control	--	100^{a}

Means followed by a common letter within a column are not significantly different at $p \leq 0.05$
[1]Sheath blight lesion spread was recorded at 5 days after incubation by
Relative Lesion Height method.

Table 4. Efficacy of PGPR strains on suppression of rice sheath blight in a detached leaf assay (Assay 4).

Strain	Identification	Sheath blight lesion spread[1]
ABU 361	*Bacillus simplex*	91.6[b]
ABU 371	*Bacillus megaterium*	100[a]
ABU 402	*Bacillus weihenstephanensis*	93.3[b]
ABU 457	*Bacillus simplex*	100[a]
ABU 524	*Bacillus simplex*	100[a]
ABU 871	*Bacillus simplex*	93.3[b]
ABU 882	*Bacillus megaterium*	100[a]
ABU 890	*Bacillus simplex*	100[a]
ABU 891	*Bacillus simplex*	91.2[b]
ABU 1025	*Bacillus simplex*	100[a]
Control	--	100[a]

Means followed by a common letter within a column are not significantly different at $p \leq 0.05$
[1]Sheath blight lesion spread was recorded at 5 days after incubation by Relative Lesion Height method.

Table 5. Efficacy of PGPR strains on suppression of rice sheath blight in a detached leaf assay (Assay 5).

Strain	Identification	Sheath blight lesion spread[1]
ABU 1053	*Bacillus simplex*	87.5[abc]
ABU 1240	*Bacillus mycoides*	66.6[c]
ABU 1419A	*Bacillus simplex*	100[a]
ABU 1627	*Bacillus mycoides*	75[bc]
ABU 1645	*Bacillus simplex*	87.5[abc]
ABU 1687	*Bacillus simplex*	88.7[abc]
ABU 1930	*Bacillus simplex*	87.5[abc]
ABU 1966	*Paenibacillus taichungensis*	90.8[ab]
ABU 1970	*Bacillus simplex*	95.8[ab]
ABU 2002	*Bacillus simplex*	95.8[ab]
Control	--	100[a]

Means followed by a common letter within a column are not significantly different at $p \leq 0.05$,
[1]Sheath blight lesion spread was recorded at 5 days after incubation by Relative Lesion Height method.

Table 6. Efficacy of PGPR strains on suppression of rice sheath blight in a detached leaf assay (Assay 6).

Strain	Identification	Sheath blight lesion spread[1]
ABU 2017	*Bacillus simplex*	85.4[b]
ABU 2041A	*Bacillus simplex*	87.5[ab]
ABU 2099B	*Bacillus simplex*	90.4[ab]
ABU 2197	*Bacillus simplex*	97.0[a]
ABU 2213	*Bacillus simplex*	91.6[ab]
ABU 2252	*Bacillus megaterium*	95[ab]
ABU 2424	*Bacillus simplex*	95.8[ab]
ABU 2429B	*Bacillus megaterium*	87.9[ab]
ABU 2549	*Bacillus mycoides*	66.6[c]
ABU 2644	*Bacillus simplex*	90.8[ab]
Control	--	100[a]

Means followed by a common letter within a column are not significantly different at $p \leq 0.05$
[1]Sheath blight lesion spread was recorded at 5 days after incubation by Relative Lesion Height method.

Table 7. Efficacy of PGPR strains on suppression of rice sheath blight in a detached leaf assay (Assay 7).

Strain	Identification	Sheath blight lesion spread1
ABU 2772	Bacillus subtilis	45.8cd
ABU 3099	Bacillus simplex	100a
ABU 3118	Bacillus simplex	95.4a
ABU 3128	Bacillus simplex	50cd
ABU 3135	Bacillus weihenstephanensis	58.3bc
ABU 3296	Bacillus simplex	65.8b
ABU 3421A	Bacillus vallismortis	89.5a
ABU 3454	Bacillus weihenstephanensis	41.6d
ABU 3586	Bacillus mycoides	68.7b
ABU 3819	Bacillus aerophilus	100a
Control	--	100a

Means followed by a common letter within a column are not significantly different at p<0.05
Sheath blight lesion spread was recorded at 5 days after incubation by Relative Lesion Height method.

Challenged with MBI 600

Control

Fig. 1. Reduction of sheath blight lesions by *B. subtilis* MBI 600 in a detached leaf assay.

DISCUSSION

The detached leaf inoculation technique was earlier attempted for determining the morphological and pathological variability in rice isolates of *R. solani* and molecular analysis of their genetic variability (Guleria *et al*., 2007). The assay was found to be useful in determining the host specific toxin production by *R. solani* in rice (Vidhyasekaran *et al*., 1997). Use of detached leaves for assays is less time consuming compared to whole-plant assays under greenhouse conditions.

Evaluation of PGPR under laboratory conditions using detached leaf assay is the first step for identifying PGPR strains for disease management at the field level. Because the disease assay uses rice plants at late tillering stage, it requires a longer duration of time. However, the disease assay may complement the seedling-based quick screening in determining superior PGPR strains against ShB disease. Since, the pathogen is soil-borne, and subsequent spread of the disease is through infection of the foliage (Rabindran and Vidhyasekaran, 1996). Therefore, foliar application of PGPR is essential for management of ShB under field conditions. PGPR, when applied to rice leaves, produce substances, such as phenylalanine ammonia-lyase, peroxidases, chitinases, glucanases, thaumatin-like proteins, and PR proteins, which may inhibit the severity of ShB disease (Jayaraj *et al*., 2004). In our evaluation of PGPR, strains *Bacillus subtilis* MBI 600, *B. subtilis* subsp *subtilis* AP 209 and AP 52, and *B. amyloliquefaciens* AP 219 were highly effective in reducing ShB lesions on detached rice leaves.

Overall, strain MBI 600 significantly reduced ShB lesions on rice leaves and was the best strain compared to other strains tested. In our earlier studies, MBI 600 showed significant reduction in mycelial growth of *R. solani.* Also the sclerotial germination of *R. solani* was completely inhibited by MBI 600 under *in vitro* conditions, and the strain significantly improved seedling vigor. Strain MBI 600 was found to be the superior to the other tested strains and was selected for further studies. Further studies are needed on this strain to determine the growth promoting characteristics, its compatibility with commonly used fungicides, mode of action against *R. solani*, and suppression of ShB under greenhouse and field conditions.

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