

## Sheath blight disease of rice (*Oryza sativa* L.) - An overview

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(Received: July 14, 2009; Accepted: August 24, 2009)

Rice is the most widely cultivated food crop in the world. Global rice production was approximately 645 million t in 2007. Rice is being cultivated in 114 countries throughout the world, and more than 50 countries have a minimum annual production of 100,000 t. The majority of the rice (90%) is being produced in Asian countries with China and India being the major producers (IRRI, 2008). The other major rice producing countries are Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil, and Japan. In the United States, rice has been produced for 300 years and currently has an annual production of 230,808 (1000 cwt) t. Major rice producing states of the US include Arkansas, California, Louisiana, Mississippi, Missouri, and Texas.

Rice cultivation is often subjected to several biotic stresses of which diseases like blast, sheath blight, stem rot, and bacterial blight are the important ones. Sheath blight (ShB) in rice is an important soil-borne fungal disease (*Rhizoctonia solani* Kuhn) causing up to 25% of yield losses. The literature pertaining to the ShB disease on rice is reviewed here under separate headings.

### Etiology, distribution and spread

Sheath blight pathogen survives from one crop season to another through sclerotia and mycelia in plant debris and also through weed hosts in tropical environments (Kobayashi *et al.*, 1997). In temperate regions, the primary source of inoculum is sclerotia produced in previous rice crops (Kozaka, 1961). Both mycelia and sclerotia survive in infected plant debris. Mostly the survival is through sclerotia dropped in field during harvest, which will infect the crop during next season.

Changes in the magnitude and variability of temperature, precipitation and other climatic variables were found to have tremendous influence on plant diseases. Sheath blight and blast diseases in rice were found to be severe at elevated CO<sub>2</sub> concentrations (Jeger and Pautasso, 2008). Areas under progress curves of disease severity and those of percent diseased rice tillers were positively correlated to the relative initial inoculum density of ShB pathogen. Further, rice yields were linearly and negatively correlated with disease severity and percent tillers affected (Tan WanZhong *et al.*, 2007). Further, rice diseases like ShB and bacterial blight were found to be prevalent in kharif (rainy season) (Saha and Dutta, 2007). Gao YuLiang (1997) reported that vertical development of rice ShB is primarily dependent on the average daylight time within the first 5 days followed by the average RH and temperature. Sarkar *et al* (2003) reported that high temperatures and high humidity favor ShB lesion development both length wise and breadth wise in rice under laboratory conditions. Further, the lesion development was faster in sheaths inoculated with sclerotia than in already infected sheaths.

Sarkar and Gupta (2002) reported that ShB disease severity was positively correlated with sandiness of soil. Further, the disease incidence was highest in wet soils with 50-60% water holding capacity (WHC) and lowest in submerged soils with 100% WHC. Infection on plants was very high when oil cakes were applied immediately after sowing; whereas its infection was low when oil cakes were applied at 20 days after sowing. The extent of damage of rice seedlings due to ShB incidence is dependent on resistance levels among the rice

strains, average daily temperature, and frequency of rain. However, no significant relationship between incidence time and damage loss due to ShB was reported (Ding KeJian *et al.*, 1998). Pot culture studies on the susceptibility of rice seedlings to *R. solani* revealed that disease incidence and development was rampant on 20- to 30-days-old rice seedlings compared to seedlings of 30- to 40-days-old under artificially inoculated conditions (Deepti Sharma and Thrimurthy, 2006).

Isolation, pathogenicity and cross inoculation tests revealed that several plants were found to be hosts to *R. solani*. Besides, several weeds like *Cyperus rotundus*, *C. difformis*, *Cynodon dactylon*, *Echinochloa colonum*, *Setaria glauca* (*S. pumila*), *Panicum repens*, *Brachiaria*, *Commelina obliqua*, and *Amaranthus viridis* were identified as collateral hosts, and the pathogen perpetuates in these hosts in absence of rice plants (Acharya and Sengupta, 1998). Sivalingam *et al* (2006) studied the role of seed borne inoculum in rice ShB disease development and observed no correlation between degree of seed discoloration and isolation frequency of pathogen. Further, the biocontrol studies with *Trichoderma harzianum*, *T. viride*, *T. virens* (*Gliocladium virens*) and *Pseudomonas fluorescens* and also with carbendazim proved that *R. solani* was internally seed-borne in nature. However, despite its good survival in seed, the transmission by seeds to rice plants under field conditions was very poor.

#### Pathogenicity of *Rhizoctonia solani*

Rice ShB symptom production under artificial conditions depends on the method of inoculation. Of different inoculation techniques such as single grain insertion, single sclerotium insertion, and mycelial suspension injection; single sclerotium insertion was most effective with highest ShB symptoms (68.5 to 80.0%), lesion length (2.45 to 4.75 cm) and percent disease index (32.5-43.5) followed by single grain insertion technique (Chakraborty *et al.*, 2006). Maximum disease severity was observed when sheaths and leaves were inoculated with 7-day-old propagules of the pathogen (Deepti Sharma and Thrimurthy, 2004).

The amount of *R. solani* inoculum plays a major role in uniform ShB disease development. Inoculum at the rate of 0.2 mg when placed inside the leaf sheath with a few drops of sterile water, induced single, discrete and uniform-sized lesions irrespective of the inoculum type (mature, immature sclerotium, and mycelium). Use of immature

sclerotia is a simple, rapid, and highly reproducible disease production assay under greenhouse conditions (Amita Singh *et al.*, 2002). Further studies indicated that the pathogen when inoculated on inner surface of rice sheath, first colonized the surface before producing lobate, bulbous appressoria and infection cushions. The colonization of epidermal and mesophyll cells was both intra- and inter-cellular. The intra-cellular hyphae were thick and deformed whereas the surface hyphae from primary lesions penetrated the healthy tissue both by hyphal tips as well as branched lobate appressoria. Early infection on a healthy plant within 12 h is possible when mycelium of the pathogen was used instead of sclerotial bodies (Amita Singh *et al.*, 2003).

The ShB pathogen can infect the rice crop at any stage of growth from seedling to flowering by different inoculum sources. Among the different types of symptoms, ShB is the most prominent and common one. Other symptoms on rice include pre- and post-emergence seedling blight, banded leaf blight, panicle infection, and spotted seed (Acharya *et al.*, 1997). Three pathogens are found to cause ShB disease in rice. They are *R. solani* (*Thanatephorus cucumeris*), *R. oryzae-sativae* (*Ceratobasidium oryzae-sativae*), and *R. oryzae* (*Waitea circinata*). Combined inoculation with these pathogens resulted in highest disease severity. Further, ShB incidence was maximum when treated with *R. solani*, moderate with *R. oryzae-sativae*, and low with *R. oryzae*. *R. oryzae* was antagonistic to *R. solani* whereas *R. oryzae-sativae* did not show any antagonism towards *R. solani* (Akter *et al.*, 2003).

Host range studies indicated that crop plants such as *Cajanus cajan*, *Capsicum annuum*, *Curcuma longa*, *Dolichos biflorus*, *Lycopersicon esculentum*, *Panicum miliaceum*, *Paspalum scrobiculatum*, *Setaria italica*, *Sorghum vulgare*, and *Zea mays* were moderately susceptible to the pathogen. The other plants such as *Brachiaria mutica*, *Cynodon dactylon*, *Cyperus rotundus*, *Echinochloa colona*, *Eleusine corocana*, and *Phaseolus aureus* were susceptible to *R. solani*. The other plants *Dolichos lablab* var. *typicus* and *Vigna sinensis* fall under the most susceptible category (Meena and Muthusamy, 1998<sup>b</sup>).

The ShB pathogen produces several cell wall degrading enzymes (CWDEs) in improved Marcus medium under *in vitro* conditions. Immersion of rice sheaths in these enzymes resulted in breaking of callus, sheath cell, organelle, and also

in cell wall cracking and mitochondrial damage (Zhang Hong *et al.*, 2005). CWDEs include polygalacturonase (PG), cellulase (Cx), pectin methylgalacturonase (PMG), and polygalacturonic acid trans-eliminase (pectate lyase) (PMTE) in improved Marcus's medium of which the activity of PG, Cx, and PMG were significantly higher than PGTE and PMTE. These CWDEs play an important role in lesion formation and expansion (Chen XiJun *et al.*, 2006). The *R. solani* isolates that produce extra cellular cellulase, pectolytic and protease enzymes under *in vitro* conditions exhibited greater virulence over isolates devoid of enzyme production. All the isolates were obtained from areas which experienced full introduction of hybrid and high yielding rice varieties (Salam *et al.*, 2006).

Rice ShB pathogen also produces toxin that induce characteristic symptoms on rice leaves, wilting of seedlings, and inhibited rice radicle growth. A positive correlation was noted between crude toxin production and the virulence of the pathogen. The radicles and seedlings of resistant rice cultivars were more tolerant to the crude toxin compared to susceptible cultivars, indicating the scope of resistance screening through treatment of rice radicles with the crude toxin (Xu Jing You *et al.*, 2004). Studies on pathogenicity factors of *R. solani* indicated that melanin producing cultures (M+ type) are more virulent than non melanin producing cultures (M- type) (Kim Heung Tae *et al.*, 2001).

### ShB disease management

#### Host plant resistance

Presently, no strong genetic sources of resistance are reported against rice ShB disease. The rice ShB resistance among the cultivable varieties in the southern United States currently ranges only from very susceptible to moderately resistant. The yield losses were reported to be 8% in moderately resistant (cv. Jupiter) and up to 30% in very susceptible (cv. Trenasse) in rice fields with artificial inoculation (Groth, 2008). In a separate study during 2003 through 2005, following artificial inoculations with ShB pathogen, a significant increase in disease incidence and severity was observed in moderately susceptible and very susceptible cultivars. Further, a yield loss of 4% was noticed in moderately susceptible cv. Francis and 21% was found in very susceptible cv. Cocodrie. (Groth and Bond, 2007).

Several screening methods for determining ShB resistance are reported. Araujo *et al.* (2007) standardized inoculation methods in 38 somaclones

of rice cultivar Metica-1 and observed a positive and significant correlation between disease severity in greenhouse and field conditions. Greenhouse inoculation with 2g of pathogen multiplied on rice grain and hull medium placed on soil surface around the plant is a reliable method for germplasm screening against ShB resistance. Jia *et al.* (2007) developed an effective and standard micro-chamber screening method in quantifying resistance to rice ShB pathogen under greenhouse conditions wherein rice seedlings were inoculated at the three to four-leaf stage with potato dextrose agar plugs containing mycelium and then covered with a 2- or 3-liter transparent plastic bottle for maintaining high humidity after inoculation. Consistent results were obtained and the resistance levels matched both under greenhouse and field conditions.

Chitinase production in rice cultivars is an important factor contributing to disease resistance against ShB. Greenhouse studies revealed chitinase activities in rice plants at 24 h after inoculation of moderately resistant cultivars whereas in susceptible cultivars, the chitinase activity was detected after 36h. Western blot analysis revealed that chitinases were induced in plant system following *R. solani* infection and they were greater in moderately resistant rice cultivars with low sheath blight disease severity compared to susceptible cultivars (Shrestha *et al.*, 2008). In a different study, 41 homozygous rice lines that were transformed with chitinase and beta-1, 3-glucanase genes for their resistance to ShB and it was observed that 92% of them were either moderately resistant or moderately susceptible. A significant correlation was obtained between ShB resistance in resistant or susceptible transgenic lines with chitinase activity (Li AiHong *et al.*, 2003).

Induction of systemic resistance in rice plants against ShB is often in practice. Seed treatment with chemicals such as salicylic acid, acetylsalicylic acid, DL-gamma-amino-n-butyric acid, gamma-amino-butyric acid, amino-iso-butyric acid, indole-3-pyruvic acid, IAA, IBA, nicotinic acid, iso-nicotinic acid, DL-norvaline, propionic acid, benzoic acid, para-aminobenzoic acid, and zinc sulfate were proved effective in inducing systemic resistance to ShB. Among them, salicylic acid + gamma-amino-n-butyric acid treatment was the most effective in reducing lesion length over control (Dantre and Rathi, 2007).

Screening of rice germplasm is a continuous process to identify definite sources of

resistance against ShB. Out of 200 rice accessions representing 15 *Oryza* species that were screened for major rice diseases, seven accessions, IRGC 81940 and 81941 (belonging to *O. nivara*) and IRGC 103303, 105165, 105268, 105270, and 105272 belonging to *O. australiensis* were resistant/moderately resistant to ShB and sheath rot. The IRGC 105272 of *O. australiensis* was found to be resistant to ShB, sheath rot, and bacterial blight diseases. These accessions can serve as donors of multiple disease resistance in an irrigated agroecosystem for widening the resistance gene pool of *O. sativa* (Ritu Bala and Goel, 2007). Verma *et al.* (2002) reported a new rice cv. NDR2030 derived from the cross Ratna/Saket//IR36, which is a mid-early cultivar with high yield potential and long-slender, translucent grains. The cultivar is resistant to gall midge, moderately resistant to white-backed planthopper, gall midge biotype 2, bacterial leaf blight and ShB. In another study, a new rice variety Giri (IR36 X Bhasamani) was found resistant to ShB, bacterial blight and tolerant to submergence (Sinha *et al.*, 2004).

#### Agronomic practices

Incidence of ShB in rice fields is dependent on the method of planting and plant population density. Investigations at farmers' fields and experimental fields (Taizhou institute of agricultural science, China) revealed that square method of transplantation resulted in optimum high-yield density, higher leaf area index and dry matter production. This method of transplantation also contributed to increased ShB resistance and higher grain yields (Yang *et al.*, 2008). Sparse planting resulted in lower ShB occurrence and greater lodging resistance in rice. The other important effects of sparse planting included fewer number of stems/m<sup>2</sup>, more stems/hill, delay in date of maximum tillering stage, heading time, ripening time, greater number of pods per head and more pods on secondary rachis-branches (Sugiyama *et al.*, 2007). Planting of rice seedlings far from the bund resulted in reduced ShB incidence since bunds have weed hosts of *R. solani*. Both vertical and horizontal spread of the disease in the field increased from the source of infection and with the increase of plant age (Sarkar and Chowdhury, 2003). Submergence of the crop had a negative effect on disease progress and resulted in reduced ShB disease development (Das and Dath, 1997). Maximum survival of ShB pathogen was reported in 50% soil saturation whereas maximum survival of fungal bio-agents like *T. viride* and *T. harzianum* was reported at 100% soil saturation. Control of

ShB as well as increase in plant growth by these bioagents was effective under submerged conditions (Bhagawati and Roy, 2005).

Soil amendment with organic fertilizers has a definite role in managing rice diseases. Organic fertilization with both animal manures and composts resulted in enhanced growth and yield of rice. Besides, the incidence of rice diseases like ShB, blast and pests like brown plant hopper, stem borer and leaf folder was reduced remarkably (Luong and Heong, 2005). Of various soil organic amendments (*Azadirachta indica*, *Pongamia pinnata*, *Gliricidia maculata*, *Chromolaena odorata*, *Prosopis juliflora*, and *Terminalia bellirica*), *A. indica* at 150 kg/ha as oil cake was most effective in reducing the ShB incidence (66.35% reduction over control), followed by *G. maculata* (as leaves), *Pongamia pinnata* (as oilcake), and *P. juliflora* (as leaves). Yield levels were significantly enhanced with soil amendments, and the greatest increase in yield was obtained with *A. indica* oilseed cake (3200.60 kg/ha vs. 2200.72 kg/ha for the control) (Kumar *et al.*, 2006). Low population densities of the pathogen were observed in rice fields amended with mustard and groundnut oilcakes. Further, the population densities of fungal antagonists such as *Aspergillus* spp and *Penicillium* spp were increased in amended soil. Other beneficial effects include congenial conditions for multiplication of fungal bioagent, *T. harzianum* and thickening, swelling and lysis of pathogen hyphae due to bacterial activity (Sarkar *et al.*, 2002). Greenhouse studies indicated that the bioagent *T. harzianum* was highly effective when the soil is amended with neem cake. Further, the ShB disease incidence and severity was less in clay loam soils compared to sandy loams (Khan and Sinha, 2005b). Application of 50% organics (as decomposed rice straw) in combination with 50% inorganic fertilizers increased rice yields by 23% and also harbored higher microbial communities over control and for plots that received 100% inorganic fertilizers. Further, the ShB incidence was delayed and the beneficial *Trichoderma* spp was higher in plots that received 100% organic manures when compared to application of 100% inorganic fertilizers (Luu Hong Man *et al.*, 2001). Pot and field studies on the effects of organic soil amendments in rice revealed that the mean soil fungal and bacterial population increased by 2 weeks and 10 weeks after addition of soil amendments. However, the populations of both fungi and bacteria decreased at 14 weeks after addition of soil amendments. Even the population levels of *R. solani* showed a steady decline by 2 and 10 weeks after addition of soil

amendments but no significant reduction was observed at 14 weeks after addition (Surulirajan and Janki Kandhari, 2006).

Inorganic nutrient management is also a major factor determining rice ShB disease. Detailed investigations on comparative studies between plots under site-specific nutrient management (SSNM) and farmer's field practices (FFP) revealed that ShB and leaf folder are major N- dependent variables whereas ShB, grain discoloration, brown spot, and red stripe were major yield reducing factors (Hill *et al.*, 2005). Tang QiYuan *et al* (2007) reported that plant variety and nitrogen fertilizers are the major factors influencing ShB disease and concomitant yield losses in rice, both during wet and dry seasons. Varieties with taller stature, fewer tillers, and lower leaf N concentration, such as IR68284H, generally had lower ShB lesion height, ShB index, and consequently lower yield loss from the disease. Disease intensity and yield loss increased with increasing N rates, but the magnitude of yield loss varied among varieties.

Among different plant nutrients, silicon (Si) plays an important role in imparting resistance against blast, brown spot, and ShB diseases of rice. The Si mediated resistance is due to a mechanical barrier caused by its polymerization *in planta*, accumulation of phenolics and phytoalexins, and activation of some pathogenesis-related proteins. Further, the prevalence of these diseases is more severe in rice grown in Si depleted soils (Rodrigues and Datnoff, 2005). Field studies indicated that application of complete silicon fertilizer (mixture of silicon, nitrogen, phosphorus and potassium) and organic fertilizers increased early rice yields by 12 and 21%, late rice yields by 8 and 29% respectively. Besides, the incidence of rice diseases such as blast, ShB and stem borer were reduced significantly (Wang MeiQing, 2005).

#### Plant extracts

The use of botanicals in the management of rice ShB is gaining importance of late. Different plant extracts are being used all over the world and among them, neem formulations are very effective in controlling the ShB incidence as well as in increasing grain yields. Biswas (2007) reported that field application of neem formulations, 0.03% (300 ppm azadirachtin) and 0.15% EC (1500 ppm azadirachtin) @4.5 ml/L during afternoon hrs was very effective in reducing disease incidence as well as in increasing grain yields. Greenhouse studies on the efficacy of neem products revealed that neem

oil, its saturated fraction and its stabilized formulations were effective in containing the disease incidence as well as in reduction of percent infected tillers (Janki Kandhari and Devakumar, 2003).

Besides, certain plant extracts such as *Odiyana wodier*, *Lawsonia alba*, *Ocimum sanctum*, and *Pongamia glabra* were found to be effective both in reducing the mycelial growth (70 to 85% inhibition) and sporulation of *R. solani* under *in vitro* conditions. Further, field studies with *O. wodier* and *O. sanctum* were very effective (26 and 28% ShB severity) over control (42%) (Karthikeyan and Chandrasekaran, 2007). The plant extract of *Gaultheria* spp formulated as Biotos was found to be highly effective at 0.25% concentration and was superior both in controlling ShB severity (9.7%) and in increasing grain yields (9859 kg/ha). Further, the efficacy of Biotos was significantly superior over neem-based botanicals such as Achook and Tricure (Biswas, 2006). Other effective plant extracts include *Allium sativum*, *Prosopis juliflora*, *Gynandropsis pentaphylla*, *Leucos aspera*, and *Vitex negundo*. (Sundarraaj *et al.*,1996). Meena and Muthusamy (1998) reported that palmarosa oil (@ 0.05 and 0.1% concentrations) effectively inhibited the mycelial growth and sclerotial production of rice *R. solani*.

Leaf extracts of certain plant species were also used for effective management of rice ShB. Among them, the leaf extract of *Pithecellobium dulce* was highly effective in inhibiting mycelial growth of test pathogen (2.5 cm over 8.9 cm in control). Both the leaf extracts of *P. dulce* and *Prosopis juliflora* were equally effective in inhibiting sclerotial number, dry weight, and germination of the pathogen and also in controlling ShB with a disease incidence of 32.3 and 33.3%, respectively, over 76.2% in control (Meena *et al.*, 2002). Shylaja and Ranakausar (1997) reported that the leaf extracts of *Clerodendron viscosum*, *Lantana camara*, and *Vitex negundo* were highly inhibitory to both mycelial growth and sclerotial production of *R. solani*, whereas the other tested leaf extracts like *Citrus aurantiifolia* and fenugreek were not effective.

#### Fungicides

Presently, ShB disease management is majorly achieved through systemic fungicides and also with certain non-systemic fungicides (Pal *et al*, 2005). The resistance gain by pathogen to these systemic fungicides is of concern, thus demanding an evolution of newer fungicides and screening of

certain commonly used fungicides before evolving a comprehensive and compatible integrated disease management (IDM). Moreover, host plant resistance to ShB range only from very susceptible to moderately susceptible levels in rice (Groth & Bond, 2007), thus chemical management has become a necessary component for an effective IDM.

Application of fungicidal mixtures and pesticides for the control of pests and diseases is common in rice. The compatibility of these chemicals is a pre-requisite for effective management of these biotic stresses. Plant hopper is an economically important pest and the general practice is to target both ShB disease and plant hoppers in rice at a time. Field studies indicated that combined application of the insecticide imidacloprid (Confidor 200 SL) at 0.25ml/L and the fungicide validamycin (Rhizocin 3L) at 2.5 ml/L were high compatible and effective in reducing plant hopper and ShB incidence besides contributing to yield increase (Bhanu *et al.*, 2007). Fungicidal combinations are popular in management of rice diseases. Greenhouse and field studies with the fungicide Lustre (37.5SE) (flusilazole + carbendazim) against ShB revealed that application of the triazole mix could reduce disease severity and increase yields. Further, it was proved that the test fungicide was a safe combination fungicide without any phytotoxic symptoms. Its prophylactic application gave better results than as a curative application (Reddy and Muralidharan, 2007).

Use of fungicides with a broad spectrum of activity against more than one diseases is common in rice. Apart from blast, ShB, sheath rot and brown spot are the major economic diseases and a broad spectrum fungicide against all these rice diseases is economical. Among different fungicides screened under laboratory and field conditions (from 2002 to 2004), Tilt 25 EC (propiconazole) at 0.1% was highly effective against all these diseases. Whereas, Bavistin 50 WP (carbendazim) and Contaf 5 EC (hexaconazole) at 0.1% concentration were effective against ShB and sheath rot. Among other fungicides, Rhizocin 3 L (validamycin) at 0.25% was effective against ShB. Laboratory studies revealed that Tilt 25EC followed by Contaf 5EC were effective against all the test pathogens (Lore *et al.*, 2007). In a separate study on the evaluation of seed treatment against rice diseases, Vitavax 200 (carboxin + thiram) application (0.3% of seed weight) reduced the incidence of brown spot, blast, bakanae, foot rot and seedling blight in seed beds. Brown spot, narrow brown spot,

blast, ShB and sheath rot diseases are the diseases that are controlled in transplanted fields. Highest weight of healthy seeds per panicle (17.5g), highest number of healthy seeds per panicle (158.6), and highest seed yield (18.07%) increase over control) were recorded in Vitavax 200-treated seeds (Kabir *et al.*, 2006).

The effective fungicides at field level are Akonazole 250 EC (propiconazole) and Folicur EW 250 (tebuconazole) in reducing percent tiller infection, relative lesion height, and percent disease index (PDI) over control. Besides, a significant improvement in grain yields was reported with these fungicides (Mian *et al.*, 2004). The fungicide Monceren (pencycuron 250 SC) was also effective against ShB both in terms of disease reduction as well as increase of grain yields. Other effective fungicides include RIL 010/F1 25 SC, RIL 010/F1 50 SC, Rhizolex 50 WP, Rhizocin 3L, Folicur (tebuconazole) 250 EW, Contaf (hexaconazole) 5 EC, and Tilt (propiconazole) 25 EC at higher concentrations and were equal with Bavistin (carbendazim) 50 WP. Shield (clopuralid) 2.62 SC was the least effective one (Lore *et al.*, 2005). Biswas (2005) reported that field application of Tilt/Result (propiconazole) at 0.10% as sprays twice was effective in reducing ShB severity and improving grain yield over others.

Certain new fungicidal formulations were also found effective against rice ShB. Among them, Amistar 25 SC @ 1.0 ml L<sup>-1</sup> (30.6%) and RIL-010/FI 25 SC at 0.75 ml L<sup>-1</sup> (30.1%) showed a high degree of efficacy in reducing the disease severity and were superior over the standard fungicides (validamycin at 2.5 ml L<sup>-1</sup>). Highest grain yields were also reported in these fungicide treatments (Ranjan Nath *et al.*, 2005). The fungicide pencycuron (Monceren 250 SC) was most effective when sprayed at 35 and 55 days after transplanting. A ShB disease severity of 2.7 and 4.7% was observed after its first and second application in successive years of study during 2001-'02 (Chowdhury and Sarkar, 2006).

Strobilurins are new group of fungicides that are showing promising results in rice ShB disease control. The biofungicidal activity of strobilurins was reviewed and comparisons were drawn between its efficacy and the existing recommendations such as carbendazim, validamycin, and other triazoles. Strobilurins were very effective both in terms of disease reduction as well as in increasing grain yields (Biswas, 2006).

Ichiba *et al.* (2000) worked on the respiratory activity of metominostrobin against ShB pathogen and concluded that mycelial cells of pathogen induce an alternate respiratory pathway in response to blockage of cytochrome pathway. However, the alternate pathway of the pathogen could also be suppressed by some flavonoids, suggesting that metominostrobin is to be used in conjunction with plant components especially when the fungicide is applied in a prophylactic manner.

The other new fungicidal formulations that show promising activity against rice *R. solani* include O, O-diaryl O-ethyl phosphorothionate compounds (BG-8, BG-11, BG-14 and BG-19). These compounds when tested at 250, 500, and 1000 ppm under *in vitro* conditions against *R. solani* were found effective. Complete control of the pathogen was attained with application of BG-8 and BG-19 at 500 and 1000 ppm (Janki Kandhari *et al.*, 2005). In a separate study, the fungicides G/FT-3 (O, O-di (2, 4, 5-trichlorophenyl)-S-methyl phosphorothionate) and G/FT-9 (O, O-di (2, 4, 6-trichlorophenyl)-O-methyl phosphorothionate) were found to inhibit the mycelial growth of *R. solani* under *in vitro* conditions at 25-50 and 1-2 ppm respectively. *In vivo* studies on rice cv. Pusa Basmati-1 revealed that G/FT-3 and G/FT-9 caused ShB disease reduction of 52.8% and 43.9% at 100 and 4 ppm respectively (Janki Kandhari and Gupta, 1999).

### Biological control

Biological control of plant pathogens though gaining popularity in majority of crops, its utilization in rice ecosystem is still at its infancy due to varied reasons. Rice, being a crop that is grown under inundated conditions; the survival, growth and establishment of biological control agents is questionable. However, effective management strategy of ShB disease is feasible only when the biocontrol agents those are in vogue in rice based cropping systems survive, establish, proliferate and control sheath blight pathogen and also have a synergistic growth promoting effect on the crop. Besides, the biocontrol agent should be able to induce systemic resistance thereby contributing to the disease control.

### Fungal bioagents

Among the fungal antagonists, *Trichoderma* spp and *Gliocladium* spp are widely used in the management of rice ShB disease. These fungal antagonists are either applied to rice seed, soil, root dip and foliar spray for managing the disease. In pot culture studies, seed treatment of

the bioagent *T. viride* resulted in ShB disease reduction. Further, the efficacy of *T. viride* was comparatively more than the bacterial bioagent *Bacillus subtilis* (Das *et al.*, 1998). Foliar application of *Trichoderma* spp also was found to be very effective in reducing ShB severity. Studies on field application of *T. harzianum* as talc + CMC based formulation proved that disease severity was reduced by 52%. The bioagent was found effective when applied at 7 days compared to simultaneous application with ShB pathogen (Khan and Sinha, 2006<sup>a</sup>). The optimum dose of the bioagent was found to be 4 or 8 g/L and increased grain yields were also reported (Khan and Sinha, 2007). Spray application of the bioagent was highly effective on rice seedlings that received 60 kg N + 60 kg P + 40 kg K/ha (30 kg N and whole of P and K as basal and remaining 30 kg N at 20 and 40 days after transplanting) both in terms of reduction in ShB incidence, severity, and increased yields (Khan and Sinha, 2006<sup>b</sup>). Further, the rice leaf isolate of *Trichoderma* spp was more effective compared to the rhizosphere isolate of *T. virens* (Khan and Sinha, 2005a). According to Lakshmi Tewari and Rajbir Singh (2005), soil application of *T. harzianum* was not effective both under greenhouse and field conditions. On the other hand, mixed mode of application of bioagent as soil treatment, root dipping, and foliar spray was found to be very effective in reducing ShB severity over control. However, foliar application of the bioagent alone was also effective under field conditions. In a separate study, Nagaraju *et al.* (2002) reported that application of *T. viride* as root dip + spray was effective in reducing ShB severity by 59% under field conditions.

Combined applications of bioagents also were proved effective in controlling ShB both under greenhouse and field conditions. Mathivanan *et al.*, (2005) reported that combined applications of *T. viride* and *Pseudomonas fluorescens* was effective without any negative effects in reducing rice ShB besides increasing number of productive tillers, higher grain and straw yields. However, individual applications of bacterial and fungal antagonists separately had more beneficial effects. Sarmah (1999) reported that combined application of *G. virens* and *B. subtilis* was more effective in ShB disease reduction (73%) over their individual applications. Further, lower doses of bioagents ((2.5g/kg of *G. virens* and 10<sup>8</sup> cells/ml of *B. subtilis*) were necessitated in combined application compared to their individual applications. Tang Jia Bin *et al.*, (2002) examined cellulase activity of *Trichoderma* spp and proved that *T. hamatum*,

*T. aureoviride* and *G. virens* were effective. Field studies indicated that the fungal bioagents exhibited good antagonism, and a disease control effect of 32% was obtained with fungal antagonist mixture besides positive effects on seed setting rate and 1000-grain weight of rice plants. In a separate study, Tang JiaBin *et al.*, (2001) evaluated 800 strains of *Trichoderma* spp and reported that six strains were highly inhibitory to the growth of pathogen in dual culture studies. Among the fungal antagonists, T3 was found superior in reducing the pathogen growth by 53%. Bhagawati (2005) proved that ShB disease suppression at field level can be obtained by soil application of *T. harzianum* and *T. viride* at a pH range of 5.1 to 6.0. A concomitant increase in plant growth and yield was obtained. Further, it was reported that population levels of *Trichoderma* spp are high and that of *R. solani* are low in acid soils.

Among other fungal bioagents that are effective against rice ShB, *Helminthosporium gramineum* is an important one. The culture filtrates and crude toxin of *H. gramineum* were highly inhibitory to *in vitro* growth of *R. solani*. The biologically active metabolite of the crude toxin is identified as "ophiobolin A" by spectroscopic analysis and was found to significantly inhibit the mycelial growth of *R. solani* at all concentrations tested. Field studies indicated that the crude toxin was highly effective in reducing the rice ShB disease incidence and severity without any adverse effects on growth and yield attributes (Duan GuiFang *et al.*, 2007). Application of avirulent strains of ShB pathogen was also found effective. Field studies with three avirulent strains of *R. oryzae* (*Waitea circinata*) isolates on rice cultivar Swarna revealed that ShB incidence was low in terms of relative lesion height, tiller infection, and severity index when the inocula of the bioagent, *R. oryzae* (isolate no.545) were broadcasted to the field at five days after inoculation with *R. solani* pathogen (Akter *et al.*, 2005).

### Bacterial bioagents

Among the bacterial biocontrol agents, plant growth-promoting rhizobacteria (PGPR) offer a promising means of controlling plant diseases besides contributing to the plant resistance, growth and yield in rice (Mew and Rosales, 1992). Of different PGPR, fluorescent *Pseudomonads* and *Bacillus* spp group of bacteria offer an effective control of ShB besides inducing growth promoting effects and systemic resistance. Bacteria isolated from rice seeds and rice ecosystem were able to effectively suppress ShB besides producing growth promoting effects. Further, these bacterial

antagonists should be applied only after maximum tillering stage of the crop since ShB pathogen is rarely rampant during flooded conditions (Lai Van E *et al.*, 2001). Seed treatment with these antagonistic bacteria resulted in increased root and shoot length of seedlings. Foliar sprays with these antagonists resulted in reduced ShB incidence (Sharma *et al.*, 2004). Yi TuYong *et al* (2000) reported antagonistic activity of endophytic and epiphytic bacterial strains isolated from healthy rice seeds against rice ShB pathogen. The strains S-11, S-13, S-14 and S-18 effectively inhibited mycelial growth of pathogen. Field application of the strain S-18 at  $3 \times 10^9$  cfu /ml resulted in reduced ShB incidence. Marine bacteria also have antagonistic abilities to control different plant pathogens. Nie YaFeng *et al* (2007) reported that 11 strains of marine bacteria isolated from sea mud and water of Lianyungang sea area of China were found effective. The bacterium has ability to inhibit plant pathogens like *Alternaria brassicae*, *Magnaporthe grisea* and *Botrytis cineria*. Further, the extracellular substance of the bacterium has good ShB controlling efficacy in pot and field experiments.

Strains of *P. fluorescens* were found to inhibit the rice ShB pathogen under *in vitro* conditions. All the strains of the bioagent (biovar 2) produced siderophores on King's B media. The volatile metabolites, extra cellular secretions and antibiotics of these isolates were inhibitory to *R. solani*. All the antagonists could reduce germination and caused lysis of sclerotial bodies (Kazempour, 2004). The population densities of the strains were increased on rice root system (Kazempour, 2007). Rhizosphere isolates of *P. fluorescens* (PF-3 and PF-4) were also inhibitory to chilli damping-off pathogen *Pythium* spp. These isolates also produce salicylic acid, siderophores and hydrogen cyanide (HCN) that are responsible for inhibition of test pathogens (Muthukumar and Bhaskaran, 2007). Besides, some rhizosphere isolates of *P. fluorescens* (PfMDU2 strain) also produce  $\beta$  1, 3-glucanase. A significant relationship between the antagonistic activity of the bacterium against *R. solani* and its level of  $\beta$  1, 3-glucanase, salicylic acid and HCN production was noticed (Nagarajkumar *et al.*, 2004). Rhizosphere isolates of *P. fluorescens* (GR1, GR25, GR27, WR49, WR55, and WR62) from chick pea and wheat crops were also inhibitory to mycelial growth of rice ShB pathogen. Even the sclerotial bodies of the rice pathogen were inactivated completely when they were pretreated with bacterial cell suspensions for 1 minute to 4 weeks (Pande and Chaube, 2003). The isolates of *P. fluorescens*



were found to be compatible with one another under *in vitro* conditions. Li XiangMin *et al.* (2007) reported that strains of *Pseudomonas*, Pf7-14 (natural resistant to nalidixic acid) and P13-R (spontaneous rifampicin resistant mutants of P13) that were highly antagonistic to rice *R. solani*. are compatible with each other under *in vitro* conditions.

Efficacy of *P. fluorescens* strains under greenhouse and field conditions depend on time of application. Field studies indicated that spraying of *P. fluorescens* at 7 days before pathogen inoculation resulted in maximum reduction in ShB severity (59.6-64.4%) over simultaneous application and at 7 days after inoculation. Further, with inoculation at 7 days before pathogen inoculation, an increase in 1000-grain weight (27.2-29.5%) was reported (Rajbir Singh and Sinha, 2005<sup>a</sup>). The *Pseudomonas* treated rice plants show increased chitinase activity at 2 days after inoculation. This increased induction of pathogenesis-related chitinase is attributed to its role in suppressing ShB disease incidence and development (Radjacommare *et al.*, 2004). Ren XiaoPing *et al.* (2006) concluded that the optimum spraying time of the bioagent was during the first day of inoculation of ShB pathogen on rice plants. The mode of application of *Pseudomonas* spp also determines their efficacy in controlling ShB disease. Ren XiaoPing *et al.* (2006) worked on crude extracts of antagonistic bacterium, *P. aeruginosa*, against *R. solani* in rice and reported that the biocontrol effect was dependent on the concentration of extracts and the treatment time. The duration of colonization of the antagonist on rice plants is directly related to the initial concentration applied. Rajbir Singh and Sinha (2005<sup>b</sup>) reported that ShB in rice fields could be effectively controlled with foliar sprays of *P. fluorescens*. Increased grain yields and 1000-grain weight are also reported with foliar application of the bioagent (Pfr1). In contrast, Kazempour (2004) reported that seed coating of *P. fluorescens* (B41) was found to be comparatively more effective than soil drenching and foliar sprays in reducing ShB disease in rice under greenhouse conditions. However, field studies indicated that the bioagent was highly effective when applied as seed coating, soil drenching, and as seed coating + foliar sprays (with 10.5, 11.75 and 18.75% disease intensity, respectively, against 52% in control plots). Pathak *et al.* (2004) reported that dual treatment of *Pseudomonas* strain GRP3 as seed bacterization followed by root dipping resulted in inhibition of mycelial growth and sclerotial germination of *R. solani*. The ShB lesion length was reduced up to 46%. The results were significantly superior

compared to single application methods of the bioagent and control. Additionally, the peroxidase activity and phenol levels in dual treated plots were higher in plants treated with GRP<sub>3</sub> compared to the control. This increase is attributed to the control by bacterial bioagent that induces systemic resistance in host plants.

Enhanced efficacy of *Pseudomonas* spp was reported against ShB disease when the bioagents are used in conjunction with other bacterial and fungal bioagents. Combined applications of *P. fluorescens* with *T. viride* were found to be effective in rice ShB control as well as in promoting seedling growth (Mathivanan *et al.*, 2006). Talc based formulations of two *P. fluorescens* strains (PF1 and PF7) when applied through seed, root, soil and foliar sprays significantly reduced ShB and leafhopper incidence under greenhouse and field conditions. The bacterial mixture performed better than individual strains, showing a reduction of 62% ShB and 47 to 56% leafhopper incidence (Commare *et al.*, 2002). In a separate study, Nandakumar *et al.* (2001) reported that PGPR strains of *P. fluorescens* (PF1, FP7, and PB2) when applied in combination as bacterial suspension or as talc based formulations through seed, root, foliar, and soil application significantly reduced the ShB incidence (45%) under greenhouse and field conditions over their individual applications (29% reduction). A significant increase in yield was obtained with application of bioagent mixture over their individual applications (25.9% and 17.7% increase respectively over control).

*Bacillus* spp are important gram positive PGPR in the biocontrol of rice ShB disease. The bacterium produces endospores and microscopic studies revealed that isolates of *B. subtilis* and *B. megaterium* exhibited effective inhibition against the pathogens of ShB and bakane diseases of rice (Luo Jin Yan *et al.*, 2005). The fermented product of *Bacillus* strain Drt-11 was highly antagonistic to rice ShB pathogen, causing reduced sclerotial germination (40-60% inhibition over control), reduced hyphal growth and colony diameter (by 14%) besides increased rice seedling growth (Chen Min and Kang Xiao Hui, 2006). The bacterial antagonist (*B. subtilis* A30) produces an antagonistic substance named P1 which is both thermostable and proteinase-stable one. Further, the antagonistic substance had a negative ninhydrin reaction and positive ninhydrin and biuret reactions after acid hydrolysis. The bacterial strain is highly antagonistic to rice ShB and blast pathogens (He QingFang *et al.*, 2002). The bacterium

(*B. subtilis* strain AUBS1) also produces phenylalanine ammonia-lyase (PAL), peroxidase (PO) and certain pathogenesis-related proteins (PR) in rice leaves when applied against ShB disease. Application of bioagent also resulted in accumulation of thaumatin-like proteins, glucanases and chitinases (Jayaraj *et al.*, 2004). Increased antagonistic abilities of *B. subtilis* (BS-916) were reported against *R. solani*, when the bioagent was implanted with N<sup>+</sup> at 150x2.6x10<sup>13</sup> to 250x2.6x10<sup>13</sup> N<sup>+</sup>/cm<sup>2</sup>. An increase in inhibition zone against ShB pathogen was noticed with the mutants to an extent of 4.3 to 31% under *in vitro* conditions. The control effect of the mutants is estimated to be 3.2 to 19% over that of BS-916 (Li DeQuan *et al.*, 2006).

The efficacy of *Bacillus* spp against rice ShB disease is dependent on the antagonist population threshold in the soil. For effective suppression the population levels of the antagonist should be higher than 1 × 10<sup>6</sup> cfu/g during early infection of *R. solani* within 6-7 days (Li Xiang Min *et al.*, 2003). Mode of application of the bacterial bioagent and the type of formulation also affects its efficacy under greenhouse and field conditions. Floating pellet and water-soluble granule formulations of *B. megaterium* were found effective against rice ShB disease. Of these, foliar spraying of the bioagent was more effective than the floating pellet formulation in reducing the percent ShB affected tillers (Kanjamaneesathian *et al.*, 2007). Wiwattanapatapee *et al* (2004) reported that the floating pellet formulation of *B. megaterium* consisting of hydrogenated vegetable oil, lactose, microcrystalline cellulose, and a disintegrant, cross-linked sodium carboxy-methyl-cellulose showed promising result in suppression of rice ShB lesions in greenhouse experiment. The effervescent, fast-disintegrating granules, containing endospores of *B. megaterium* when either broadcasted or sprayed, reduced ShB infection in rice under greenhouse conditions. Further, the bacteria remained viable in effervescent granular form (10<sup>9</sup> cfu/g) even after one year of storage at room temperature. Even the number of viable and virulent bacteria after applying into water and spraying on rice seedlings in greenhouse were also satisfactory (10<sup>9</sup> and 10<sup>6</sup> cfu/g respectively) (Wiwattanapatapee *et al.*, 2007).

*Bacillus* spp exhibited synergistic effect when used in conjunction with other bio-pesticides. When used along with fungal bioagents such as *T. viride*, *B. subtilis* resulted in ShB disease reduction effectively in pot culture studies (Das *et al.*, 1998). When applied in combination with *Gliocladium virens*

it effectively controlled ShB disease reduction (73%) at lower doses of both the bioagents (2.5g/kg of *G. virens* and 10<sup>8</sup> cells/ml of *B. subtilis*) (Sarmah, DK., 1999). Chen Min and Kang Xiao Hui (2006) reported that the fermented product of *Bacillus* strain Drt-11 when used in combination with commercial biofungicide Jिंगgangmeisu WP (20%) yielded significantly higher efficacies in rice ShB control than their individual applications.

Other bacteria showing antagonistic activity against ShB pathogen include *Streptomyces* spp and *Serratia marcescens*. Antifungal metabolites of *Streptomyces* spp (PM5, SPM5C-1 and SPM5C-2) were highly effective against the mycelial growth of rice ShB and blast pathogens under *in vitro* conditions. A complete inhibition was obtained at concentrations of 25, 50, 75 and 100 µg/ml. Greenhouse studies indicated that spraying of SPM5C-2 @500 µg/ml on rice significantly decreased ShB and blast disease development by 82 and 76% respectively (Prabavathy *et al.*, 2006). The antifungal activity of *Serratia marcescens* was reported by Someya *et al* (2005). Culture filtrates of the bioagent showed enhanced biocontrol activity when combined with low concentrations of fungicides like flutolanil, pencycuron and validamycin in terms of reducing sclerotial viability of ShB pathogen.

### Integrated disease management

Integrated disease management (IDM) of rice ShB is gaining momentum and encompasses all the available control methods with each method compensating the deficiencies of others. Among the available IDM practices, combined use of chemical, cultural, biological and host plant resistance is a common phenomenon. However, host plant resistance to ShB range only from very susceptible to moderately susceptible levels in rice (Groth and Bond, 2007), thus chemical management has become a necessary component for an effective IDM.

Combined applications of bioagent with chemical fungicides are an important IDM package against ShB. The use of fungal bioagents in controlling rice ShB in an IDM is gaining importance. Among the fungal bioagents, *Trichoderma* spp are important biocontrol agents that are effective against major soil borne diseases. Application of *T. harzianum* with soil organic amendements such as FYM, wheat straw, dhaincha (*Sesbania aculeata*), saw dust and neem cake worked effectively in managing rice ShB and also in increasing grain yields (Khan and Sinha, 2006<sup>c</sup>). Combined field

applications of *T. viride* (5kg) and validmycin (2L)/ha was found to be effective in controlling ShB and sheath rot diseases of rice besides enhancing crop yield (Daroga Singh *et al.*, 2007). Spray application of the spore suspension of *T. viride* (Tv3235) along with carbendazim (0.1%) and soil applications of FYM (1%) + saw dust (1%) showed maximum reduction in ShB severity, percent disease incidence and significant increase in grain yields over control (Surulirajan and Janki Kandhari, 2005).

*Trichoderma* spp were found to be compatible with majority of fungicides used in ShB management. *In vitro* studies revealed that fungal bioagents, *T. harzianum* and *G. virens*, are compatible with captan and are effective against ShB pathogen in rice. Integrated field evaluation proved that *Azolla pinnata* at 5t/ha as green manure along with FYM at 2.5 t/ha was highly effective in reducing the sheath blight disease incidence (14.63%) and increasing winter rice yield (40.29q/ha). FYM alone and *Sesbania aculeata* + FYM are the next best treatments. The interactive effects of seed/root dip treatment and amendments showed the best results in disease reduction and in yield increase (Gogoi and Ali, 2005). The bioagent *T. harzianum* was highly compatible with Hinosan (edifenphos) at 0.05% concentration. Field studies indicated that the bioagent was effective when combined with Contaf (hexaconazole), Hinosan, Rhizolex (tolelofoxmethyl), and Validacin (validamycin). Hinosan is suggested as the best fungicide for combined application with *T. harzianum* due to its compatibility (Ali and Pathak, 1997).

The PGPR offer a promising means of controlling plant diseases besides contributing to the plant resistance, growth and yield in rice (Mew and Rosales, 1992). Of different PGPR, *Pseudomonads*

and *Bacillus* spp were found to be very effective as a supplement in IDM. Greenhouse and field studies against rice ShB pathogen with different bacterial bioagents isolated from farmyard manure, rice seed, rice phyllosphere, and rice rhizosphere proved that three bacteria, PF-9 (*Pseudomonas fluorescens*), B-44 (*Bacillus* sp), and Chb-1 (chitinolytic bacterium) are compatible with carbendazim (Bavistin) at 500 and 1000 ppm concentrations. Among the three bioagents, PF-9 was most effective in reducing disease severity either alone or in combination with one spray of 0.1% Bavistin, followed by combination of PF-9 and B-44 (Laha and Venkataraman, 2001). The bacterial bioagent, *B. subtilis* (Bs-916) when applied along with jinggangmycin was found to colonize the root system effectively. Further, the population density of BS-916 was maintained in its presence without any decline (Chen ZhiYi *et al.*, 2003). In a separate study, it was found that the ShB disease was effectively controlled when jinggangmycin was mixed and sprayed with a growth regulator (Yi-Sui-Su) at the booting stage. A synergistic effect of the combination was noticed both in terms of reduction in disease severity as well as increase in growth and yield (Xu WeiLiang *et al.*, 1999).

Other IDM packages that were found effective against ShB are combined use of botanicals, fungicides and organic amendments. Janki Kandhari (2007) reported Achook (azadirachtin), a neem based chemical performed better with a ShB disease incidence of 65% compared to control (83%). Ashrafuzzaman *et al* (2005) reported that ShB disease development was least and mean filled grains per panicle, 1000-gram weight, straw and grain yields were higher in pot culture studies with combined doses of ash, bleaching powder, poultry manure and Bavistin over control.

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