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Seed Health and Seed-Associated Microorganisms for Rice Disease Management

T.W. Mew and B. Cottyn, Editors

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Contents

Microorganisms Associated with Rice Seed

Diversity of bacterial communities associated with rice seed in the tropical environment

B. Cottyn, E. Regalado, J. Swings, and T.W. Mew

Harvested rice grains were collected from farmers in Iloilo and Rizal provinces (Philippines) during the wet seasons of 1995 and 1996, respectively. Bacterial isolations from seeds yielded 942 isolates. The isolates were characterized by BOX-PCR (polymerase chain reaction) fingerprinting of total genomic DNA and were found to represent 443 fingerprint types (FPTs). Most FPTs were found only once and about 1.5% of the defined FPTs occurred in more than half of the examined samples. Isolates representative for each defined FPT were identified by cellular fatty acid methyl ester (FAME) analysis, Biolog GN and GP MicroPlates, and API 20E and 50 CHE systems. Gram-negative bacteria comprised 55% and Gram-positive bacteria 45% of the total number of rice seed isolates. The predominant bacteria were Enterobacteriaceae, *Bacillus* spp., and nonfluorescent Pseudomonas spp. Other frequently isolated bacteria were *Burkholderia* spp., *Xanthomonas* spp., *Curtobacterium* spp., *Microbacterium* spp., *Streptococcus* spp., and *Micrococcus* spp. Eight percent of the total number of isolated nonpathogenic bacteria exhibited in vitro antifungal activity against *Rhizoctonia solani* and *Pyricularia* grisea. Two percent of the isolates were pathogens identified as *Burkholderia glumae, B. gladioli,* and *Acidovorax avenae* subsp. *avenae.* Another 4% of the isolates induced sheath browning on 50–90% of the inoculated plants and were identified as *Bacillus pumilus, Paenibacillus* spp., *Pseudomonas* spp., and *Pantoea* spp. This study revealed a high genetic diversity of bacteria associated with rice seed.

Current integrated pest management (IPM) strategies in tropical rice production emphasize the use of environmentally sound ways to control diseases, and biological control has the potential of being an alternative to help reduce the need for chemicals (Schaenly et al 1998). The diverse communities of nonpathogenic plant-associated microorganisms are considered a largely untapped resource for suppressing disease development in the field (Blakeman and Fokkema 1982). It is anticipated that a better understanding of the indigenous microbial diversity of rice ecosystems can contribute to more effective disease management (Schoenly et al 1998).

Seed is of particular interest because of its importance as planting material and its potential as a vehicle for transmitting beneficial or deleterious bacteria. In contrast to the rice rhizosphere. where the interest in biological nitrogen fixation has led to ample characterization of free-living and endophytic diazotrophic bacteria (Oyaizu-Masuchi and Komagata 1985, Stoltzfus et al 1997), studies on rice seed microflora have commonly been restricted to seedborne pathogens (Ou 1985, Coto and Ohata 1956).

In this study, we used the BOXA1R primer corresponding to the box A-subunit sequences (Martin et al 1992) for PCR (polymerase chain reaction)-based DNA fingerprinting of rice seed bacteria. Whole-cell fatty acid analysis, Biolog GN and GP MicroPlate systems, and API systems (BioMerieux, La Balme-les-Grottes, France) were used to identify the isolates. Rep-PCR has been demonstrated to be useful for DNA fingerprinting of a large variety of bacteria and for studying microbial diversity in natural ecosystems (Schneider and de Bruijn 1996, Versalovic et al 1991). The phenotypic typing methods of whole-cell fatty acid methyl ester (FAME) analysis and the Biolog Microplate system have been used in both taxonomic studies and identification analyses (Jones et al 1993, Moss 1990, Welch 1991).

The aim of this study was to characterize the viable aerobic bacterial populations associated with rice seed.

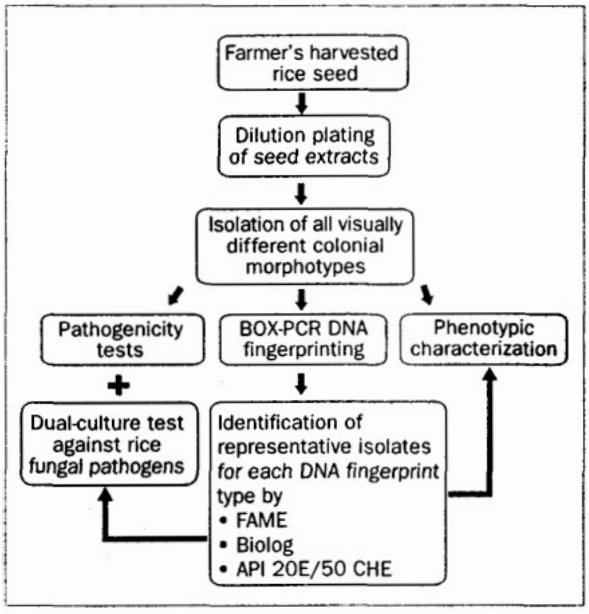

Fig. 1. Overview of the polyphasic approach used to characterize culturable bacteria associated with rice seed.

Materials and methods

During the wet seasons of 1995 and 1996, 1-kg samples of harvested rice were purchased from 11 fanners in Iloilo Province and from I2 farmers in Rizal Province (Philippines), respectively. Bacteria were isolated from seeds as described before (Cottyn et a1 2001). Figure 1 shows a flowchart of the procedures used to characterize the rice seed bacteria. For the Iloilo samples, batches of 10 g of seed were partially crushed with a sterilized mortar and pestle and suspended in 100 mL of sterile phosphate-buffered saline solution (PBS; 137 mM NaCl, 2.7 mM KCl, 0.01 M Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) with 0.025% Tween 20 (Sigma Chemical Co., St. Louis, Mo., USA). For the Rizal samples, batches of 50 g of seed were soaked in 100 mL of sterile distilled water with 0.025% Tween 20 on a Lab Line Orbit Shaker at 130 rpm for 3 h. Aliquots of 0.1 mL from IO-fold serial dilutions of the seed suspensions in sterile saline (0.85% NaCI) were plated in duplicate on King's medium B (KMB) and tryptic soy agar (TSA; Difco Laboratories, Detroit, Mich.. USA) supplemented with 0.01% cycloheximide (Sigma Chemical Co., St. Louis, Mo.). The plates were incubated at 28 °C for 4 d. All visually distinct colony types, with two to three arbitrarily picked for abundant types, were isolated from each sample. All isolates were maintained at −70°C in nutrient broth (NB; Difco Laboratories, Detroit, Mich.) with 15% glycerol.

All isolates were tested for pathogenicity by inoculation of 21-d-old rice seedlings of cultivar IR24 grown in the greenhouse under natural light with day/night temperatures of about 35/25 °C and with relative humidity ranging from 40% to 65%. Four seedlings per isolate were inoculated by injecting the culm about 2 cm above the soil with 0.1 mL of an aqueous suspension of an overnight-grown TSA culture. The seedlings were examined for symptoms 3 and 10 d after inoculation. Localized browning around the point of inoculation was considered a negative reaction. Elongation of a brown necrotic zone of tissue away from the point of inoculation, often extended up to the third leaf, was scored as a positive reaction. Isolates that produced symptoms on at least two out of four seedlings were inoculated two more times on four plants. Bacterial isolates were considered pathogens if all plants inoculated showed a positive reaction. Isolates that caused symptoms on at least 50% of inoculated plants were considered pathogens with low disease potential.

All bacterial isolates were tested by the dual-culture method as described by Pamplona et al (this volume) for antagonistic activity against four rice fungal pathogens: *Rhizoctonia solani, Pyricularia grisea, Saroclaclium oryzae,* and *Fusarium moniliforme.*

For genomic DNA isolation, bacteria were grown in 8.0 mL NB on a rotary shaker at 150 rpm for 24 h at 28 °C. Extraction of DNA was done as described by Jones et al(1989) and Pitcher et al (1989). The DNA concentrations were estimated visually by comparison to lambda DNA standards in an agarose gel.

The BOX-PCR assay was performed as described by Cottyn et al (2001). After the amplification process, 7 µL of each reaction mixture were electrophoresed on gels composed of 0.8% Synergel (Murfreesboro, Tenn., USA) and 0.8% agarose (United States Biochemical) in 0.5x TBE (Tris borate-EDTA) at 75V for 14 h. As a standard, a 1-kb marker (Life Technologies, Inc.) was run in the 2nd, 16th, and 30th lane. The gels were stained with ethidium bromide and photographed using Polaroid Type 57 film. The photographs were scanned on a flatbed scanner (Sharp JX-610) at 200 dpi resolution. Normalization of BOX-PCR patterns and cluster analysis were done using the GelCompar software version 4.0 (Applied Maths, Kortrijk, Belgium). Similarity between the DNA fingerprints was calculated using the Pearson correlation coefficient and clustering was done by the unweighted pair group method using arithmetic averages (UPGMA).

In addition, the fingerprints were interpreted by visual examination. We defined a fingerprint type (FPT) as a set of strains with identical or nearly identical BOX-PCR DNA patterns. Patterns were considered nearly identical when variation was limited to two or three faint DNA fragments.

The following phenotypic features were examined for all isolates: colonial and cellular morphology, Gram stain, fluorescent pigment production on King's medium B, Kovac's oxidase reaction, nitrate reduction, and reaction on Hugh and Leifson's oxidation-fermentation medium.

The bacterial isolates were grown on trypticase soy agar (TSA; BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md., USA) at 28 °C for 24 h for FAME-analysis. Extraction and preparation of the cellular fatty acid methyl esters were performed according to Sasser and Wichman (1991). The generated profiles were identified by using the Microbial Identification System (MIS version 4.15; Microbial ID, Inc., Newark, Del., USA).

Further identification was done using the Biolog GN/GP MicroPlate systems (Biolog Inc., Hayward, Calif., USA). Isolates identified by Biolog with a low similarity coefficient as belonging to the Enterobacteriaceae were further investigated by API 20E and 50 CHE galleries according to the manufacturer's instructions (API Systems, Biomérieux, La Balme-les-Grottes, France).

Whole-cell protein profiles were generated for those isolates that were thought to belong to the genus *Burkholderia.* The obtained protein profiles were visually compared with the patterns in the database of the genus *Burkholderia* created by Vandamme et al (1997).

Results and discussion

A total of 942 bacterial isolates, comprising 55% Gram-negative and 45% Gram-positive bacteria, were isolated from the 23 rice seed samples collected from farmers in Iloilo and Rizal (Philippines). On the basis of BOX-PCR fingerprinting of genomic DNA, 443 FPTs were distinguished among the seed-associated bacteria. If isolates within one FPT can be considered members of a single population, then at least 443 bacterial populations occurred in the seed samples. No FPT was found in all 23 samples. The majority of FPTs (70%) were not shared among samples and about 1.5% of the defined FPTs occurred in more than half of the examined samples.

On the basis of FAME and BOX-PCR analysis, Enterobacteriaceae were the most diverse group of isolates among the Gram-negative bacteria found in rice seed. *Pantoea* populations, followed by *Enterobacter* populations related to *E. cloacae* and *E. sakazakii,* dominated the group of *Enterobacteriaceae.* Identifications at the species level, however, remained doubtful for many isolates. The most frequently found enterobacterial morphotype revealed a BOX-PCR pattern that was highly similar to the pattern generated for the *Pantoea stewurtii* subsp. stewartii type strain LMG 2715 (previously *Erwinia stewartii*).

Several studies have demonstrated that pseudomonads can be commonly isolated from healthy and discolored rice seeds (Cottyn et al 1996, Xie 1996, Ziegler and Alvarez 1990). In this study, mainly nonfluorescent pseudomonads were found in the seed samples. They were homogeneous in fatty acid composition but revealed a high genetic diversity on the basis of BOX-PCR analysis. Obtained FAME-MIS identifications as *Pseudomonas oryzihabitans* and *P. stutzeri* were congruent with the phenotypic characteristics determined for the isolates; however, no clear identifications were obtained for the other nonfluorescent pseudomonads by the methods applied.

Also, *Xanthomonas* spp. were frequently found in the seed samples and were not pathogenic on rice in our pathogenicity tests. These nonpathogenic xanthomonads may be confused with *X. oryzae* based on similar colony appearance but certainly did not belong to *X. oryzae* in both FAME and BOX-PCR analyses. They possess the three characteristic fatty acids of the genus *Xanthomonas:* 11:0 iso, 11:0 iso 3OH, and 13:0 iso 3OH; however, their fatty acid profiles did not fit to any recognized *Xanthonmonas* species. Hence, they appear to occupy a unique position within the genus *Xanthomonas.* The occurrence of nonpathogenic xanthomonads has been reported from rice seeds as well as from other crops (Jones et al 1989, Vauterin et al 1996).

Other Gram-negative bacteria isolated were identified as *Acitiovorax avenue, Acinetobacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Azospirillum* spp., *Brevundimonas vesicularis, Burkholderia* spp., *Chryseobacterium* spp., *Herbaspirillum rubrisubalbicans, Methylobacterium* spp., *Pseudomonas aeruginosa, P. putida, P. citronellolis, P. resinovorans, Sphingomonas* spp.. *Sphingobacterium* spp., and *Stenotrophomonas maltophilia.*

An unexpected finding was the large diversity of bacilli and coryneform bacteria carried by the rice seed. The abundance of Gram-positive bacteria associated with rice seed has hardly been explored in the past. The most prevalent *Bacillus* spp. were identified as *B. coagulans, B. subtilis,* and *B. pumilus*. Others were identified as *B. cereus, B. megaterium, B. sphaericus, B. licheniformis, B. gibsonii,* and *Paenihacillus* spp. Coryneform isolates were identified by FAME-MIS as *Clavibacter michiganense, Curtobacterium flaccumfaciens, Cellulomonas turbata, Microbacterium* spp., *Rrevibacterium* spp., and *Arthrohacter* spp. This group of seed-associated coryneform isolates revealed a large complexity of BOX-PCR DNA patterns that often shared few bands in common among the different genera. Gram-positive cocci isolated from rice seed were identified as *Staphylococcus* spp., *Micrococcus* spp., and *Kocuria* spp.

Among the 942 isolates from seeds, 2% consistently caused symptoms on all inoculated plants and 4% caused variable symptoms on 6 to 11 inoculated plants. The remaining isolates induced no symptoms or induced a hypersensitive-like reaction localized at the point of inoculation. Two pathogenic isolates were identified by both FAME-MIS and Biolog as *Acidovorax avenue* subsp. *avenue,* the causal agent of bacterial brown stripe (Ou 1985). The majority of the pathogens though were identified as *Burkholderia glumae* and *B. gladioli* based on comparison of SDS-PAGE protein patterns to the database of *Burkholderia* generated by Vandamme et al (1997). In pathogenicity testing, these strains caused wilting of IR24 seedlings. *B. glumae* is recognized as an important rice pathogen causing both seed rot (Goto and Ohata 1956) and seedling rot (Uematsu et al 1976). On the other hand, reports on *B. gladioli* as a rice pathogen are limited and come only from Japan (Ura et al 1996). *B. plantarii,* another *Burkholderia* species known as a rice pathogen causing seedling blight (Azegami et al 1987). was not found in the seed samples. Four percent of the total number of isolates caused variable symptoms of sheath necrosis on 50–90% of inoculated plants and were considered pathogens with low disease potential. They were identified as *Bacillus pumilus, Paenibacillus* spp., *Pseudomonas* spp., and *Pantoea* spp.

In this study, 8% of the isolated nonpathogenic bacteria showed in vitro antifungal activity. None of the isolates inhibited *Fusarium moniliforme* or *Sarocladium oryzae.* Thirty-two isolates inhibited the growth of *Rhizoctonia solani* only, 18 isolates inhibited *Pyricularia grisea* only, and 25 isolates suppressed the growth of both fungal pathogens. Rice isolates with antifungal activity belonged to various taxa (Table 1). However, in vitro inhibition of mycelial growth does not guarantee that the strains will be effective biocontrol agents and further work to establish their potential in vivo is needed.

In conclusion, the results of this study revealed a high diversity of seed-associated bacteria despite the known limitations of the traditional cultivation technique. BOX-PCR analysis of the collection of rice seed isolates revealed a large variety of bacteria with unique fingerprints and groups of bacteria with nearly identical fingerprints that were isolated from different samples. The results also suggested that considerable differences in composition of bacterial communities exist among samples. The function of this genetic diversity and its consequence for deployment strategies, however, is not clear. Further research that elucidates the mechanisms eliciting this genetic diversity is needed.

Table 1. Rice seed bacteria with in vitro antifungal activity.

Rice seed isolates[a]	No. of antifungal isolates	No. of isolates suppressing growth of[b]	
		Rhizoctonia solani	*Pyricularia grisea*
Pantoea spp. (B)	17	11	9
Enterobacter cloacae (B)	3	3	1
Klebsiella mobilis (B)	2	2	1
Nonfluorescent pseudomonads	9	3	7
Pseudomonas putida (B)	2	2	2
Stenotrophomonas maltophilia	1	1	0
Xanthomonas spp.	2	2	0
Acinetobacter baumannii	3	2	2
Agrobacterium spp.	1	1	0
Bacillus subtilis	19	17	11
Bacillus cereus	2	2	1
Bacillus coagulans	1	1	1
Bacillus sphaericus	1	1	0
Paenibacillus spp.	1	0	1
Microbacterium spp.	2	2	0
Brevibacterium spp.	1	1	1
Cellulomonas flavigena	1	1	0
Staphylococcus spp.	2	1	2
Micrococcus spp.	2	1	2
Actinomycetes	3	3	2

[a]Identifications were obtained by FAME-MIS (version 4.15); (E) = Biolog GN Microplate system (Microlog version 3.50). [b]Inhibition of fungal growth was determined by dual-culture test on pigment production medium (PPM) (20 g of proteose peptone, 20 g of glycerol, 5 g of NaCl. 1 g of KNO_3, and 15 g of agar per liter, pH 7.2) incubated at 28 °C and scored for inhibition after 2 to 3 d. Each fungal x bacterial combination was done in three replicates. Twenty-five of the 75 isolates with antifungal activity suppressed both *R. solani* and *P. grisea*.

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Rice seed: source of bacteria antagonistic against rice pathogens

G.L. Xie, R.S. Pamplona, B. Cottyn, J. Swings, and T.W. Mew

More than 4,000 Gram-negative bacteria were isolated from 446 batches of l·kg rice seed samples obtained from 22 provinces in the Philippines during 1993·95. They were initially characterized by colony morphology, pathogenicity, and biochemical tests. Six hundred and fifty-two strains were further identified by Biolog, from which 133 were selected for fatty acid methyl ester (FAME) analysis. Sixteen different species or types of Pseudomonas and 24 species of 17 other genera were identified. The most predominant species observed were *P. putida* and *P. fulva.* About 17% of the strains of Pseudomonas and 2% of the nonpseudomonads were antagonistic to one or more fungal or bacterial pathogens of rice. Some *P. putida* strains showed both antagonistic and plant growth-promoting (PGP) effects. Thus, rice seed not only harbors pathogens but is also a source of biological control and PGP agents.

Rice seed harbors a dozen plant pathogenic bacteria (Goto et al 1988, Mew and Ezzuka 1987, Ou 1985). These bacterial pathogens cause a range of rice diseases. Some are very destructive whereas others have no effect on rice crop growth. *Xanthomonas oryzae* pv. *oryzae* and *Acidovorax avenue* subsp. avenue, the causal organisms of bacterial blight and bacterial brown stripe, respectively, are known to be widely distributed throughout rice-growing countries worldwide (Goto 1992, Mew and Misra 1994, Mew et al 1990). *Pseudomonas fuscovaginae,* the causal organism of bacterial brown sheath rot, was first reported in Japan (Tanii et al 1976) and later found to be carried by rice seed in many countries (Zeigler 1987). Initially, *P fuscovaginae* was thought to cause the disease only in the temperate environment because the predisposing factors of bacterial brown sheath rot appear to be related to low temperature at 20 °C in the northern part of Japan (Hokkaido Island) and also at high altitudes. Later, the bacteria were reported to he isolated from seed from subtropical and tropical regions (Cottyn et al 1996b, Fang and Ren 1992, IRRI 1991, 1992, Zeigler 1987). *P fuscovaginae* was isolated from discolored rice seeds as well as from apparently healthy seeds (Cottyn et at 1996a. Xie 1996). *Burkholderia glumae,* the causal organism of bacterial grain rot, was also reported as a minor disease in Japan in 1967. But when rice production became mechanized and when seedlings were raised indoors in seedboxes, bacterial grain rot emerged as the most important seedborne disease in the country and *B. glumae* has become an important pathogen (Goto 1992, Goto et al 1988). Three decades after the first report of the disease caused by *B. glumae,* its occurrence in subtropical and tropical areas outside Japan was noted (Fang and Ren 1992, Mew and Misra 1994, Nieves Mortensen et al 1992).

These bacterial pathogens are known to be seedborne. Formerly believed to be of limited occurrence, they are now distributed worldwide (Goto et al 1988, Mew and Misra 1994). As *B. glumae* and *A. avenue* subsp. avenue are reported to be associated with sheath and grain discoloration, an earlier attempt was made to establish the pathogenic bacteria associated with rice seeds in the tropics (Cottyn et al 1996a,b). All these bacteria were frequently isolated from rice seed, with the exception of A. avenue subsp. avenue, and others seemed to cause no distinctive symptoms. It is from this study that we found many bacteria associated with rice seed that appear to show an antagonistic effect to some of the fungal pathogens of rice. So far, many plant pathogenic bacteria have been reported from rice seed, yet no information suggests that, among the microbial community of rice seed, those that possess antagonistic ability are also a potential source of biological control agents for disease management. Seed dressing with antagonistic bacteria for the control of plant pathogens is reported in several crops, including rice (Anderson and Liberta 1986,

Dileepkumar and Dube 1992, Garagulya 1988, Lee and Ogoshi 1986, Sakthivel and Gnanamanickam 1986, Zablotowicz et al 1992). *P. fluorescens* and *P. aeruginosa* isolated from rice have been evaluated for their potential as biological agents against *Rhizoctonia solani,* the causal organism of sheath blight (Gnanamanickam and Mew 1992, Mew and Rosales 1986). More recently, *Bacillus subtilis,* a soil isolate, was used on a larger scale to control the said fungal disease in China (Chen zhiyi, personal communication).

Surveys of antagonistic bacteria from rice seed are rare, but seed could be an important source of naturally occurring biological control agents that are valuable natural resources in the management of rice pathogens (Gnanamanickam and Mew 1992, Mew and Rosales 1986, Sakthivel and Gnanamanickam 1986). The present study therefore aimed to determine the extent of the antagonistic bacteria associated with rice seed in the tropics using seeds collected from the Philippines as a case study.

Materials and methods

Standard reference strains of phytobacteria and fungal pathogens and their maintenance

Fifty-two bacterial reference strains were used for comparative purposes in the identification of the rice seed isolates and were provided by the BCCM^{TM}/ LMG Culture Collection, Laboratory for Microbiology, Gent, Belgium. Cultures of fungal pathogens (*Rhizoctonia solani* and *Sarocladium oryzae*) were obtained from the culture collection maintained at the Entomology and Plant Pathology Division at IRRI, Philippines, and were maintained in potato dextrose agar (PDA) for routine laboratory testing. All rice seed isolates were maintained in peptone sucrose medium for short-term use and a selection of isolates was lyophilized for long-term storage. During this study, the senior author spent five months at the Laboratory for Microbiology, University of Gent, Belgium, to identify the selected rice seed isolates.

Rice seed sampling and processing

Rice seeds were sampled during the period between harvesting of the wet-season crop (late September to late October) and sowing of the dry-season crop (December to January of the following year). Four hundred and forty-six seed samples (1 kg each) were collected from 22 provinces in the Philippines from I993 to 1995. If moisture content was above 14.5%, the sample was dried under sunlight for 1 d. Each seed sample was maintained in a paper box and all samples were kept at 25 °C at 60% relative humidity before isolation. Isolation was done within 1 mo after sampling.

Isolation of bacteria from rice seeds

Two procedures were used to isolate the bacteria: seed washings from germinating seed and extracts from crushed seed. For germinating seed, 50 g seed from each sample was soaked in 100 mL of sterile distilled water with 0.025% Tween 20 and shaken for 48 h at 30 °C until the seed began to germinate. One milliliter of the suspension was taken to make tenfold serial dilutions and 0.1 mL was plated on King's medium B (KMB) (Mew and Misra 1994) and nutrient agar medium (NA) in four replicates. Representative and unique colonies were picked up after 3 d of incubation at 28 °C. They were purified on peptone potassium medium (PPM) (Mew and Misra 1994) agar plates and maintained on agar slants for further tests. For crushed seed, 50 g seed was crushed and suspended in 100 mL of sterile distilled water with 0.025% Tween 20 for 1 h under aeration. As with the germinating seed, tenfold serial dilutions were plated on King's medium B and NA in four replicates. Representative colonies with unique characteristics were chosen and purifed on PPM agar plates and maintained on agar slants for further tests.

Pathogenicity test

The inoculum of the tested bacterial strains was prepared from 48-h-old culture grown on PSA. The inoculum concentration was adjusted to 10^{x} cfu mL^{-1}. Prior to sowing, IR24 seeds were surface-disinfected by soaking them in 70% ethanol for 1.5 min. The seeds were then washed in sterile water 2 to 3 times. Seeds were then sown in seedling boxes until they were 21 d old and transplanted in pots filled with paddy soil collected from the field. The soil was sun-dried and pasteurized by steam. Pathogenicity was tested on 35-d-old plants by injecting the inoculum into the sheath. Symptoms were observed during a period from 3 d until 2 wk after inoculation. Negative control plants were injected with sterile distilled water.

Test for bacteria antagonistic against rice pathogens

Three hundred and three nonpathogenic bacterial isolates from rice seeds were tested for their ability to inhibit growth of three bacterial pathogens, *A. avenue* subsp. *avenae* (causing bacterial brown stripe), *B. glumae* (causing grain rot or glume blight), and *P. fuscovaginae* (causing bacterial sheath brown rot),

and two fungal pathogens, *R. solani* (causing sheath blight) and *S. oryzae* (causing sheath rot).

Against pathogenic fungi. The target fungi were grown on PDA plates and incubated for 48 h at 28 °C. A 4-mm-diameter agar disk was cut from the periphery of the fungal colony grown on the PDA plate and was transferred to the center of the PPM agar plate. One loop (4-mm diameter) of each test bacterial culture was transferred onto the PPM plate, four per plate in equal distances. The inhibition zones were measured 3 d after inoculation at 30 °C.

Against pathogenic bacteria. The target bacteria were transferred to the nutrient broth and shaken for 24 h at 28 °C. The concentration was adjusted to 10^{10} cfu mL^{-1}. The suspension was mixed with the melted PPM (1 mL per 50 mL medium) at 45 °C. The medium was poured into sterilized petri dishes (20 mL per dish). One loop (4-mm diameter) of the tested bacterial culture was spotted onto the surface of **PPM** plates, five bacterial cultures per plate, after it solidified. The inhibition zones were measured 2 d after inoculation at 28 °C.

Test of growth promotion effect of the bacteria

Bacterial strains showing an antagonistic effect against the rice pathogens were chosen to evaluate whether they promoted seed germination and seedling vigor. Ten grams of IR8 seed was soaked overnight (a simulation of the farmers' practice of soaking rice seed in water overnight before sowing) in the bacterial suspension prepared from 48-h-old cultures grown on PSA plates of tested bacterial strains. The seeds were then drained and sown in 10 rows in seedling boxes filled with 5 cm of sun-dried paddy soil prepared as described above. Seed soaked in sterile distilled water served as a check. Seed germination and color of the seedlings were observed at 7 d after sowing. Two weeks after sowing, 300 seedlings per bacterial culture were randomly selected and pulled out from the soil to measure shoot and root length together with the check. Growth promotion was measured in terms of total number of seed germinated, seedling color (greenness of the shoot), and seedling vigor as well as shoot length and root length.

Identification of bacteria from rice seeds

After purification of the strains, the identification process started with Gram staining and checking of fluorescence under the 365-nm long-wave ultraviolet light. Bacterial isolates from rice seeds were separated into several representative groups for further identification. Standard bacteriological procedures (Lelliot and Stead 1987, Mew and Rosales 1986) were used for all strains.

Biolog. The Gram-negative bacteria were further identified by a numerical taxonomic method, Biolog (Biolog Inc., 3447 Investment Blvd., Suite 3, Hayward, Calif. 94.545, USA). The Gram-negative (GN) Microplates with 96 wells were inoculated with a bacterial suspension (optical density at 590 nm of 0.2.5). The plates were incubated at 30 °C for 48 h. The Biolog GN database (version 3.5) was used to determine the identity of the isolates (Biolog Inc. 1993, Xie 1996).

Gas chromatographic analysis of fatty acid methyl esters (FAME). Pure cultures were grown on nutrient agar for 24 h at 28 °C. Grown cultures were transferred onto trypticase soy agar (TSA) plates containing 3% trypticase soy broth (TSB) and 1.5% Bacto-Agar (Difco) for 24 h at 28 °C. A loopful of cells was harvested with a sterile loop (4-mm diameter) and transferred to a test tube covered with a Teflon-lined screw cap. Extraction and preparation of FAME were performed following the method of Stead (1989). FAME profiles were obtained by gas-liquid chromatography using a model 5980a gas chromatograph (Hewlett-Packard Co., Avondale, Penn., USA), an automated sampler, a flame ionization detection system, and an integrator. FAME fingerprints were identitied by using a microbial identification system software package (MIS version 4.1.5 obtained from Microbial ID, Inc., Newark, Del., USA) and a calibration mixture of known standards.

Results

Isolation frequency of *Pseudomonas* spp. from rice seed

More than 4,000 bacterial isolates were isolated from the samples, of which 2,915 isolates were identified as belonging to the genus *Pseudomonas.* After preliminary characterization, 652 Gram-negative bacterial isolates were selected for the Biolog test. Biolog identified 16 different species or types of *Pseudomonas.* Aside from the nine species previously reported from rice plants (Cottyn et al 1996b), seven additional species or types of *Pseudomonas* were found in rice seed in the Philippines. *P. putida* Al, which was isolated from 9 out of 10 seed samples, was the predominant species. Three fluorescent species—*P. fulva. P. resinovorans,* and *P. putida* B I—were found in about 25-45% of the seed lots (Fig. 1). *P. fuscovaginae,* the causal organism of bacterial sheath brown rot, was isolated from 9.8%. of the seed samples.

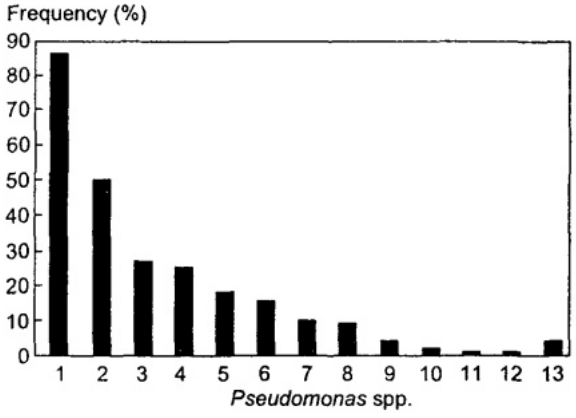

Fig. 1. Isolation frequency of *Pseudomonas* spp. from 446 seed samples of rice in the Philippines (1 = *P. putida* A1, 2 = *P. fulva*, 3 = *P. putida* B1, 4 = *P. resinovorans*, 5 = *P. aeruginosa*, 6 = *P. viridilivida* A, 7 = *P. fuscovaginae*, 8 = *P. fragi*, 9 = *P. fluorescens* C, 10 = *P. corrugata*, 11 = *P. mendocina*, 12 = *P. pseudoalcaligenes*, 13 = *P. tolaasii* + *P. fluorescens* B + *P. maculicola* + *P. fluorescens* A + *P. marginalis*).

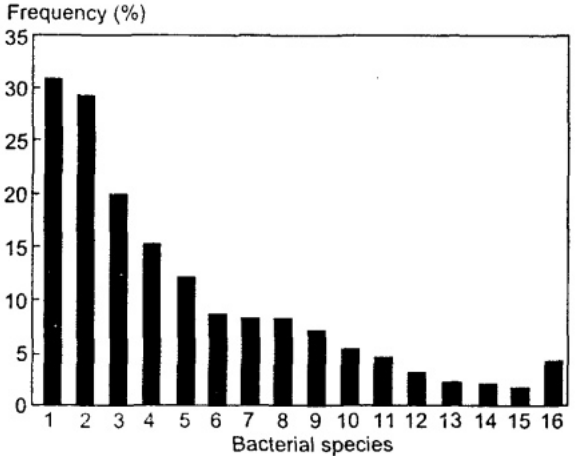

Fig. 2. Isolation frequency of nonpseudomonads from 446 seed samples in the Philippines (1 = *Acinetobacter baumannii* genospecies 2,2 = *Ac. calcoaceticus genospecies* 13, 3 = Ac. *calcoaceticus genospecies* 1, 4 = Gilardi pink Gram-negative rod, 5 =*A. avenae* subsp. *avenae*, 6 = *Enterobacter cloacae*, 7 = En. *aerogenes* and En. *gergoviae*, 8 = *X. O. oryzae* + *oryzicola*, 9 = *Stenotrophomonas maltophilia*, 10 = *Brevundimonas vesicularis*, 11 = *Pantoea agglomerans*, 12 = *Burkholderia glumae*, 13 = *Flavimonas oryzihabitans*, 14 =*X. campestris* pv. *sygonii*, 15 = *Burkholderia cepacia*, 16 = *Agrobacier rhizogenes* + *Escherichia hermanii* + *Gluconobacter cerinus* + *Klebsiella pneumoniae* + *Kluyvera ascorbata* + *Serratia marcescens* + *Sphingobacteriurn thalpophilum* (less than 1% for each species).

Isolation frequency of nonpseudomonads from rice seed

Twenty-four species of nonpseudomonads were identified. Four species each belonged to the genera *Acinetobacter Enterobacter,* and *Xanthomonas. Acinetobacter baumannii* genospecies 2, *Acinetobacter calcoaceticus* genospecies 13, *Acinetobacter calcoaceticus* genospecies 1, and Gilardi pink Gram-negative rod showed a higher isolation frequency, ranging from about 15% to 31% (Fig. 2). A. a. subsp. *avenae* (formerly *P. avenae*), the causal organism of bacterial brown stripe, had a relatively higher frequency (about 13%), whereas *B. glumae* (formerly *P. glumae*), the causal organism of bacterial grain rot, had a frequency of 2.9%.

Antagonistic effect of nonpathogenic bacteria on selected rice pathogens

After the characterization of bacterial isolates from rice seeds, including the pathogenicity test, 303 representative nonpathogenic bacteria were tested for antagonism to the two fungal pathogens and the three bacterial pathogens of rice. The highest number of antagonists (29%) was observed against *B. glumae,* followed by those against *P. fuscovaginae* (Fig. 3). The percentage of antagonists against the two fungal pathogens *S. oryzae* and *R. solani* was 13.2% and 19.8% of the total isolates tested, respectively. A. a. subsp. *avenae* had the least number of antagonists.

Two hundred and eight out of 303 strains tested were *Psedomonas* spp. and 95 were other species. About 12–1996 of the total strains antagonistic

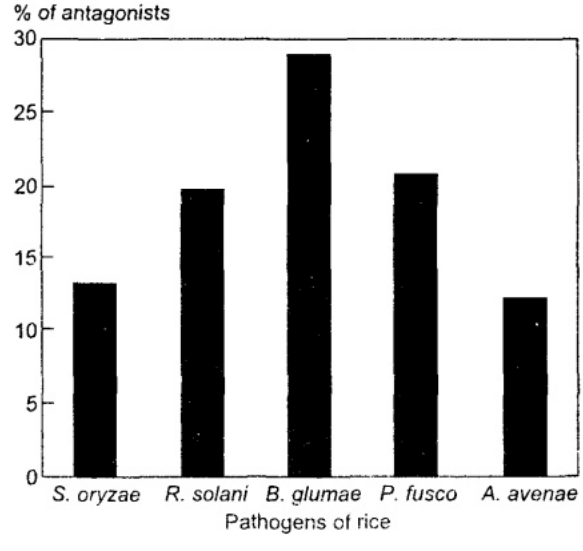

Fig. 3. Percentage of 303 bacterial isolates from rice seeds in the Philippines showing antagonism to five major pathogens of rice.

against the two fungal pathogens belong to *Pseudomonas* spp. and only 1% of the antagonists belongs to other species (Table 1). Within each species, *P. aeruginosa* and *P. resinovorans* showed

Table 1. Some nonpathogenic antagonistic bacterial strains from rice seeds showing an inhibition zone against *R. solani* and *S. oryzae,* the causal organisms of sheath blight and sheath rot of rice, respectively.

Species	Number of strains tested	Against *R. solani*		Against *S. oryzae*	
		% of antag.[a]	Inhibition zone	% of antag.	Inhibition zone
A. c. genospecies 13[b]	18	5.6	11.7	5.6	11.1
A. b. genospecies 2[c]	15	6.7	15.0	6.7	12.0
E. gergoviae[d]	13	7.7	11.2	7.7	11.6
P. aeruginosa	15	60.0	12.3–16.7	46.7	11.3–16.0
P. fluorescens C	6	50.0	15.0–16.3	0	0
P. fulva	24	29.2	11.0–14.7	8.3	11.6–13.0
P. maculicola	3	33.3	16.0	33.3	11.3
P. putida A1	101	15.8	11.0–15.3	17.0	10.0–15.2
P. putida B1	20	10.0	12.3–13.0	5.0	0
P. resinovorans	28	57.1	11.0-18.0	28.6	10.0–14.1
P. stutzeri	3	33.3	11.0	0	0
P. viridilivida A	8	25.0	14.0–15.2	12.5	15.0

[a]Percentage of antagonists to total strains in a species tested. [b]*Acinetobacter calcoaceticus* genospecies 13. [c]*Acinetobacter baumannii* genospecies 2. [d]*Enterobacter gergoviae, P. = Pseudomonas.*

Table 2. Some nonpathogenic antagonistic bacterial strains (antag.) from rice seeds showing an inhibition zone against *Pseudomonas fuscovaginae, Acidovorax avenae* subsp. *avenae,* and *Burkholderia glumae,* the causal organisms of bacterial sheath brown rot, bacterial brown stripe, and bacterial grain rot of rice, respectively.

Species[a]	No. of strains tested	Against *P. fuscovaginae*		Against *A. a.* subsp. *avenae*		Against *B. glumae*	
		% of antag.	Inhibition zone (mm)	% of antag.	Inhibition zone (mm)	% of antag.	Inhibition zone
A. c. genospecies 13	18	11.2	10.2–11.0	5.6	9.1	11.2	7.8– 9.7
A. b. genospecies 2	15	6.7	9.0–11.5	6.6	8.4	6.7	8.2
E. gergoviae	13	7.7	11.2	7.7	8.6	15.4	9.1–10.7
P. aeruginosa	15	33.3	10.0	33.3	7.6–10.2	53.3	7.0–11.3
P. fluorescens C	6	16.7	10.0–11.6	0	0	0	0
P. fulva	24	20.8	7.4–11.7	16.7	7.1–11.0	37.5	7.6–10.0
P. maculicola	3	33.3	11.0	0	0	33.3	11.3
P. putida A1	101	31.7	7.6–11.6	18.0	7.0–10.0	40.6	9.3–10.5
P. putida B1	20	15.0	7.3–10.0	10.0	8.1– 9.2	35.0	7.6–11.0
P. resinovorans	28	35.7	9.0–11.8	14.3	7.3–11.1	50.0	9.6–11.0
P. stutzeri	3	33.3	11.0	0	0	0	0
P. viridilivida A	8	12.5	8.2	12.5	9.1	25.0	8.3–9.7

[a]*A. c. = Acinetobacter calcoaceticus, A. b. = Acinetobacter baumannii, E. = Enterobacter, P. = Pseudomonas.*

the highest number of antagonists and the largest inhibition zones against *R. solani* and *S. oryzae.* Among the species, there was a relatively higher number of antagonists and larger inhibition zones against one of the two fungal pathogens. Three antagonistic bacterial strains out of 95 strains of nonpseudomonads tested were observed in species *Ac. c.* genospecies 13, Ac. c. genospecies 2, and En. *gergoviae,*. The largest inhibition zone (18 mm in diameter) was found in strain 9409 of *P. resinovorans.* Eleven strains were antagonistic to both *R. solani* and *S. oryzae,* with inhibition zones of 10–16mm in diameter.

A higher number of bacterial strains antagonistic against the three bacterial pathogens was observed from *P. aeruginosa, P. putida* A1, and *P. resinovorans.* Strains from other *Pseudomonas* spp. showed more antagonists against one of the three pathogens (Table 2). The inhibition zones against the bacterial pathogens were smaller than those against the two fungal pathogens. Fifteen strains were antagonistic against one of the fungal pathogens and against more than one of the bacterial pathogens. They belong to four species: *P. aeruginosa, P. putida* Al, *P. resinovorans,* and *P. fluorescens* C. The lowest number of antagonists was observed from nonpseudomonads against the three bacterial pathogens. Strain 10707 of *P. aeruginosa* was antagonistic against the five pathogens. Strain 9409 of *P. resinovorans* was antagonistic against four of the pathogens, except *A. a.* subsp. *avenae,* against which the lowest number of antagonists was detected.

Effect of some nonpathogenic bacteria on rice seed germination and seedling vigor

Ten nonpathogenic bacterial strains that were antagonistic to *R. solani* were tested for their effects on seedling growth. When the rice seeds were treated with a suspension of 10^9 cfu mL^{-1} of strain 10866 *(P. putida* Al) for 24 h, seed germination was uniform and the seedlings remained greener than the control (this was at 7 d after sowing in seed germination boxes with sterile distilled water only). Strain 10866 increased seedling vigor (Seshu et al 1988). Bacterization with bacterial strain 10866 resulted in significantly increased root length and root dry weight, although shoot length and shoot dry weight were not significantly different from those of the control (Table 3). Bacterial strain 10826B of *P. fulva* inhibited the growth of seed fungi in the same way that strain 10866 did. However, the other parameters did not differ significantly from those of the control. This indicates that only strain 10866 (out of the 10 bacterial isolates tested) promoted root growth aside from being antagonistic to *R. solani.*

Discussion

In a survey of Gram-negative bacteria associated with rice seeds, we collected more than 446 kg of rice seeds representing 446 seed lots from 22 rice-growing provinces in the Philippines. Two main methods. germinating isolation and crushing isolation, were used to isolate the bacteria from all seed samples. Each method has its advantages and disadvantages (Nieves Mortensen et al 1992). We have found that, with the germinating isolation method, the more fluorescent and last-growing bacteria were easily isolated even if they occurred in low density. The number of species and amount of colonies of fluorescent and fast-growing bacteria were significantly higher than those obtained by using other methods (Cottyn et al 1996b, Xie 1996). However, more colonies of the slow-growing bacteria, such as *B. glumae, A. avenue* subsp. *avenae, X. oryzae* pv. *oryzae,* and *X. oryzae* pv. *orpicola,* could be recovered with the crushing isolation method. For isolation of pathogenic bacteria, less than 5 g of rice seed (sometimes, even individual seeds are reported) was usually used with the crushing method (Cottyn et al 1996a, Mew and Rosales 1986, Nieves Mortensen et al 1992, Sharada et al 1992). In our case, we used more than 50 g of rice seed for each isolation. This provided greater chances of obtaining different bacterial species. Since one of our objectives was to survey all possible Gram-negative bacteria from discolored and nondiscolored rice seeds, it was necessary to use several isolation methods on available media with a relatively large amount of rice seeds.

The traditional methods of bacterial identification rely on some biochemical and physiological tests (Atlas and Bartha 1993, Goto 1992, Schaad 1988). These tests, though useful, are time-consuming and laborious (Janse 1995, Jones et al 1992). When handling a large number of bacteria, the use of traditional methods is difficult. Fortunately, some numerical taxonomic methods, such as Biolog (Biolog Inc. 1993, Cottyn et al 1996b, Grimont et al 1996, Jones et a1 1992) and FAME (Moss et al 1972,

Table 3. Effect of two nonpathogenic strains of *Pseudomonas* spp. from rice seeds on growth of rice seedlings and the major pathogens of rice.

Character	Control (10826B)	*P. fulva* (10866)	*P. putida* Al
Root length (cm)	6.50 b	7.12 b	15.90 a
Shoot length (cm)	5.24 ns	5.48 ns	6.22 ns
Root dry weight (g)[a]	0.22 b	0.23 b	0.41 a
Shoot dry weight (g)	0.27 ns	0.24 ns	0.23 ns
Uniform germination	No	No	Yes
Color of roots	Light brown	Light brown	White
Color of leaves	Yellow-green	Yellow-green	Green
Infection of fungi (%)	5 a	2 b	1 b
inhibition to (mm):			
Rhizoctonia solani	0	8.3	11.3
Sarocladium oryzae	0	0	0
Pseudomonas fuscovaginae	0	0	0
Burkholderia glumae	0	0	0
Acidovorax avenae subsp. *avenae*	0	0	0

[a] Root or shoot dry weight of 100 seedlings of IR8 with three replications was measured 1 wk after sowing. in a row. treatment means followed by a common letter are not significantly different by LSD at the P <0..05 level. ns = nonsignificant.

Oyaizu and Komagata 1983, Stead 1989, Vauterin et a1 1991, 1996), provide rapid and reliable approaches for identifying some bacteria. In our case, several thousand bacteria were isolated from rice seeds. We initially grouped the bacteria roughly into several groups based on colony morphology and results of simple bacteriological and pathogenicity tests. Biolog and FAME were used for further identification of the bacteria. Jones et al (1992) reported that the Biolog system (version 2.0) correctly identified more than 95% of the bacterial strains when about 900 strains of *Agrobacterium, Pseudomonas,* and *Xanthomonas* were tested. Similar results were obtained by Cottyn et al (1996b), who identified 204 bacteria from rice seeds. After comparing FAME and API 20 results with those of Biolog (version 2.0), they concluded that the Biolog system was the best method for identifying bacteria from rice. Our study used a more advanced version of the Biolog system (version 3.5). Compared with FAME (version 4.15), we found that Biolog supported a better differentiation of the bacteria from rice seeds, especially *Pseudomonas* spp. Several reports have shown that FAME supported good identification in *Xanthomonas* spp. (Stead 1989, Vauterin et al 199 I, 1996). A comparison between FAME versions 3.0 (Cottyn et al 1996a) and 4.15 (Xie et al 1996) showed that FAME has improved a lot inasmuch as many species were identified correctly. But the main disadvantage of FAME version 4.15 is that some *Pseudomonas* species have not been included in it. Several species closely related to *P. putida* could therefore not be differentiated.

Each system, however, he it Biolog, FAME, API (Cottyn et al 1996b), or Biotype-100 (Grimont et al 1996), relies on a computer-aided database to identify bacteria. The database contains a limited number of bacterial species, whereas the bacteria associated with rice seeds are very diverse. Because many bacterial species have not yet been included in the database, some pathogenic and nonpathogenic bacterial isolates could not be identified by any of the systems. These systems should be further improved to meet the requirements in this special field. Definitive phenotype differentiation of bacterial strains may require the use of more than the 95 tests available in the Biolog GN MicroPlate system (Cottyn et al 1996b).

We have isolated and characterized more than 4,000 Gram-negative bacteria from the 446 seed samples. Forty-two species or types belonging to 18 genera were identified in our study, more than half of which have not been recorded in rice (Balows et al 1992, Cottyn et al 1996a,b, Goto et al 1988, IRRI 1991, 1992, Mew and Misra 1994, Mew et al 1990, Willems et al 1992). Some isolates still have to be differentiated using other methods in spite of the 80 standard reference strains used. *P. tolaasii, P. pseudoalcaligenes,* and *P. viridilivida* A—reported to be bacterial pathogens of other crops in some countries (Balows et al 1992, Goto 1992)—were also isolated from rice seeds and found to be nonpathogenic to rice plants. However, their pathogenicity on the original crops has to be confirmed. Species of *Pseudomonas* were predominant among the 42 species or types, with 16 classified under the genus *Pseudomonas,* although several species were recently transferred to other genera. Many bacterial species have been recovered from rice plants (Cottyn et al 1996a,b, Jaunet et al 1995, Mew and Rosales 1986, Ou 1985). Our data clearly demonstrated the great diversity of bacteria associated with rice seeds. About 91% of the total bacterial isolates were nonpathogenic, whereas 9% were pathogenic and opportunistic. About 80% of the nonpathogenic bacteria from rice seeds neither affected the growth of rice plants nor inhibited the spread of pathogenic organisms. They coexisted with the rice seeds or rice plants. However, about 20% of the nonpathogenic bacteria were antagonistic to one or more pathogenic fungi or bacteria. Nine species or types of *Pseudomonas* and three species of nonpathogenic bacteria were involved in antagonistic relationships. *P. aeruginosa, P. fluorescens* C, and *P. putida* Al reportedly served as biological control agents against fungal pathogens (Gnanamanickam and Mew 1992, Mew and Rosales 1986. Pierce and Schroth 1994). In our study, some strains of these three species from rice seeds were observed to be antagonistic to several fungal and bacterial pathogens. *P. resinovnrans. P. putida* B1, and *P. viridilivida* A, with a relatively higher isolation frequency, also inhibited the growth of some pathogenic fungi and bacteria. The data showed that most of these antagonistic bacteria belonged to *Pseudomonas. P. resinovorans,* reported to be Isolated from a special soil (Balows et al 1992, Willems et al 1992), was found to be widely distributed as a rice seed contaminant in the Philippines. It holds promise as a good biological control agent because some isolates were antagonistic to both fungal and bacterial pathogens.

There is no detailed report on rice bacteria with both antagonistic and PCP effects. Dileepkumar and Dube (1992), however, reported that bacterization of chickpea and soybean seeds with a fluorescent strain of *Pseudomonas* isolated from the tomato rhizoplane increased seed germination and plant growth and

yield and reduced the number of wilted chickpea plants. Although we did not test many strains for a PGP effect (aside from the antagonistic effect), one (isolate 10866) exhibited both antagonistn to the pathogens and a PGP effect. This fluorescent isolate was identified as *P. putida.* It significantly promoted growth of the roots and inhibited growth of the fungal pathogen. This indicates that rice seeds not only harbor pathogenic bacteria but also contain a large number of antagonists and PGP strains. Rice seed is a very important source of biological control agents. It is a challenging task for scientists to manage seed-associated biological control agents with an antagonistic effect on pathogens and with a PGP effect on the crop for disease management and crop production.

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Burkholderia spp. associated with rice

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Burkholderia species such as *B. glumae, B. plantarii, B. gladioli* pv. *gladioli,* and *B. cepacia* are commonly isolated from rice plants, seeds, seedlings, bed soil, rhizosphere, weeds, or irrigation water in Japan. Some of them cause serious problems. *B. glumae* and *B. plantarii,* the causal organisms of bacterial seedling rot and bacterial seedling blight, respectively, cause serious damage on rice seedlings grown in nursery boxes. The abovementioned diseases occur mainly in northern Japan, where seedlings are grown in greenhouses and are subject to unexpected high temperature and moisture. *B. glumae* causes bacterial grain rot in fields and sometimes forms brown lesions on leaf sheaths and leaf blades. Grain rot occurs mainly in the southern part of Japan. *B. gladioli* pv. *gladioli* sometimes causes symptoms similar to those caused by *B. glumae*. *B. cepacia* is isolated from rice bed soil. It does not show a definite pathogenicity on rice, although it retards the growth of seedlings in nursery boxes.

The ecology of *B. glumae* and *B. plantarii* has been studied. The pathogens are seedborne and mainly inhabit the intercellular spaces of hull parenchyma. They proliferate rapidly during the process of seedling raising and cause disease symptoms. They decrease rapidly in population after seedlings are transplanted in rice fields. They persist, however, in the basal parts of rice plants.

B. glumae causes grain rot. Recent studies revealed that the pathogen proliferates rapidly first on anthers. On the other hand, *B. plantarii* is isolated from weeds and reservoirs that have no rice fields upstream, which indicates that *B. plantarii* can survive in the fields.

Chemicals such as oxolinic acid, kasugamycin, and copper hydroxide are being used in the process of seedling raising, and the diseases are relatively well controlled.

The occurrence of plant diseases is greatly influenced by agricultural practices as well as by meteorological factors, plant disease resistance, etc. This means that bacterial species that have been unknown or less important can emerge as important pathogens. The emergence of *Burkholderia glumae* as an important rice pathogen in Japan is one such example. It is considered to have emerged because of the introduction of the seedling-raising system using nursery boxes, which is concomitant with mechanization of seedling transplanting. Other species such as *B. plantarii, B. gladioli* pv. *gladioli,* and B. cepacia have also been isolated from rice plants, seeds, seedlings, bed soil, rhizosphere, weeds, or irrigation water. Some of them have been found to cause diseases. These *Burkholderia* species associated with rice, the diseases, their ecology, and how they emerged as important pathogens will he briefly reviewed below.

Burkholderia spp.

Burkholderia glumae

B. glumae was first isolated as the pathogen of bacterial grain rot of rice in Japan in the 1950s (Goto and Ohata 1956). At that time, the pathogen or disease was not so important. However, the rice seedling-raising system using nursery boxes became widespread in the 1970s and the pathogen was found to cause bacterial seedling rot on seedlings grown in nursery boxes (Uematsu et al 1976a,b). In 1983, bacterial grain rot was severe (Mogi et al 1984). It has now become one of the most important rice pathogens in Japan, causing grain rot mainly in the southern part and causing seedling rot mainly in the northern part, where greenhouses are needed for raising seedlings. It causes grain rot in Southeast Asia also.

The symptoms it causes on rice seedlings are characterized by conspicuous chlorosis in the basal parts of the third and sometimes the second leaves, followed by withering and brown rot. Diseased seedlings form patches on nursery boxes, which have to be thrown away, and the supply of seedlings for transplanting may run short. In fields, the pathogen causes grain rot and sometimes sheath rot (Yasunaga et al 1986).

B. plantarii

B. plantarii was isolated from a diseased rice seedling and bed soil as the pathogen of bacterial seedling blight of rice in Japan in 1982 (Azegami et al 1983, 1987). Its virulence against seedlings is very strong and it, as well as *B. glumae,* causes severe damage to seedlings in nursery boxes. The disease occurs mainly in the northern part of Japan, although the pathogen has been isolated from all over the country. The wide distribution of some good-tasting varieties produced in limited areas where apparently fine seeds are produced spreads the pathogen because these varieties are susceptible to the disease through spread of the pathogen with seeds.

The symptoms B. plantarii causes on rice seedlings closely resemble those caused by B. glumae. The soft rot symptom, however, is not as conspicuous as the one caused by B. glumae.

B. giadioli pv. gladioli

B. gladioli pv. *gladioli* was isolated from nursery bed soil in 1982 and it slightly retarded seedling growth, especially when seeds were submerged in water (Azegami 1994). It was also isolated from harvested rice seeds and caused brown rot on rice grains (Miyagawa and Kimura 1989). In 1993, when there was a cool summer and much rain in Japan, B. gladioli pv. gladioli was isolated from brown lesions on sheaths of rice in the caldera of Mt. Aso, Kumamoto Prefecture, and, when injected into rice sheaths at the booting stage, it formed brown lesions on the sheaths and caused grain rot (unpublished data of the author). B. gladioli was isolated from similar lesions in Fukuoka Prefecture also in 1995-96 (Ura et al 1996). Although reports that B. gladioli or B. gladioli pv, gladioli forms lesions on rice plants are limited, the species or the pathovar is commonly isolated from plants or rhizosphere. So, it is possible that some strains of B. gladioli or B. gladioli pv. gladioli are sometimes responsible for brown lesions or grain rot, which are considered to be caused by *B. glumae.*

B. cepacia

B. cepacia was isolated from rice seedlings and nursery bed soil. It slightly retarded seedling growth, especially when seeds were submerged (Azegami 1994); however, it has not caused serious problems.

B. vietnamiensis

B. vietnamiensis was isolated as an N_2-fixing bacterium from roots of rice growing in rice fields in Vietnam (Gillis et al 1995).

Pathogenesis

The pathogenesis of the diseases caused by *Burkholderia* spp. has not been totally clarified. However, toxic substances considered to be involved in pathogenesis have been isolated from *B. glumae* and *B. plantarii. B. glumae* produces toxoflavin and fervenulin, which produce chlorotic spots on leaves of rice seedlings and reduce the growth of leaves and roots of rice seedlings (Sato et al 1989). It also produces calcium oxalic acid (Matsuda et al 1988) and polygalacturonase (Iiyama et al 1994, 1998). which are considered to be involved in pathogenesis. The genes and proteins related to toxin production have been studied (Suzuki et al 1998a,b, Yoneyama et al 1998).

B. plantarii produces tropolone, which inhibits the growth of seedling roots and produces symptoms similar to those caused by the pathogen (Azegami et al 1985). Tropolone was first isolated as a natural product from a *Pseudomonas* sp., probably belonging to the present *Burkholderia* sp.. which was found among fungal colonies isolated from Bermuda grass and has been shown to have antibacterial and antifungal activity (Lindberg et al 1980, Lindberg 1981, Trust 1975). *B. vandii* (Urakami et al 1994), first identified as *P. gladioli* pv. *gladioli* as the pathogen of bacterial brown rot of Vanda sp. (Kijima et al 1986), also produces tropolone and produces symptoms on rice seedlings similar to those caused by *B. plantarii* (Azegami 1989. 1994).

Ecology of *B. glumae* and *B. plantarii*

Entry of pathogens into grains and seedlings These pathogens are seed-transmitted (Goto and Watanabe 1975, Kato et al 1991). On grains, they are observed mainly in the intercellular spaces of parenchyma beneath the inner epidermis of hulls and

are considered to enter hulls mostly through the stomata on the inner epidermis (Azegami et al 1988b. Tabei et al 1989).

On seedlings, they are observed mainly in the intercellular spaces of parenchyma and are considered to enter through stomata in the surfaces of coleoptiles or leaf sheaths and through wounds, and thus secondarily infect seedlings (Azegami et al 1988a).

Behavior of pathogens and occurrence of diseases

For the ecological study, selective media have been devised for *B. glumae* (Tsushima et a1 1986, Kawaradani et al 1998). A semiselective and a selective medium containing 100 ppm tropolone have been devised for *B. plantarii* (Azegami 1994, Takeuchi 1995). Polymerase chain reaction primers for detecting the pathogens have been designed (Tsushima et a1 1994, Takeuchi et al 1997).

In the process of seedling raising, seeds are soaked in cold water for several days, incubated at 30 to 32 °C for a few days to synchronize and promote germination, and then sown, usually on artificially made granulated soil enriched with nitrogen, phosphorus, and potassium. The nursery boxes are kept at 30 to 32 °C for a few days and transferred into greenhouses. The pathogens can proliferate rapidly in the process. When high temperature prevails, the pathogens cause serious problems. Seedling blight is more severe on artificial bed soil than on natural soil (Iguchi 1988).

After transplanting, the populations decrease rapidly. They persist, however, in the basal parts of rice plants (Matsuda and Sato 1987, Azegami 1990). Again, the pathogens, especially *B. glumae,* become easily detected in upper leaf sheaths and grains after the booting stage. The occurrence of grain rot is greatly influenced by rainfall at the time of panicle emergence (Seki 1958). No resistant variety for seedling rot has been found.

From studies using pathogens transformed with bioluminescence genes, it has been found that the pathogens grow especially well on anthers and grow well on dead tissues (Azegami 1996a,b). Rainfall at the hooting stage or flowering stage will enhance the opportunity for the pathogens to reach anthers and proliferate. When rice plants at the flowering stage were spray-inoculated with the transformants, anthers became luminous in 12 h and hulls became luminous 2 d after inoculation, suggesting that the transformants actively or passively migrate into hulls. The transformants were translocated upward with the growth of rice plants and by capillarity (Azegami 1997).

B. plantarii has been isolated from weeds and a maize plant in fields (Tanaka et al 1992, 1994, Tanaka and Kato 1999, Azegami et al 1993, Sato and Matsuda 1997) and irrigation reservoirs that have no rice fields upstream, indicating that the species can overwinter in fields (Miyagawa and Satou 1997, Miyagawa and Okuda 1998) as well as on harvested rice seeds.

Control

Oxolinic acid, kasugamycin, copper hydroxide, and copper hydroxy nonylbenzenesulfonate have been registered for the diseases caused by *B. glumae* and *B. plantarii.* They are applied before or at seeding, or in fields, and are effective.

Biological control agents have been sought. Two bacterial isolates (Torigoe et al 1990, Sumida and Takaya 1995, Nakaho et al 1997, Miyagawa and Okuda 1997) have been patented for the purpose, and one of them is now being evaluated for commercial adoption (Okuda 1999).

New trials to control the diseases are under study. The pool seedling-raising method is among them, in which nursery boxes are immersed in water, and it has been shown to be very effective (Katsube and Takeda 1997, Hayashi et al 1997). The oxidative potential water, pH about 2.5, has also been effective (Yamashita et al 1997).

Conclusions

The diseases caused by *B. glumae* and *B. plantarii* became important after new cultural methods such as the machine-transplanting system and the seedling-raising system using nursery boxes became widespread and mercurials were banned. High temperature, high moisture, artificially granulated soil free from antagonists, excessive nutrients, and distribution of some good-tasting varieties produced in some limited areas would favor the widespread and severe occurrence of the diseases.

The occurrence of such newly emerged or emerging diseases will be easily reduced if the newly adopted agricultural practices are replaced. Direct-sowing of rice seeds, adopted by way of trials to save costs and labor, will reduce diseases. However, the method has not spread as widely as was expected, and it cannot be forced on farmers only because of the occurrence of diseases.

These diseases are now relatively well controlled with effective chemicals. However, strains of *B. glumae* resistant to oxolinic acid have been found (Yamashita et al 1998) in some areas. As effects of chemicals do not always last long, and the reduction of agricultural inputs in agroecosystems is vital today for sustainable agriculture, alternative and ecologically sound control measures have to be available when needed. To attain this, ecological studies, a search for biological control agents, and new trials should be encouraged.

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Fungi associated with rice seeds

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A survey of seedborne fungi occurring in irrigated lowland rice in Nueva Ecija Province, Philippines, during the 1995 wet season (n = 191) revealed that *Trichoconis padwickii* infection averaged 23.4% (range 7.2–40.2%), that of *Curvularia* spp. averaged 7.4% (range 3.8–19.7%), *Drechslera oryzae* 1.8% (range 0.3–12.2%), *Sarocladium oryzae* 0.9% (range 0.0–3.3%), *Gerlachia oryzae* 0.1% (range 0.0–0.3%), and *fusarium moniliforme* 0.6% (range 0.2–1.6%). The potential "lethal seed infection" for Nueva Ecija averaged 4.6% (range 2.3–12.3%) mainly because of *O. oryzae, F. moniliforme, T. padwickii,* and *Curvularia* spp. Iloilo Province (n = 264), representing rainfed lowland rice, had 7. *padwickii* infection averaging 21.9% (range 9–35%), *Curvularia* spp. 7.0% (range 2.7–17.0%), *O. oryzae* 4.9% (range 0.1–22.0%), *S.* oryzae, 0.7% (range 0.0–2.1%), *G. oryzae* 0.3% (range 0.0–1.0%), and *F. moniliforme* 1.2% (range 0.0–10.1%). The potential lethal seed infection for Iloilo averaged 8.3% (range 4.5–15.4%) because of *O. oryzae, F. moniliforme, T. padwickii, Curvularia* spp., and *S. oryzae.* A total of 53 fungal species were recorded, 15 of which were reported to cause lesions and diseases on different parts of the rice plant.

The rice plant is affected by 56 fungal pathogens that cause diseases on the leaves, leaf sheath, stems, roots, or grains (Ou 1985). Thirty-three distinct diseases have been reported as caused by fungi. The majority are reported to be seedborne (Richardson 1979, 1981), such as the important rice diseases rice blast *(Pyricularia grisea* syn. *P oryzae),* brown spot *(Bipolaris oryzae* syn. *Drechslera oryzae),* stackburn disease (Alternaria padwickii), leaf scald *(Microdochium oryzae* syn. *Gerlachia oryzae),* bakanae *(Fusarium moniliforme),* and sheath rot (Sarocladium *oryzae)* (Kato et al 1988, Mew et al 1988).

In the Philippines, many seedborne fungi cause no distinct disease or discoloration except for false smut *(Ustilaginoiclea virens),* kernel smut *(Tilleria barclayana),* and sometimes brown spot, which in severe cases can cause diagnostic "sesame-type" lesions on the rice hulls. These seedborne fungi are detected and identified in routine seed health tests of incoming and outgoing seed lots at the Seed Health Unit of the International Rice Research Institute (IRRI) (Table 1, Mew et al 1998, Mew and Merca 1992).

The objective of this study is to quantify live fungal infection of farmer seed lots from Nueva Ecija Province, representing irrigated lowland rice about 100 km north of Manila, and considered as a major part of the rice granary of Central Luzon, and Iloilo Province, representing rainfed lowland rice in the Central Visayan Islands about 600 km south of Manila. We also aim to assess the potential loss of or reduction in planting value of seed lots (lethal seed infection). Methods used were the standard blotter test using 200 seeds per seed lot, 21 °C, 12 h near ultraviolet (NUV)/12 h darkness for 7 d, and the between-paper germination test using 400 seeds per seed lot, 28 °C, 12 h light, 12 h dark, and 14 d (ISTA 1993), after which all abnormal and dead/nongerminated seeds were incubated by the blotter method to determine lethal seed infection (LSI). Lethal seed infection is defined as infection that caused death to seeds or germinating seedlings because of a high degree of seed infection.

Materials and methods

In Nueva Ecija Province, 10 farmers each from 20 barangays (the smallest political unit in the Philippines) of 10 towns were selected for the collection of 1 kg of farmers' rice seeds. In Iloilo

Table 1. Fungi detected on rice seeds, data from IRRI's Seed Health Unit. 1983-97.

Species	Incidence[a]	Species	Incidence
Alternaria padwickii	+++	*D. longirostrata*	+
Bipolaris oryzae	+++	*D. maydis*	+
Curvularia lunata	+++	*D. rostrata*	+
C. oryzae	+++	*D. sacharri*	+
Fusarium semitectum	+++	*D. sorokiniana*	+
F: moniliforme	+++	*D. turcica*	+
Microdochium oryzae	+++	*D. tetramera*	+
Phoma spp.	+++	*Fusarium avenaceum*	+
Sarocladium oryzae	+++	*F. decemcellulare*	+
Alternaria longissima	++	*F. equiseti*	+
Aspergillus clavatus	++	*F. fusarioides*	+
A. flavus-oryzae	++	*F. graminearum*	+
A. niger	++	*F. larvarum*	+
Cladosporium sp.	++	*F. longipes*	+
Curvularia affinis	++	*F. nivale*	+
C. pallescens	++	*F. solani*	+
Epicoccum purpurascens	++	*F. tumidium*	+
Nakataea sigmoidea	++	*Graphium* sp.	+
Nigrospora oryzae	++	*Humicola* sp.	+
Penicillium sp.	++	*Leptosphaeria* sp.	+
Pinatubo oryzae	++	*L. sacchari*	+
Tilletia barclayana	++	*Masonomyces clarifomis*	+
Pithomyces maydicus	++	*Melanospora zamiae*	+
Rhizopus sp.	++	*Microascus cirrosus*	+
Ustilaginoidea virens	++	*Monodictys levis*	+
Acremoniella atra	+	*Nectria haematococca*	+
Alternaria tenuis	+	*Nigrospora sphaerica*	+
Annellophragmia sp.	+	*Papularia* sp.	+
Botrytis cinerea	+	*Penicillifer fulcer*	+
Cephalosporium sp.	+	*Periconia* sp.	+
Cercospora janseana	+	*Pestalotia* sp.	+
Chaetomium globosum	+	*Phaeotrichoconis crotolariae*	+
Chramyphora sp.	+	*Phyllosticta* sp.	+
Colletotrichum sp.	+	*P. glumarum*	+
Corynespora sp.	+	*Pyrenochaeta* sp.	+
Cunninghamella sp.	+	*P. oryzae*	+
Curvularia cymbopogonis	+	*Pyricularia grisea*	+
C. eragrostidis	+	*Septoria* sp.	+
C. inaequalis	+	*Spegazzinia deightonii*	+
C. intermedia	+	*Stachybotrys* sp.	+
C. ovoidea	+	*Stemphylium* sp.	+
C. stapeliae	+	*Sterigmatobotris macrocarpa*	+
Cylindrocarpon sp.	+	*Tetraphloa aristata*	+
Darluca sp.	+	*Trichoderma* sp.	+
Diarimella setulosa	+	*Trichosporiella* sp.	+
Diplodia sp.	+	*Trichotecium* sp.	+
Drechslera cynodontis	+	*Trichosponiella* sp.	+
D. dematioideum	+	*Tritirachium* sp.	+
D. halodes	+	*Ulocladium* sp.	+
D. hawaiensis	+	*Verticilium albo-atrum*	+

[a]+++ = frequent, ++ = moderate, + = low. Source: Mew et al(1998).

Province, 10 farmers each from 30 barangays of 10 Iloilo towns were also selected. All the farmers' seed samples were placed in paper bags, properly labeled, and brought to IRRI's Seed Health Unit in Los Baños, Laguna. Initial moisture content was measured and the seeds were fumigated to kill all storage insects. From each farmer seed sample, working samples were prepared using a riffle divider for seed health analyses. Seedborne fungi were determined by the standard blotter test using 200 seeds per seed lot, incubated at 21°C: 12 h NUV/12 h darkness for 7 d, and examined in a stereo microscope and compound microscope lor detection and identification.

Results

Blotter test results (Table 2) of the Nueva Ecija farmer seed survey (n = 191) of 10 towns with 10 farmers from 20 harangays revealed that the highest

infection caused by *A. padwickii* (syn. *Trichoconis padwickii*) is 23% (range 7.2% in brgy. 2 to 40%, SEM ± 6.97, in brgy. 15); *Curvularia* spp. 7% (range 3.8% in brgy. 4 to 19%, SEM ± 5.4, in brgy. 2); S. *oryzae* 0.9% (range 0% in brgy. 9 to 3% in brgy. 2); *Gerlachia oryzae* 0.1% (range 0% in brgy. 4 and 5 to 0.5% in brgy. 12); *D. oryzae* 1.8% (range 0% in brgy. 8 to 12.0%, SEM ± 5.45, in brgy. 10); *F. moniliforme* 0.6% (range 0.2% in brgy. 1 and 11 to 1.6% in brgy. 2).

For lethal seed infection, the average of all 20 barangays is 4.6% (range 2.25% in brgy. 5 and 6 to 12%. SEM ± 3.04, in brgy. 10). Examination of individual fungi causing LSI (Fig. 1) revealed that *F. moniliforme* affected 83% of the Nueva Ecija seed lots (range 0.25–10%), followed by A. padwickii affecting 86% of the seed lots (range 0.25-4.25%), Curvularia spp. 73% (range 0.25–2.75%), and *D. oryzae* affecting 50% of the seed lots (range 0.25–27.75%). whereas the other minor fungi causing LSI were *E solani, F. equisite, F. semitectum, Phoma* spp., *S. oryzae,* and the storage fungi.

The Iloilo farmer seed survey (n = 264) of 10 towns with 10 farmers each from 30 barangays revealed blotter test results (Table 3) of A. padwickii having an average of 22% (range 9% in brgy. 12 to 35%, SEM ± 3.98, in brgy. 20); Curvularia spp. 7% (range 3.7% in brgy. 26 to 17%, SEM ± 6.08, in brgy. 19); *S. oryzae* 0.67% (range 0% in brgy. 25 to 2% in brgy. 1); *Gerlachia oryzae* 0.3% (range 0–1% in brgy. 8); D. oryzae 4.9% (range 0.1% in brgy. 26 to 22%, SEM ± 7.71, in brgy. 23); *F. moniliforme* 1.24% (range 0% in brgy. 25 to 10%, SEM ± 9.55, in brgy. 3); Phoma spp. 0.9% (range 0.1% in brgy. 15 to 3.796, SEM ± 1.46, in brgy. 7).

The LSI for the Iloilo farmer seed survey is 8.25% (range 5% in brgy. 11 to 15.4%, SEM ± 3.7, in brgy. 7). Based on each fungal species causing LSI (Fig. 2), *F. moniliforme* affected 98% of the Iloilo farmer seed lots (range 0.25–27%), followed by A. padwickii affecting 83% (range 0.25–796). *D. oryzae* affecting 68.2% (range 0.25–17.5%), and S. oryzae affecting 22% (range 0.25–6.75%). The other fungi causing lower levels of LSI were *Gerlachia oryzae,* the Fusarium group, Phoma, Pinatubo oryzae, and the storage fungi.

When comparing LSI from the two provinces surveyed, Iloilo had a higher average (8.25%) than Nueva Ecija (4.6%) and generally had a higher maximum range of LSI except for *D. oryzae* (1 7.5%) compared with the Nueva Ecija seed lots (27.7%).

The between-paper test is suitable for the germination of rice seeds and is normally used to assess the amount of normal seedlings. It is also

Table 2. Mean and standard error of the mean (SEM) of fungi detected by blotter tests on farmers' seeds from 10 towns of Nueva Ecija Province, 1995 wet season (n = 191).

Barangay	n	LSI[a]		TP		CURV		SO		GO		DO		FM	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	10	3.73	± 0.32	8.30	± 1.96	8.40	± 2.06	0.40	± 0.16	0.10	± 0.10	0.80	± 0.24	0.20	± 0.13
2	10	6.25	± 1.05	7.20	± 2.27	19.70	± 5.36	3.30	± 1.13	0.20	± 0.20	0.80	± 0.25	1.60	± 0.61
3	10	3.83	± 0.49	21.40	± 1.70	7.50	± 0.72	0.20	± 0.20	0.20	± 0.13	1.00	± 0.14	1.20	± 0.38
4	10	2.94	± 0.29	16.50	± 2.12	3.80	± 0.63	0.40	± 0.16	0.00	0.00	0.30	± 0.15	1.10	± 0.27
5	10	2.25	± 0.41	22.70	± 3.41	7.20	± 0.89	0.80	± 0.29	0.00	0.00	1.00	± 0.68	0.20	± 0.13
6	7	2.25	± 0.62	21.96	± 2.10	5.17	± 0.40	1.97	± 0.63	0.14	± 0.14	0.71	± 0.42	0.46	± 0.20
7	10	3.08	± 0.48	16.56	± 4.26	5.22	± 0.83	1.00	± 0.31	0.11	± 0.10	0.22	± 0.13	0.22	± 0.13
8	6	2.46	± 0.48	31.83	± 4.46	5.67	± 0.71	1.33	± 0.66	0.33	± 0.21	0.00	0.00	0.17	± 0.16
9	7	3.57	± 0.74	29.7.1	±5.54	6.71	± 1.26	0.00	0.00	0.00	0.00	1.14	± 0.55	1.00	± 0.69
10	10	12.25	± 3.04	17.90	± 1.88	8.40	± 1.81	0.30	± 0.21	0.00	0.00	12.20	± 5.44	0.80	± 0.35
11	10	3.68	± 0.51	31.20	± 3.10	9.00	± 2.06	1.40	± 1.18	0.10	± 0.10	1.70	± 0.30	0.20	± 0.13
12	10	4.38	± 0.80	25.10	± 2.46	5.80	± 1.18	1.90	± 0.67	0.50	± 0.26	1.30	± 0.39	0.30	± 0.15
13	10	4.90	± 0.67	33.50	± 5.17	5.90	± 0.88	0.90	± 0.40	0.10	± 0.10	1.80	± 0.32	0.40	± 0.16
14	9	4.64	± 0.66	25.61	± 3.55	6.56	± 0.95	1.07	± 0.16	0.14	± 0.17	2.23	± 0.33	0.42	± 0.14
15	9	5.11	± 0.49	40.22	± 6.96	5.78	± 1.34	0.22	± 0.14	0.11	±0.11	1.00	± 0.33	0.22	± 0.14
16	9	8.39	± 1.44	31.89	± 5.90	11.22	± 1.57	0.33	± 0.16	0.00	0.00	7.22	± 2.05	0.56	± 0.17
17	10	3.08	± 0.45	16.80	± 2.68	5.20	± 0.84	0.70	± 0.26	0.00	0.00	0.90	± 0.37	0.90	± 0.27
18	10	5.08	± 0.71	17.90	± 2.85	6.50	± 1.08	0.60	± 0.16	0.00	0.00	0.40	± 0.16	0.40	± 0.16
19	10	5.28	± 0.98	15.80	± 3.22	6.50	± 0.87	0.30	± 0.15	0.00	0.00	0.20	± 0.13	0.30	± 0.15
20	10	4.48	± 0.47	36.10	± 3.99	8.40	± 0.80	0.60	± 0.22	0.00	0.00	0.60	± 0.26	0.40	± 0.22
Mean		4.58		23.41		7.43		0.89		0.10		1.78		0.55	

[a] LSI = lethal seed infection. TP = *Trichoconis padwickii,* CURV = *Curvularia* spp., SO = *Sarocladium oryzae,* GO = *Gerlachia oryzae,* DO = *Drechslera oryzae.* FM = *Fusarium moniliforme.*

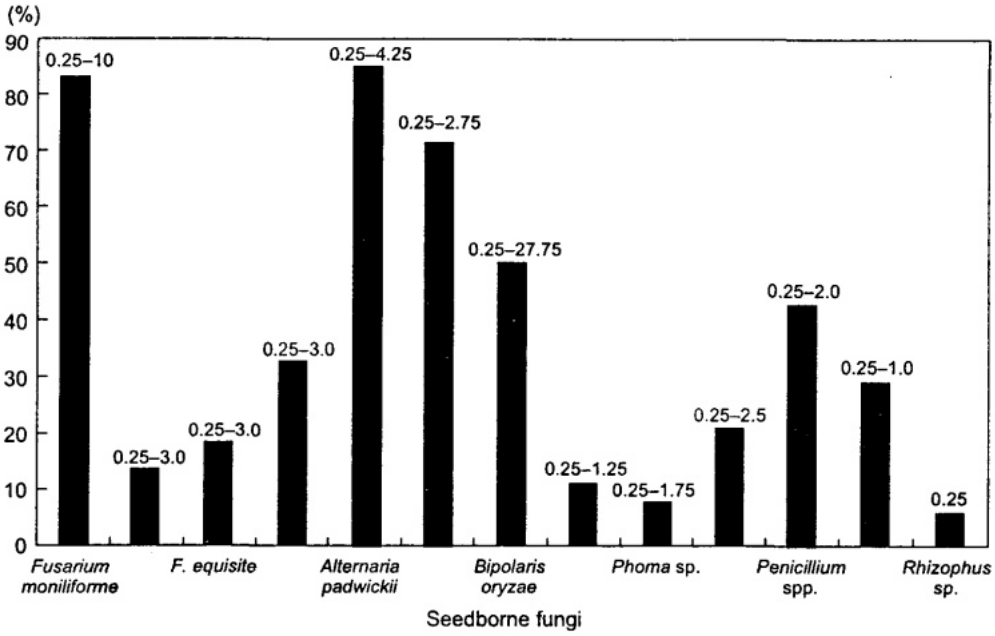

Fig. 1. Percent samples affected and range of infection causing lethal seed infection on Nueva Ecija farmers' seeds, 1995 dry season, n = 191 (between-paper blotter test, 400 seeds). Numbers above columns indicate range of infection.

suitable to some seedborne fungi that survive well in moist conditions for 14 d; hence, LSI can also be evaluated after the germination test.

Table 4 lists the seedborne fungi detected from farmers' seed lots from the provinces of Nueva Ecija and Iloilo. There are about 52 fungal species: 11 species detected frequently, 12 detected moderately, and only 29 detected at low levels. Rice seeds from a worldwide origin account for 100 species: 9 are detected frequently, 16 moderately, and 75 at low levels (Table 1).

Table 3. Mean and standard error of the mean (SEM) of fungi detected by blotter test on farmers' seed from 10 towns of Iloilo Province, 1995 wet season (n = 264).

Barangay	n	LSI[a]		TP		CURV		SO+		GO		DO		FM	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	10	7.35	± 1.55	32.90	± 3.23	8.60	± 3.52	2.10	± 0.60	0.00	0.00	1.30	± 0.15	2.30	± 0.78
2	10	6.80	± 1.39	26.80	± 4.68	6.50	± 2.04	1.30	± 0.60	0.00	0.00	2.10	± 0.57	1.00	± 0.33
3	10	7.18	± 0.99	13.10	± 1.81	5.60	± 0.86	0.40	± 0.16	0.10	± 0.10	1.00	± 0.26	10.10	± 9.55
4	10	6.63	± 0.87	22.50	± 0.77	6.50	± 0.57	0.20	± 0.04	0.20	± 0.04	1.40	± 0.10	0.80	± 0.19
5	8	7.63	± 1.61	23.00	± 4.10	9.25	± 1.94	0.63	± 0.18	0.25	± 0.16	1.38	± 0.32	3.63	± 1.73
6	10	9.40	± 2.59	26.20	± 5.46	6.00	± 3.03	0.30	± 0.21	0.30	± 0.15	4.30	± 1.45	0.50	± 0.22
7	8	15.44	± 3.67	18.63	± 3.09	8.00	± 2.34	0.63	± 0.26	0.50	± 0.19	21.25	± 4.77	1.38	± 0.84
8	6	13.42	± 2.01	26.67	± 2.64	7.00	± 3.06	0.67	± 0.33	1.00	± 0.37	22.33	± 7.71	1.50	± 0.50
9	10	5.78	± 0.99	25.70	± 3.86	8.70	± 3.02	0.60	± 0.27	0.40	± 0.16	4.00	± 1.26	0.90	± 0.28
10	10	6.90	± 1.32	18.30	± 1.55	6.80	± 0.99	0.90	± 0.28	0.40	± 0.16	8.30	± 2.04	0.60	± 0.16
11	9	5.00	± 0.42	25.89	± 5.51	5.56	± 1.33	0.44	± 0.24	0.33	± 0.17	8.33	± 1.08	0.33	± 0.33
12	7	5.19	± 0.80	9.22	± 2.33	11.00	± 2.86	0.78	± 0.36	0.67	± 0.24	2.33	± 0.71	0.78	± 0.22
13	8	8.56	± 1.09	21.50	± 5.28	5.90	± 0.77	1.00	± 0.49	0.30	± 0.15	3.20	± 0.77	1.70	± 0.47
14	7	6.71	± 1.28	24.89	± 6.31	4.33	± 1.45	0.33	± 0.17	0.11	± 0.11	5.00	± 1.78	0.67	± 0.29
15	9	11.98	± 2.80	18.00	± 3.92	5.00	± 1.22	0.10	± 0.10	0.20	± 0.13	2.10	± 0.43	0.50	± 0.17
16	7	15.13	± 2.09	16.00	± 3.82	6.50	± 2.91	0.63	± 0.26	0.13	± 0.13	5.25	± 2.45	0.75	± 0.25
17	7	8.61	± 2.00	28.86	± 7.31	7.14	± 1.55	1.71	± 0.97	0.29	± 0.18	5.00	± 1.59	2.00	± 0.49
18	a	7.47	± 2.52	17.00	± 2.59	4.50	± 0.85	1.63	± 0.46	0.13	± 0.13	4.00	± 1.55	1.50	± 0.98
19	8	5.31	± 1.16	12.89	± 3.85	17.22	± 6.08	0.56	± 0.18	0.00	0.00	1.44	± 0.29	0.56	± 0.29
20	6	4.53	± 0.46	35.30	± 3.98	2.70	± 0.47	0.70	± 0.40	0.60	± 0.16	0.90	± 0.43	0.30	± 0.15
21	4	7.57	± 1.32	26.57	± 3.56	5.14	± 2.25	2.00	± 0.87	0.57	± 0.43	9.71	± 2.75	0.29	± 0.18
22	9	12.47	± 1.61	23.89	± 1.59	4.56	± 0.56	0.56	± 0.24	0.67	± 0.33	12.44	± 1.74	0.22	± 0.15
23	7	8.93	± 2.16	33.40	± 5.05	12.40	± 3.96	0.20	± 0.13	0.10	± 0.10	10.90	± 3.98	0.90	± 0.31
24	9	11.53	± 2.16	19.56	± 5.45	8.22	± 1.68	0.22	± 0.15	0.22	± 0.15	5.67	± 1.47	0.56	± 0.24
25	4	7.80	± 0.92	14.75	± 12.43	12.75	± 8.29	0.00	0.00	0.00	0.00	1.75	± 1.44	0.00	0.00
26	8	6.07	± 0.83	11.38	± 3.20	3.75	± 1.28	0.25	± 0.16	0.25	± 0.16	0.13	± 0.13	0.50	± 0.38
27	10	7.95	± 2.06	15.60	± 2.79	6.40	± 1.54	0.10	± 0.10	0.30	± 0.15	0.70	± 0.21	1.20	± 0.79
28	8	6.25	± 1.63	17.25	± 2.37	6.75	± 4.38	0.50	± 0.27	0.13	± 0.13	0.13	± 0.13	1.13	± 0.40
29	6	5.25	± 1.19	19.14	± 2.79	5.86	± 1.77	0.43	± 0.20	0.00	0.00	0.29	± 0.29	0.43	± 0.20
30	11	8.59	± 1.86	32.45	± 4.93	2.00	± 0.52	0.18	± 0.12	0.82	± 0.23	1.00	± 0.47	0.27	± 0.14
Mean		8.25		21.91		7.02		0.67		0.30		4.92		1.24	

Table 3 continued.

Barangay	n	PH		NIG		BAC		AB	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	10	2.50	± 1.32	1.10	± 0.57	1.30	± 0.26	0.00	0.00
2	10	1.60	± 0.56	0.30	± 0.21	3.40	± 2.20	0.00	0.00
3	10	0.60	± 0.22	2.70	± 2.48	3.20	± 1.26	0.10	0.10
4	10	0.30	± 0.04	0.40	± 0.07	0.90	± 0.10	0.00	0.00
5	8	1.00	± 0.26	0.38	± 0.18	1.50	± 0.38	0.00	0.00
6	10	1.10	± 0.65	0.20	± 0.20	3.30	± 0.72	0.40	± 0.40
7	8	3.75	± 1.46	1.25	± 0.98	2.25	± 0.37	1.13	± 1.13
8	6	0.50	± 0.34	2.67	± 1.89	2.50	± 0.50	84.50	± 82.32
9	10	1.00	± 0.36	0.30	± 0.15	5.00	± 1.15	2.00	± 2.00
10	10	1.10	± 0.23	2.30	± 0.91	2.00	± 0.89	0.00	0.00
11	9	0.44	± 0.17	3.78	± 2.18	2.11	± 1.20	0.00	0.00
12	7	1.22	± 0.70	3.56	± 0.82	1.11	± 0.35	0.00	0.00
13	8	0.30	± 0.21	1.20	± 0.25	1.50	± 0.50	0.80	0.80
14	7	0.44	± 0.17	0.22	± 0.15	1.78	± 0.40	0.00	0.00
15	9	0.10	± 0.10	0.20	± 0.13	2.60	± 0.81	0.00	0.00
16	7	0.13	± 0.12	0.50	± 0.27	1.00	± 0.19	0.00	0.00
17	7	0.14	± 0.14	0.71	± 0.42	0.71	± 0.29	0.14	± 0.14
18	8	0.25	± 0.16	1.00	± 0.63	0.75	± 0.37	0.13	± 0.13
19	8	2.00	± 0.74	6.22	± 3.64	1.67	± 0.50	0.22	± 0.22
20	6	0.80	± 0.24	1.20	± 0.59	2.20	± 0.81	0.50	± 0.34
21	4	0.86	± 0.70	7.57	± 5.71	1.71	± 0.64	0.00	0.00
22	9	0.00	0.00	0.00	0.00	0.67	± 0.17	0.00	0.00
23	7	0.60	± 0.16	3.60	± 2.88	2.80	± 0.79	1.40	± 0.85
24	9	0.67	± 0.24	0.00	0.00	1.00	± 0.33	0.44	± 0.44
25	4	2.50	± 1.04	0.75	± 0.48	2.75	± 2.14	0.00	0.00
26	8	0.50	± 0.27	0.13	± 0.13	4.00	± 2.74	0.00	0.00
27	10	0.20	± 0.13	0.30	± 0.21	1.50	± 0.34	0.00	0.00
28	8	0.50	± 0.19	1.50	± 1.50	4.38	± 1.39	0.63	± 0.38
29	6	3.14	± 2.01	2.00	± 1.53	4.14	± 2.33	3.57	± 3.41
30	11	1.18	± 0.63	0.00	0.00	2.73	± 0.71	0.55	± 0.25
Mean		0.98		1.53		2.22		3.22	

[a] LSI = lethal seed infection, TP = *Trichoconis padwickii,* CURV = *Curvularia* spp. SO = *Sarocladium oryzae,* GO = *Gedachia oryzae,* DO = *Drechslera oryzae.* FM = *Fusarium monilifome,* PH = *Phoma* spp., NIG = *Nigrospora,* BAC = unidentified bacteria, AB = *Aphelenchoides besseyi,*

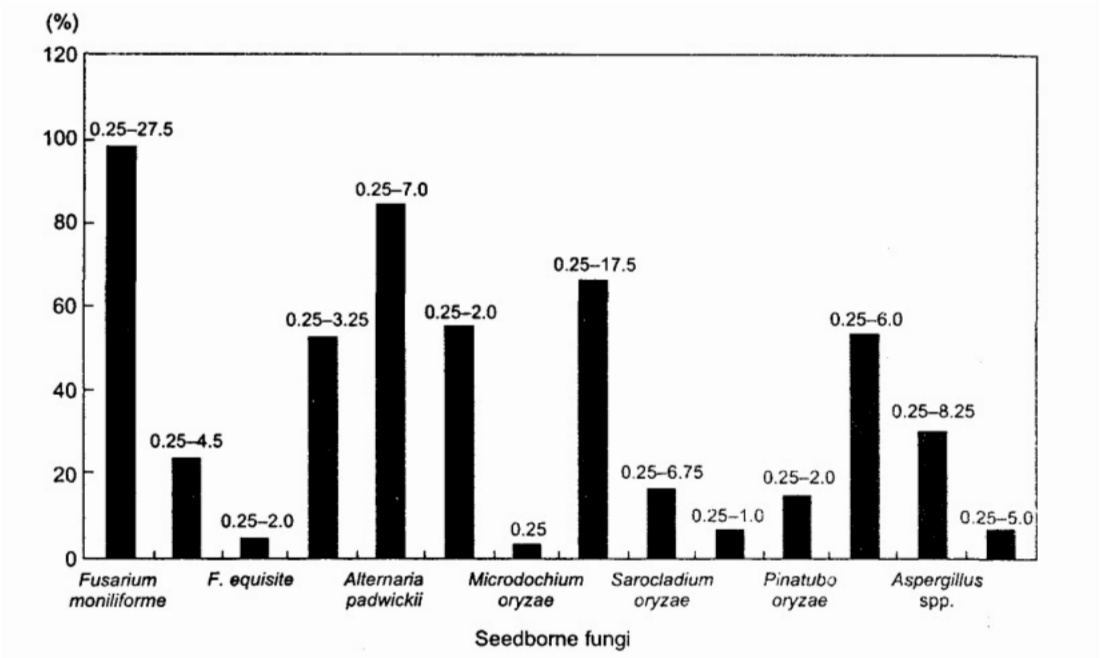

Fig. 2. Percent samples affected and range of infection by seedborne fungi causing lethal seed infection on Iloilo farmers' seeds, 1995 dry season, n = 264. Numbers above columns indicate range of infection.

Table 4. Summary of fungi detected in farmers' rice seeds from Nueva Eclja and Iloilo provinces (n = 455 seed lots, wet season 1995).

Species	Incidence[a]	Species	Incidence
Alternaria padwickii	+++	*Curvularia oryzae*	+++
Bipolaris oryzae	+++	*Fusarium semitectum*	+++
Curvularia lunata	+++	*Aspergillus* spp.	+++
Sarocladium oryzae	+++	*Penicillium* spp.	+++
Fusarium moniliforme	+++	*Rhizopus* spp.	+++
Nigrospora oryzae	+++		
Microdochium oryzae	++	*Alternaria longissima*	++
Tilletia barclayana	++	*Curvularia ovoidea*	++
Ustilaginoidea virens	++	*Cladosporium* spp.	++
Cercospora janseana	++	*Fusarium equisite*	++
Nakataea sigmoidea	++	*Pinatubo oryzae*	++
Fusarium solani	++	*Pithomyces maydicus*	++
Phoma sp.	++		
Acremoniella verrucosa	+	*Leptosphaeria* sp.	+
Alternaria tenuis	+	*Melanospora glumarum*	+
Annellophragmia sp.	+	*Microascus cirrosus*	+
Botrytis cinerea	+	*Myrothecium verrucaria*	+
Chaetomium sp.	+	*Nigrospora sphaerica*	+
Curvularia pallescens	+	*Penicillifer fulcer*	+
Curvularia affinis	+	*Phaeotrichoconis crotolariae*	+
Diarimella setulosa	+	*Pyricularia grisea*	+
Drechslera rostrata	+	*Pyrenochaeta* sp.	+
Drechslera tetramera	+	*Rhinocladiella* sp.	+
Fusarium larvarum	+	*Sterigmatobotris macrocarpa*	+
Fusarium fusarioides	+	*Trichoderma* sp.	+
Fusarium longipes	+	*Trichosponella* sp.	+
Fusarium decemcellulare	+	*Trichotecium* sp.	+
Humicola sp.	+		

[a] +++ = frequent, ++ = moderate, + = low.

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Application of Rice-Associated Biological Control Agents for Disease Control

Plant growth-promoting bacteria: mechanisms of growth promotion and disease control

K. Tsuchiya

Practices of disease control by using biocontrol agents, mainly categorized into antagonists, inducers of cross protection, or plant growth-promoting bacteria (PGPB), have been targeted to economically important soilborne diseases. The induction of host resistance with microorganisms is considered to be the most successful measure for achieving biocontrol of plant diseases. Mechanisms of disease suppression are thought to be explained by systemic host resistance induced by previously inoculated strains but not by competition or antibiosis. Cross protection is the prevention of disease development by the use of an organism similar to the real pathogen. Preinoculation of nonpathogenic *Fusarium oxysporum* for control of fusarium diseases is one well-known example.

Plant growth-promoting bacteria can suppress diseases by inducing a systemic resistance (ISR) in the plant against both root and foliar pathogens. Specific Pseudomonas strains have been shown to induce systemic resistance in various plants, as evidenced by an enhanced defensive capacity upon challenge inoculation. Bacterial determinants such as lipopolysaccharide (O-antigenic side chain), salicylic acid, and siderophores are known to act as an inducing determinant of ISR in the systems between *Pseudomonas* spp. and tobacco, radish, carnation, or *Arabidopsis.*

Continuous efforts to search for good PGPB strains are needed. Selection of biocontrol agents should focus on finding strains that occupy the same ecological niche as the pathogen. Recent advances in molecular technologies have improved procedures for evaluating PGPB activity through detection or characterization methods. Little is known, however, on the role of PGPB in antibiosis, induced resistance, and growth promotion. Future progress in using PGPB for disease control in agriculture will require additional basic and applied research.

Research on and practical application of microorganisms for biological control of plant diseases have accelerated sharply since the 1980s with an increase in public concern about the side effects of agricultural chemicals as well as global trends that aim at low-input and sustainable agriculture. So far, most research on and practices of disease control by using biocontrol agents (BCA), mainly categorized into antagonists, inducers of cross protection, or plant growth-promoting bacteria (PCPB), have targeted economically important soilborne diseases for which chemical control was inefficient. Similar efforts have also been made on aerial diseases and seedborne diseases.

Simultaneously, research on the mechanism in disease control or growth promotion, rhizosphere ecology, enhanced performance, and risk assessment of BCA or PCPB and commercializ,ation of biofungicide have been developed.

This chapter summarizes fundamental and practical research on BCA/PGPB for disease control, plant growth promotion, and application for seed health.

Biocontrol agents and disease control

On the identification and evaluation of beneficial bacteria for biological control agents of plant diseases or plant growth-promoting bacteria, there is no clear separation between growth promotion and biological control induced by them, which should be thought of as both sides of the coin.

Useful microbial BCA including PGPB have been reported so far, of which *Pseudomonas* spp., *Bacillus* spp., and *Streptomyces* sppp. were selected. Among these. fluorescent or nonfluorescent pseudomonads were reported as predominant applicants not only for research but also for practical

use, of which *P. fluorescens, P putida, P.* (=*Burkholderia*) *cepacia,* and others were used as agents.

As for the targeted plant diseases, on the other hand, they were economically important soilborne diseases that were inefficiently controlled by chemicals. In the biological control of soilborne diseases, bacterization of plant materials with those BCA was common. Bacterization has been applied to control damping-off caused by *Rhizoctonia solani* with *P. cepacia,* common scab potato caused by *Streptomyces scabies* with *P. fluorescens,* take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* with *P. fluorescens,* bacterial wilt of tomato with *P. putida,* and so on. Nonpathogenic, weakly virulent, or avirulent mutants of plant pathogenic pseudomonads have also been reported as alternative agents. A nonpathogenic mutant of *P.* (=*Burk-holderia*) *glumae* was suppressive to a causative agent of bacterial grain rot disease of rice. The binary microbe system was reported to be effective in controlling diseases. Ice-nucleation activity (INA) of naturally occurring INA bacteria has been reported on various plants.

In addition to disease suppression, plant growth promotion by fluorescent pseudomonads has been reported on various plants and some chemical compounds were revealed as activators for these mechanisms. Bacteria of the genus *Azospirillum* are nitrogen-fixing organisms that live in close association with plants in the rhizosphere and they exert a growth-promoting effect on the roots and shoots of their respective host plants by inoculation to the roots.

On the other hand, rapid advances in biotechnology during the past 10 years have produced genetically modified microorganisms to improve the ability of BCA.

Mechanisms of growth promotion/ disease control

Various mechanisms are involved in the suppression of diseases as well as growth promotion, which includes the role of antibiotics, siderophores, hydrogen cyanide, induced resistance, and growth-promoting substances.

Antibiosis based on the production of antibiotics by BCA has been considered as one of the major mechanisms in the biological control of plant pathogens, even though the efficacy of the antibiotic compounds in vitro was not always reproducible under practical application, such as the production of pyrrolnitrin or 2,4-diacetyl phloroglucinol (Phl) in culture by efficient BCA of *Pseudomonas* spp.

Mechanisms of disease suppression are thought to be explained by either antagonistic interaction by competition between a nonpathogenic strain and pathogen in infection or growth in the rhizosphere, in the rhizoplane, or in the root tissue.

Mechanisms of disease suppression are also thought to be explained by systemic host resistance induced by previously inoculated strains but not by competition or antibiosis. Induction of host resistance with microorganisms is considered to be the most successful measure for biocontrol of plant diseases.

Cross protection is the prevention of disease by the use of an organism similar to the real pathogen. Preinoculation of nonpathogenic *Fusarium oxysporum* for control of Fusarium diseases is one of the well-known examples of practical use.

Cross protection is the protection of a normally susceptible host from disease produced by inoculating an avirulent pathogen, strain, or isolate closely related to the pathogen: for example, a different *forma specialis* or an avirulent strain, which thereby becomes resistant to infection by a second, usually related, virulent pathogen in the same host. Protection may be by competition or by induced resistance.

Induced resistance is a form of cross protection in which the increased resistance of the host is caused by stimulation of the host defense system after inoculation with an isolate of the pathogen, which is avirulent or does not cause disease on that host: for example, a different forma specialis.

Plant growth-promoting bacteria can suppress diseases by inducing a systemic resistance (ISR) in the plant against both root and foliar pathogens. Specific Pseudomonas strains have been known to induce systemic resistance in various plants, as evidenced by an enhanced defensive capacity upon challenge inoculation.

Bacterial determinants such as lipopoly-saccharide (0-antigenic side chain), salicylic acid, and siderophores are known to act as an inducing determinant of ISR in the systems between *Pseudomonus* spp. and tobacco, radish, carnation, or *Arabidopsis.*

Formulation and commercialization of PGPB

To optimize the commercial we of BCA, attention must be paid to the application method. In the combination of sweet potato and Fusarium wilt, for

example, a bio-powder containing bud cells was as effective as a chemical. On the other hand, pelleted seeds incorporated with antagonistic *Pseudomonas* spp. have been developed and applied to control sugar beet damping-off disease.

Application of BCA/PGPB for seedborne pathogens

Perhaps the most efficient strategy for achieving biological control is the protection of a fixed infection court, such as a seed. The treatment of seeds with microorganisms that are beneficial to plant growth and/or that achieve disease control has been the subject of considerable investigation for many years, often with mixed results.

Seed inoculants, particularly with nitrogen-fixing rhizobial bacteria, have been used with some success to produce nitrogen gains in legumes and cereals. Others, such as *Bacillus subtilis* applied as a seed treatment, substantially increased the yields of carrots, oats, and peanuts.

Stimulation of research activity

Research activities on biological control agents or plant growth-promoting rhizobacteria have been stimulated by various kinds of international joint symposia and workshops or symposia organized by scientific societies.

Biocontrol of plant diseases has been performed extensively so far under intensive agronomic systems in the past two decades by adapting positively to introduce various modern techniques in addition to using traditional experiences. Although recent advances in molecular technologies have also improved procedures for evaluating PGPB activity through methods for detection or characterization, future progress in using PGPB to increase the applicability of disease control in agriculture will require additional fundamental and practical research.

Continuous efforts to search for good PGPB strains are needed, of which selection of biocontrol agents should focus on finding those that occupy the same ecological niche as the pathogen, such as the roots, phylloplane, and vascular system, together with the development of new assays for selection. The application of new techniques (polymerase chain reaction, restriction fragment length polymorphism, and random amplified polymorphic DNA) is also necessary, which will help not only the rapid identification of bacterial species but also specific bacterial characteristics importantly involved in either biocontrol or growth-promoting mechanisms. These techniques will also be essential for risk assessment of forthcoming genetically modified PGPB after the establishment of guidelines for their introduction into an agronomic situation. In this respect, PGPB and BCA thus obtained should be recognized and maintained as important genetic (or gene) resources that will be useful for future studies worldwide.

To increase the survival and rhizosphere colonization of PGPB strains, the importance of improving their rhizosphere or spermosphere competence, which is characterized by bacterial phenotypes, should he noted. More studies are necessary from various aspects.

Formulation and industrial fermentation technology to commercialize PGPB are also indispensable, for which integration with chemical control by combination of specific BCA with certain compatible chemicals will be one strategic approach.

The required research mentioned above cannot be accomplished without more collaboration from the public as well as private sector; therefore, more stimulation from international and domestic research institutions are necessary to facilitate what has to be done.

Methods of evaluating rice seed-associated biological control agents and plant growth-promoting bacteria

R. Pamplona, H. Barrios, L. Fernandez, and T.W. Mew

Protocols were developed to assess rice seed-associated bacteria for their potential use as biological control agents. These procedures aimed to establish the characteristics of candidate bacterial isolates from isolation up to application as a seed treatment to test plants under greenhouse conditions. The tests were as follows: in vitro antagonism test against *Rhizoctonia solani,* in vitro antagonism test against fungal pathogens that produce spores (*Pyricularia grisea, Sarocladium oryzae,* and *Fusarium moniliforme*), and seed treatment using bacterial antagonists for plant growth promotion and tapping naturally occurring microbial populations on the seed surface as a seed treatment for plant growth promotion. Of 718 bacterial isolates from seeds from Iloilo, Philippines, 32 were antagonistic to *R. solani,* 27 to *I? grisea,* 1 to *S. oryzae,* and 0 to *F. rnoniliforme.* Among these antagonists, 10 were found to be antagonistic to *R. solani* and *P. grisea* and 1 against *S. oryzae* and *P. grisea.* Ten of the antagonistic isolates induced root and shoot growth and increased dry weight in 7- and 14-d-old seedlings compared with seeds soaked in water when used as a seed treatment. Induction of roots and shoots was observed on 7-d-old seedlings soaked in seed wash from seeds collected from Rizal, Philippines, although variations were noted among seed lots collected in October 1996, April 1997, and October 1997.

A series of protocols were developed to assess rice seed-associated bacteria for their potential use as biological control agents. These were composed of different standard procedures used to establish the characteristics of a bacterial isolate as a biocontrol agent and at the same time as a plant growth-promoting bacterium. Thus, the process would allow selection of candidate bacterial isolates useful for seed treatment and as bacterial spray against fungal pathogens in aerial plant parts. On the other hand, studies on the use of seed wash were geared to harnessing the microorganisms that occur naturally on the rice seed, either on the surface or inside the seed, and using them to enhance seed germination and seedling growth. These protocols were used to assess bacterial isolates obtained from rice seeds collected from Iloilo and Rizal provinces in the Philippines.

Materials and methods

In vitro antagonism tests

Purified bacterial isolates obtained from rice seeds were tested against four rice fungal pathogens in vitro: *Rhizoctonia solani* strain LR- I, *Pyricularia grisea* strain po-6, *Fusarium moniliforme,* and *Sarocladium oryzae.* The protocols for in vitro antagonism tests against *R. solani* and sporulating fungi are given below. There were three replications per isolate. The isolates that failed to grow on pigment production medium (PPM) were not tested anymore. Antagonists were chosen according to the ability of an isolate to produce an inhibition zone. Inhibition zone diameter measurements were taken and recorded for all antagonistic isolates.

Plant growth-promotion tests

The antagonistic isolates were then chosen and used as a seed treatment. Each isolate was grown in PPM agar in flat bottles and incubated for 48 h at 30 °C. To each bottle, sterile distilled water was added, followed by shaking to harvest bacterial cells. Bacterial concentration was determined using a spectrophotometer. A suspension of each isolate was adjusted to 9–12% light transmittance by diluting the suspension and passing it through ultraviolet light at 420-nm wavelength. Serial dilution of the adjusted suspension was also done for further confirmation.

The resulting bacterial concentration of the suspension was approximately 1 x 10^9 cfu mL^{-1}.

Seeds of a high-yielding semidwarf IRRI variety, IR72, were used as the test variety. The seeds were soaked in a suspension of each isolate for 24 h. Seeds soaked in water served as a control. Two hundred soaked seeds from each isolate were then planted in 3 kg of sterile soil in trays in the greenhouse where they were grown and maintained for assessment at 7 and 14 d. Separate batches for each of the assessment dates (7 and 14 d) were prepared but the test seeds planted were taken from the same batch of seeds soaked in each of the isolates. Percent gemination, shoot and root length, and dry weight of the shoot and root were taken 7 d after planting (DAP). Shoot and root length and dry weight of the shoot and root were also assessed at 14 DAP. The number of germinated seeds was counted at 7 DAP. Percent germination was computed as the number of germinated seeds over the total number of seeds planted multiplied by 100. Seven- and 14-d-old seedlings were uprooted from each planting tray by washing the soil out with water. Root and shoot lengths of intact seedlings were measured using the millimeter scale. The root tissue was separated from the shoot by cutting. The cotyledon was included in the shoot tissues. Tissues were wrapped in aluminum foil and oven-dried at 50 °C for 7 d and then weighed. Plant growth promoters were the ones that had longer root and shoot lengths and were heavier based on dry weight measurements against the control. Analysis of variance (ANOVA) and mean comparisons were done in IRRISTAT using the random complete block design (RCBD) model with the different isolates as treatments with four replications.

Effect of seed wash on seed germination and seedlingdevelopment

Ten grams of IR64 seeds were soaked in 100 mL of sterile distilled water in a 250-mL flask and incubated for 24 h with shaking at 100 rpm. At the end of the incubation period, the liquid portion was decanted into a sterile 250-mL flask and was used as the soaking medium for seeds from the same solution. For SS1 and SS2 treatments, seeds were surface-sterilized with 66% ethanol (700 mL ethanol + 300 mL distilled water) for 1 min, rinsed three times with distilled water, and blotted dry on a sterile paper towel prior to soaking. About 200 seeds were recovered after incubation and were sowed in plastic trays (previously surface-sterilized with 95% ethanol) containing about 3 kg of fine soil. Seeds were allowed to germinate and grow in the greenhouse. The number of germinated seedlings was counted on the 7th day after sowing. About 20–30 seedlings were carefully picked per tray, with the aid of a mild flow of running water so as not to damage any plant part. Shoot and root lengths of 20 seedlings per tray were measured and recorded. ANOVA and mean comparisons were done in the SAS program using the RCBD model with four treatments and three replications for each response variable: % gemination, shoot length, and root length. Analysis was done for each seed lot.

Methodology

The following steps explain the isolation of potential biological control agents from seeds at the pre- and postgermination stages.

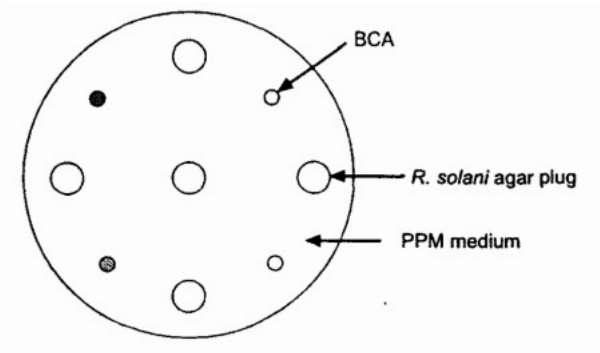
1. Weigh 50 g from the 2-kg seed sample from a given seed lot. Each seed lot corresponds to a farmer (name) from which the seed sample was obtained.
2. Soak the 50-g sample in 50 mL of sterile distilled water and then shake it for 2 h at 100 rpm to obtain the pregermination bacterial population. In addition, the postgermination bacterial population is obtained by soaking another 50-g sample, also in sterile distilled water, and incubating it in a growth chamber at 30 °C for 3 d. Do dilution plating of samples of seed washing from the two set-ups and observe until bacterial colonies appear. Purify the isolates using tryptic soy agar and store at –86°C.
3. Pathogenicity testing for these isolates is done by injecting a suspension of a 48-h-old culture of the isolate on 14- and 21-d-old rice plants. Note down the appearance of symptoms such as yellowing, browning, or wilting on each plant that corresponds to the isolate injected. Isolates that induced such plant responses are considered pathogenic and are not considered anymore for the BCA bioassay.
4. Assay the nonpathogenic isolates for their potential as biocontrol agents in vitro via the dual-culture test through a method previously described by Mew and Rosales (1986). Test the isolates against four rice fungal pathogens, *Rhizoctonia solani, Pyricularia grisea, Fusarium moniliforme,* and *Sarocladium oryzae.* Incubate the set-up at 30 °C for 48–72h. Note down and measure inhibition zones. Those isolates that

exhibit inhibitory reactions in any of the pathogens, especially those that are inhibitory to *R. solani* and *P. grisea,* will be tested further for their capability to promote plant growth.

5. Prepare a suspension of the chosen isolates (48-h-old culture at 10^9 cfu mL^{-1}). Soak test seeds in the suspension for 24 h and then plant them on sterile soil in trays. Measure % germination and hypocotyl and radicle lengths 7 d after planting. The control treatment is seeds soaked in sterile water. Set aside those isolates that will fare better against the control for the said parameters. Do not consider those that will give the same measurements as the control and those that inhibited seed development.

In vitro antagonism test for Rhizoctonia solani

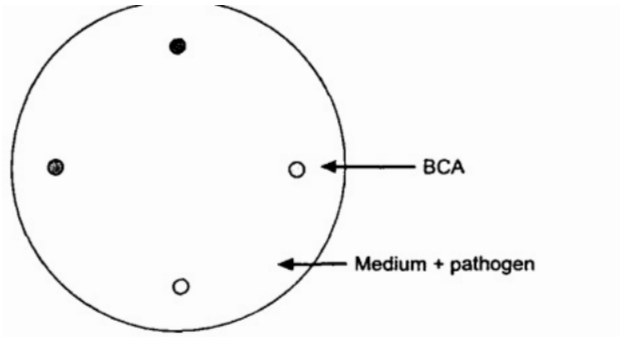
1. Place five 48-h-old agar plugs of pure culture of R. solani on the surface of PPM agar in petri plates. (Pour PPM agar onto sterile petri plates at least one night prior to use.) Place four plugs on four equidistant areas of the plate and one plug at the center.
2. Put a loopful of a 48-h-old pure culture of the bacteria in between the agar plugs. Place four different bacterial isolates on each plate.
3. Incubate for 24 to 48 h at 30 °C until inhibition zones appear.
4. Measure the inhibition zone diameter.



Dual-culture test for sporulating fungi (e.g., P. grisea, F. moniliforme, S. oryzae)

1. Melt 1 L of PPM agar and maintain at 45 °C.
2. Prepare a 50-mL spore suspension of the test pathogen. The test pathogen should be in pure culture and in a stage of active sporulation. Adjust the spore suspension at 50,000 spores mL^{-1}.
3. Add the spore suspension to melted PPM and shake carefully to evenly distribute the spores in the melted medium. Avoid vigorous shaking to prevent formation of bubbles.
4. Set aside to allow the "seeded" medium to congeal.
5. Place a loopful of a 48-h-old pure culture of the bacterial isolate on four equidistant areas on the surface of the medium.

All methods are to be used in an isolation hood under aseptic conditions. Allow the seeded medium to harden for at least 5 h after pouring for moisture to evaporate from the medium so as to prevent the formation of spreader bacterial colonies.



Effect of seed wash on seed germination and seedling development

1. Weigh 10 g from a 2-kg seed lot. Each seed lot corresponds to a farmer (name) from which the seed sample was obtained.
2. Label this sample as sample I[1] . Soak this in 100 mL of sterile distilled water in a 250-mL Erlenmeyer flask and then shake for 24 h at 100 rpm.
3. Separate the seeds from the seed washing. Transfer the seed washing of sample 1 into a sterile 250-mL Erlenmeyer flask. Also plant the seeds of sample 1 in sterile soil in a tray. Assess % germination and hypocotyl and radicle lengths after 7 d.
4. Surface-sterilize 10 g of seed of sample 2[2]. To surface-sterilize, use 10% ethanol and then submerge the seeds for 3 min and rinse three times using sterile distilled water. Dry the seeds with sterile paper towels.

[1]Sample name is arbitrary and is only used here to describe the procedure.
[2]Sample 2 serves as test seeds. Seeds from the same seed lot or other test cultivars can be used for this study.

5. Soak sample 2 in the seed washing of sample 1 and then shake the set-up for 24 h.
6. Plant the seeds of sample 2 in sterile soil in a tray. Assess % germination and hypocotyl and radicle lengths after 7 d. Compare results with sample 1. Discard the seed washing.

Plant growth-promotion test for bacterial antagonists

1. Prepare a 100-mL suspension of a 48-h-old pure culture of the antagonistic bacteria grown in PPM agar in flat bottles.
2. Expose the suspension to 420-nm ultraviolet irradiation using a spectrophotometer and adjust the turbidity to 9–12% light transmittance level.
3. Do serial dilution of the bacterial suspension to confirm the approximate bacterial cell concentration. An approximate concentration of 10^9 cfu mL^{-1} is the optimum level that should be applied for seed treatment.
4. Soak 10 g of test seeds in the bacterial suspension for 24 h. Seeds soaked for 24 h in water will serve as a control.
5. Plant the BCA-treated seeds in 2 kg of sterile soil in plastic plant boxes and maintain in the greenhouse. Prepare two sets of planting boxes with sterile soil, one to be planted with 100 BCA-treated seeds and assessed 7 d after sowing (DAS) and the other to be planted also with BCA-treated seeds but assessed at 14 DAS. For the 7- and 14-d set-ups, use the same set of seeds as in step 4.
6. Obtain % germination of seedlings on the two set-ups (7 and 14 DAS) at 7 DAS. Percent germination is obtained by dividing the number of germinated seeds by the total number of seeds planted in each set-up.
7. Upon assessment at 7 and 14 DAS, uproot 20 seedlings at random from each set-up by passing a gentle flow of water into the planting boxes to loosen the soil and carefully removing the seedlings. Also wash off soil particles adhering to the root and shoot tissues of the seedlings.
8. Measure the root and shoot length of 7- and 14-d-old seedlings using the millimeter scale.
9. Separate the root tissue from the shoot tissue of each seedling (7 and 14 DAS). Regard the cotyledon as part of the shoot.
10. Wrap the 20 shoot and root tissues of 7- and 14-d-old seedlings with aluminum foil and put them in an oven set at 50 °C for 7 d.
11. Weigh the shoot and root tissues.
12. Compare the root and shoot length and root and shoot dry weight values of BCA-treated seeds with the roots and shoots soaked in water. Perform a statistical analysis of all the obtained values to validate the results. Values above those of the control are regarded as plant growth promoters, whereas values lower than those of the control are inhibitory to plant growth and those that are the same as those of the control have no effect on plant growth.

Results

In vitro antagonism test

Out of the 718 isolates tested from Iloilo seeds in vitro, 32 were inhibitory to *R. solani,* 27 were inhibitory to *P. grisea,* 1 was inhibitory to *S. oryzae,* and none was found inhibitory to *F. moniliforme.* Among these isolates, 10 were inhibitory to both *R. solani* and *P. grisea.* The single isolate that was antagonistic to *S. oryzae* could also inhibit *P. grisea* in vitro.

Plant growth-promotion test

Ten isolates were found to increase shoot and root length and at the same time increase dry weight of tissues as a result of assessments made at 7 and 14 DAP against the control. These were C-135, C-79, C-146, C-86, C-23, C-111, C-37, C-133, C-26, and C-69. See Figures 1–5.

Effect of seed wash on seed germination and seedlingdevelopment

Seed washings of4 and 11 seed samples collected during the 1996 and 1997 dry seasons, respectively, showed potential for enhancing seedling growth, with shoot lengths varying significantly compared with the control. In addition, seed washings of one sample from the 1996 wet-season harvest showed significant enhancement of root length. For the 1997 dry-season seed samples, 9 samples showed potential for enhancing root length; however, only 3 samples showed potential for enhancing root length and shoot length.

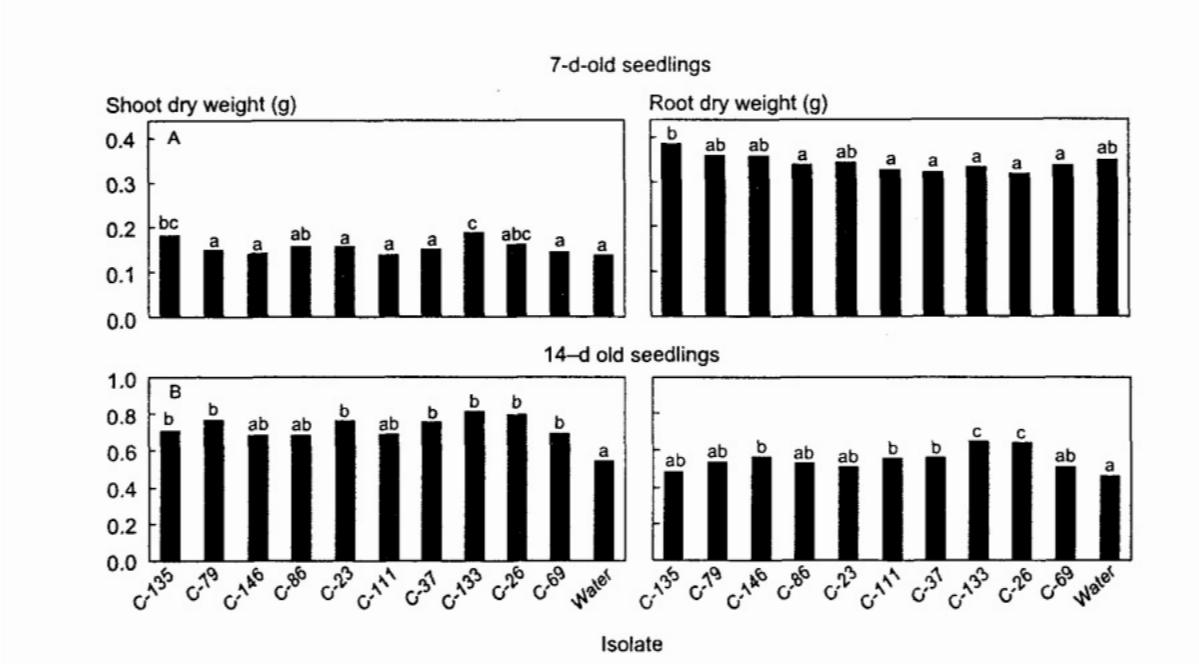


Fig. 1. Mean shoot and root dry weight of 7- (A) and 14-d-old (B) IR72 seedlings seed-treated with various biocontrol agents obtained from seeds collected from Iloilo, Philippines. Mean values sharing common letters are not significantly different at the 5% level by Duncan's multiple range test.

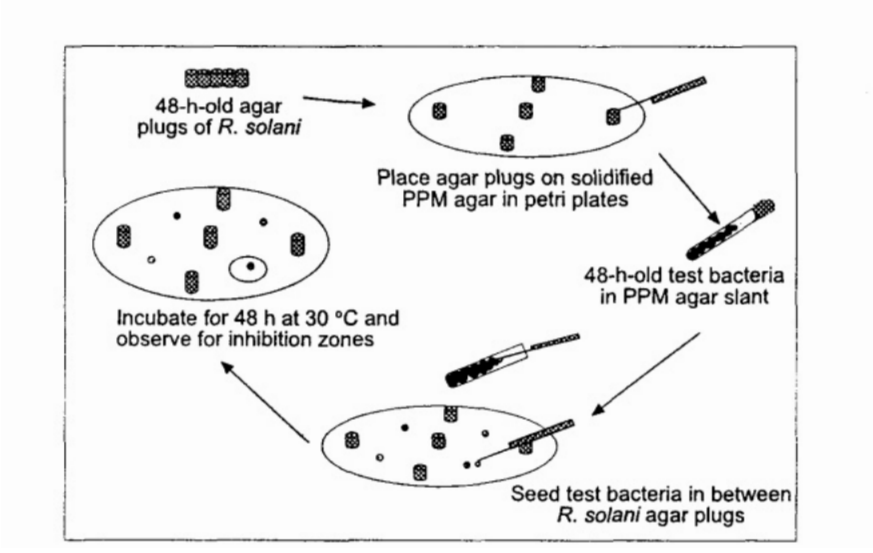


Fig. 2. Steps in in vitro antagonism test for *R. solani.* PPM = pigment production medium.

Summary and conclusions

These protocols are currently being used at IRRI in the routine assay of seeds and isolates obtained from seeds. So far, they have been useful and efficient. They could be adapted by other institutions screening for BCA on seeds or from other components of the rice ecosystem. Slight modifications in the isolation and purification procedures will have to be made according to the type of sample. Though these protocols may seem to be expensive and labor-intensive, the accuracy of the results outweighs any disadvantages.

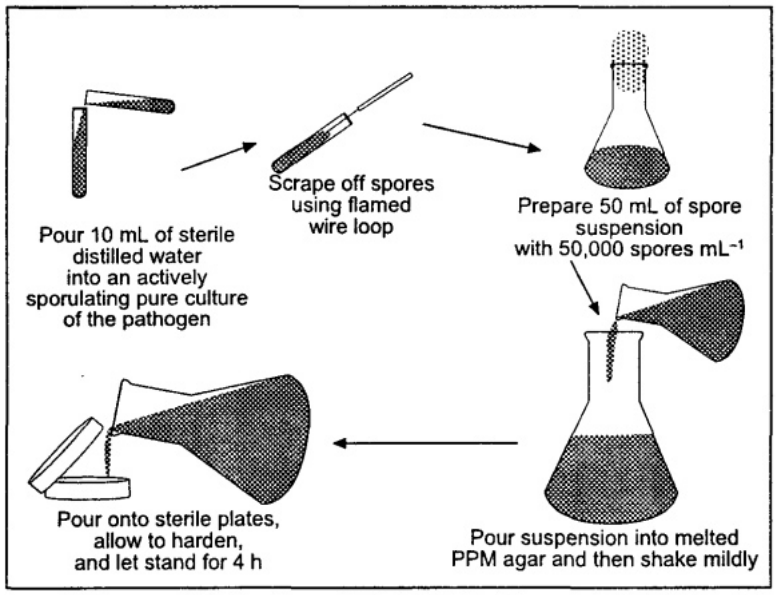


Fig. 3. In vitro antagonism test for fungi that produce spores. PPM = pigment production medium.

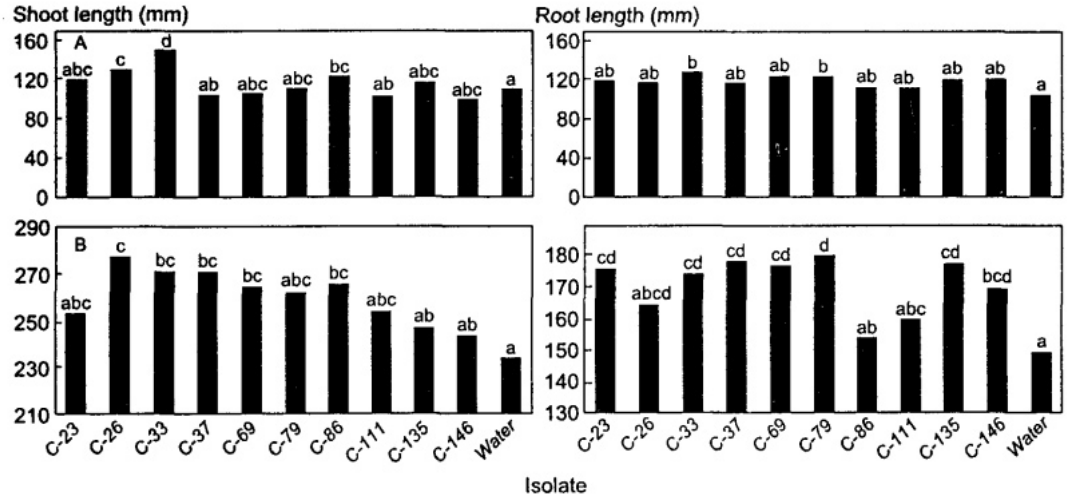


Fig. 4. Mean shoot and root length of seed-treated 7- (A) and 14-d-old (B) IR72 seedlings using various biocontrol agent Isolates from seeds collected from Iloilo, Philippines. Mean values sharing the same letters are not significantly different at the 5% level by Duncan's multiple range test.

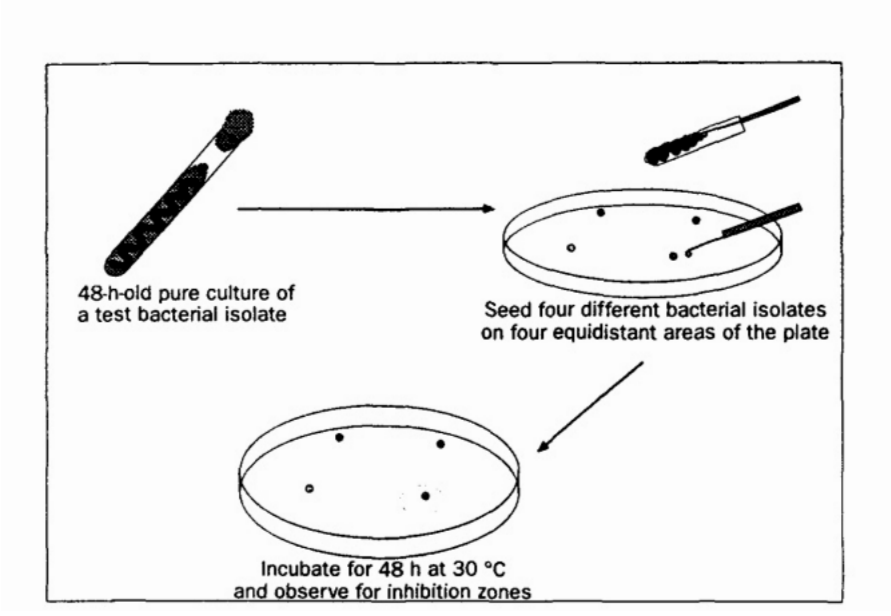


Fig. 5. Second part: seeding of spore-medium mixture with the test bacterial isolate.

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Suppression of sheath blight development in rice by rice-associated biocontrol agents

Nguyen Thi Phong Lan and T.W. Mew

A series of preliminary experiments were conducted to determine the optimum period of biological control agent (BCA) application, inoculum density of the pathogen (*Rhizoctonia solani* Kuhn), and density of BCA in relation to suppression of sheath blight (ShB) development in rice. Two strains of BCA were used: *Pseudomonas fluorescens* strain 7-14 and *Bacillus subtilis* strain B-916. The BCA were tested at five different application periods in relation to pathogen inoculation (T1 = 2 d before, T2 = 1 d before, T3 = simultaneous, T4 = 1 d after, T5 = 2 d after). Results showed that the best time for antagonist application was T2. The sprays applied 1 d after as well as simultaneously also gave good results. The results suggest that antagonists could be used for biocontrol as a preventive rather than a curative treatment. The BCA were also tested for controlling ShB development using different densities of the pathogen and biocontrol agent. In the stem segment test, a significant reduction in ShB severity was achieved with plants introduced with low or moderate (4 mm, 1–2 mm, or 2–3 mm of sclerotial diameter) levels of pathogen density but not with the highest (>3 mm of sclerotial diameter) density of pathogen. Similarly, in a greenhouse test, a 10^8 cfu mL^{-1} concentration of antagonistic bacteria substantially reduced ShB on rice when the plants were inoculated with moderate inoculum density of pathogen (5 g rice grain-hull mixture hill-'). However, at doubled inoculum density of pathogen (10 g RGH mixture $hill^{-1}$) inoculated, a density of BCA of approximately 10^{12} cfu mL^{-1} was required for a significant suppression of ShB.

In a separate test, 18 bacterial isolates from ShB lesions and four strains of antagonistic bacteria from rice seedlings (*P. fluorescens* strain 7-14), rice field soil (*6. subtilis* strain B-916), rice seeds (*P. cepacia* strain P6858), and rice plants (*P putida* strain 1821) were tested for antagonistic activity against *R. solani* in both stem segments and intact plants. Among the antagonists, more were found in intact rice plant tests than in rice stem segment tests. The results show that antagonistic bacteria from the ShB lesions were more effective in reducing *R. solani* infection and suppressing ShB.

Results from preliminary trials in Vietnam indicate that, to enable the introduction of a large enough number of bacterial cells (high enough to allow expression of their beneficial activities) in both greenhouse and field conditions, the strain should be mixed with chemical fungicide at a low rate. These results strongly suggest that a combination of antagonistic bacteria with chemical fungicide is needed for more efficient control, especially to prevent the spread of ShB under field conditions.

Sheath blight (ShB), caused by *Rhizoctonia solani* Kuhn (*Thanatephorus cucumeris* (Frank) Donk) anastomosis group AG I, is a major disease of rice in both temperate and tropical rice-producing countries. The introduction of high-yielding varieties has enhanced ShB incidence (Mew and Rosales 1986) because the microclimate and physiology of the modern varieties are conducive to disease development. Hori (1982) claimed that the characteristic of high tillering keeps humidity high in a hill, favoring both horizontal and vertical development of the disease. Yield losses from 10% to 20% have been reported in farmers' fields (Teng et al 1990). The use of chemicals and resistant varieties can reduce disease incidence, but the resistance of rice varieties to ShB is low. The use of resistant varieties and chemicals cannot markedly control sheath blight (Mew and Rosales 1986). The intensified use of chemicals poses a threat to productivity and the ecological balance. Biocontrol agents can therefore play an important role under these circumstances.

Microbial populations on plant surfaces fluctuate widely, depending on environmental factors, and are

negatively affected by low-moisture conditions. Introduced organisms most often die out quickly or are not maintained in numbers sufficient to be effective as biocontrol agents. Cook (1988) showed that, on every plant or plant population, microorganisms exist with the potential, if their numbers were higher, to protect the plant or plant population against disease. Several studies demonstrated the association of epiphytic bacterial populations comprising the bacterial antagonists from healthy or diseased leaf tissue; these antagonists showed considerable success as control agents against foliar pathogens. Leben (1985) suggested that it is best to look for and select antagonists with "special affinity" for the leaf or ones that would multiply on the leaf proper. To be effective, biological disease control depends not only on suitable biocontrol organisms but also on methods and strategies for introducing and maintaining the organisms in the crop (Stack et al 1988). Biocontrol methods and strategies involve timely manipulation of antagonist populations to suppress pathogens in various inoculum sources or on host plants. The ideal biocontrol introduces or promotes the antagonists only when and where they are needed or are most effective, and minimizes wasteful application of inoculum to nontargets. Antagonist populations need be no greater than those that adequately suppress the primary inoculum of the pathogen or the rate of disease progress. Critical evaluation of candidate antagonists and improved methods and strategies for manipulating populations of antagonists in crops have enhanced success in biocontrol (Anas and Reeleder 1988). These authors mentioned that successful biocontrol frequently involves improved application methods and well-developed strategies for timing and targeting of treatments. It has been suggested that plant-associated microorganisms may make better biocontrol agents because they are already closely associated with and adapted to the plant or plant part as well as the particular environmental conditions in which they must function (Cook 1993).

In this chapter, we describe briefly the ability of BCA to suppress ShB development, determining the appropriate application time for BCA on ShB suppression, and the interaction between inoculum density of the pathogen and density of the BCA on ShB development. We also compare the efficacy of BCA originated from the ShB lesion and other sources in suppressing ShB on rice. We also tried to find out optimum conditions for BCA function in order to obtain a more efficient control effect, particularly in the intensive irrigated rice system.

Table 1. Source of antagonistic bacteria used in the study with emphasis on the source of biocontrol agents originated from the sheath blight (ShB) lesion.

No.	Isolate no.	Origin	Antagonistic activity[a]
1	4084	ShB lesion	22.3
2	4097	ShB lesion	23.6
3	4103	ShB lesion	25.3
4	4241	ShB lesion	21.1
5	4261	ShB lesion	20.2
6	4274	ShB lesion	21.4
7	4298	ShB lesion	24.7
8	4348	ShB lesion	26.3
9	4349	ShB lesion	22.5
10	4351	ShB lesion	21.2
11	4355	ShB lesion	22.6
12	4358	ShB lesion	22.5
13	4361	ShB lesion	20.5
14	4363	ShB lesion	22.7
15	4364	ShB lesion	21.3
16	4760	ShB lesion	25.6
17	4761	ShB lesion	27.4
18	4766	ShB lesion	26.4
19	*Pseudomonas fluorescens* strain 7-14	Rice seedling, upland field	26.7
20	*Bacillus subtilis* strain B-916	Rice field soil	26.5
21	*P. cepacia* strain P-6858	Rice seeds	21.4
22	*P. putida* strain 1821	Rice plant	22.5

[a] Inhibition zone (mm), values represented by means of three replications.

Materials and methods

Strains of bacterial antagonists and ShB pathogen

The bacterial strains used in the experiments (Table 1) came from IRRI's Entomology and Plant Pathology Division. The strains were chosen for their good antagonist efficiency according to previous in vitro tests. The strains were lyophilized for maintenance. For stock production, they were transferred in pigment production medium (PPM) slants, incubated for 48 h at 28 °C, and stored at 4 °C. Before the experiments, the strains were tested again to control the stability of their antagonistic efficiency. *Pseudomonas fluorescens* strain 7-14 and *Bacillus subtilis* strain B-916 were highly efficient and were used in preliminary tests to evaluate the effects of epidemiological parameters (such as timing of application, inoculum density of the pathogen, and introduced populations of the agents). A strain of *R. solani* belonging to AG 1 was used in all the experiments. It was isolated from an infected plant in an irrigated IRRI field (Mew and Rosales 1986). The semidwarf, high-yielding cultivar IR72 was used in all the experiments.

Laboratory experiments

Using assays on stem segments, the effects of application of antagonistic bacteria at different timing of BCA application and inoculum density of the pathogen on suppression of ShB development were assessed. The stems of plants at 40 d after planting were used for these tests and three stem segments each 70 mm long were cut and placed on a petri plate, which contained 2–3 layers of blotter papers moistened with sterile distilled water. For inoculation, sclerotia of *R. solani* harvested from mature culture on a potato dextrose agar plate were used as an inoculum source that was placed on the base of each stem segment. For the application of BCA, bacterial strains were grown on PPM plates for 48 h at 28 °C. Bacterial suspension was prepared in these plates by adding 20 mL of sterile distilled water per plate and scraping the growth with a wire loop. The concentration of the bacterial suspension was adjusted to 5 x 10^9 colony forming units (cfu) per mL. Bacterial suspension was sprayed on the stem segments with an atomizer until the segments were completely covered by the droplets. For the incubation of samples, plates were incubated at 28 °C with alternating cycles of 12 h dark and 12 h light until the end of the test.

Disease was assessed 2 d after inoculation and at 2-d intervals until 8 d after inoculation based on the number of infected stem segments and % infected area.

Greenhouse experiments

Rice seeds of cultivar IR72 were sown in pots and placed in the greenhouse. Inoculation was done at 45 d after planting. *R. solani* inoculum consisted of a rice grain-hull (RGH) mixture colonized and prepared by culturing the pathogen in an RGH medium that contained three parts rice hull, one part rice grain, and 200 mL of water (Mew and Rosales 1986). The plants were inoculated approximately 45 d after planting by placing 5 g of ShB inoculum consisting of RGH at the base of each hill on the same day. For bacterial suspension application, two wild-type antagonistic bacteria strains, 7-14 *(P. fluorescens)* and B-916 *(B. subtilis),* were used in the experiment. Bacterial suspensions were sprayed in the afternoon between 1700 and 1800, until they ran off of the whole plant. The concentration of the bacterial suspension was adjusted to 1 x 10^9 cfu mL^{-1}.

The number of diseased tillers and infected area were assessed for each tiller by measuring lesion length at 5 d after inoculation and then at 5-d intervals until 20 d after inoculation.

Table 2. Timing of biocontrol agent *(Pseudomonas fluorescens* strain 7-14 and *Bacillus subtilis* strain B-916) application used for testing in laboratory and greenhouse experiments.

Treatment	Timing of bacterial application				
	2 DBI[a]	1 DBI	Simultaneous	1 DAI	2 DAI
T 1	+	-	-	-	-
T 2	-	+	-	-	-
T 3	-	-	+	-	-
T 4	-	-	-	+	-
T 5	-	-	-	-	+
Control (spray water)	-	-	-	-	-

[a] DBI = days before inoculation, DAI =days after inoculation, + = bacterial suspension spray, - = water spray.

The effect of timing of BCA application (the treatment specifications are similar to those in the laboratory experiment and are listed in Table 2) and the relationship between inoculum density of the pathogen and BCA application on ShB development and efficacy of using antagonistic bacteria isolated from the ShB lesions were also investigated in greenhouse conditions.

Experimentaldesign

Randomized complete block designs (RCBD) with three replications were used. For each treatment tested, three replications of four pots (1 hill pot^{-1}) per treatment were applied.

Disease assessment:

- The average lesion length per tiller was measured 5 d after inoculation and then at 5-d intervals. Infected area was estimated as % area infected = lesion size/plant size x 100.
- Disease progress was determined by measuring the area under the disease progress curve (AUDPC) of percentage of infected area of each plant or number of infected tillers.
- Disease incidence (DI%) was estimated as DI (%) = no. of infected tillers/no. of observations x 100.

Data analysis: data from these experiments were analyzed using analysis of variance (ANOVA) based on RCBD and the means of those parameters were compared using Duncan's multiple range test.

Results

Timing of antagonistic bacteria application and ShB lesion development

In assay plates, significantly reduced symptoms of *R. solani* were observed in all the stem segments sprayed with antagonists compared with the check. The lowest value of ShB lesion expansion was observed for the spray applied at 1 d before inoculation for both strains (Fig. 1A). The spray applied at 1 d after inoculation was lightly effective in reducing ShB symptoms. However, the spray applied at 2 d after inoculation was not effective in reducing ShB infected area for both strains. Similarly, the results from the intact plant test in the greenhouse also indicated that BCA application was highly effective at 1 d before inoculation (Fig. 1B). BCA application did not affect disease incidence in the plate test but gave good results in the greenhouse test with an intact rice plant (Table 3).

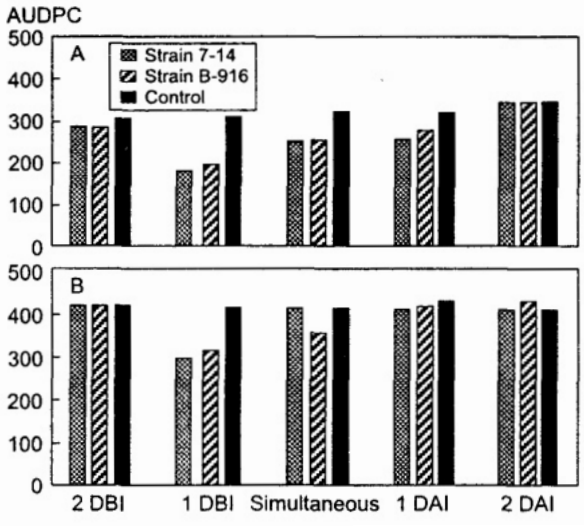

Fig. 1. Timing effect of BCA application on suppression of sheath blight development in stem segment test (A) and intact plant test (B). AUDPC = area under disease progress curve of infected area over 8 and 20 consecutive observation days under plate and greenhouse test, respectively. DBI = days before inoculation, DAI = days after inoculation.

Table 3. Effect of timing of biocontrol agent application on severity and incidence of sheath blight on rice.

Treatment	Stem segment test		Intact plant test	
	Disease incidence (%)	Percentage of infected area[a]	Disease incidence (%)	Percentage of infected area[a]
P. fluorescens strain 7-14				
2 DBI	100.0 a	281.9 b	81.5 b	429.1 a
1 DBI	100.0 a	176.8 c	68.3 c	300.8 b
Simultaneous	100.0 a	253.7 b	72.0 bc	409.5 a
1 DAI	100.0 a	250.7 b	81.5 b	414.7 a
2 DAI	100.0 a	341.3 a	100.0 a	411.7 a
B. subtilis strain B-916				
2 DBI	100.0 a	283.8 b	80.0 b	424.4 a
1 DBI	100.0 a	149.4 c	78.1 b	314.7 c
Simultaneous	100.0 a	251.7 b	77.7 b	359.4 b
1 DAI	100.0 a	279.5 b	83.3 b	414.3 a
2 DAI	100.0 a	340.2 a	100.0 a	427.6 a
Control				
2 DBI	100.0 a	303.8 a	100.0 a	430.1 a
1 DBI	100.0 a	314.9 a	100.0 a	429.7 a
Simultaneous	100.0 a	326.1 a	100.0 a	424.0 a
1 DAI	100.0 a	319.9 a	100.0 a	438.1 a
2 DAI	100.0 a	326.0 a	100.0 a	427.6 a

[a] Values represent calculated area under the disease progress curves (AUDPC) of the percentage of infected area of each plant from three replications per treatment over 8 and 20 consecutive observation days under plate and greenhouse tests, respectively. Numbers followed by the same letter within a column did not differ significantly according to Duncan's multiple range test (P = 0.01). *P.* = *Pseudomonas, B.* = *Bacillus.*

Relationship between inoculum density of the pathogen and density of BCA in suppressing ShB development

Density of the pathogen inoculum was used in the in vitro test as follows:

- TI = rice stem segment was introduced with <1 mm sclerotial diameter
- T2 = rice stem segment was introduced with 1–2mm sclerotial diameter
- T3 = rice stem segment was introduced with 2–3 mm sclerotia1 diameter
- T3 = rice stem segment was introduced with >3 mm sclerotial diameter

The ShB lesions were first observed on stem segments 2 d after inoculation in untreated segments. Lesion length increased as the incubation progressed up to 8 d after inoculation, depending on the initial inoculum density of the pathogen. Analysis of the data from the experiment indicated that average lesion length was significantly lower when the initial density of the pathogen was low. Lesion expansion over time was generally less in all treatments that were introduced with BCA than that in the unsprayed disease control. When only the BCA applied treatment was considered, BCA-introduced treatments were highly effective against ShB development at low to moderate inoculum doses of the pathogen (< 1 mm and 1–2mm of sclerotial diameter), but ineffective against *R. solani* at high initial inoculum (>3 mm of sclerotial diameter) (Fig. 2).

For the test of density of the pathogen and BCA application for ShB suppression in the greenhouse, a serial dilution of BCA suspension was saprayed on the plants that were inoculated with *R. solani* at densities ranging from 2.5 to 15.0 g of RGH mixture inoculum per hill. For the application of bacterial strains, serial dilutions of bacterial suspensions varying from 10^4 to 10^{14} cfu mL^{-1} were introduced. In the intact plant test, a high variation in ShB lesion length was observed among the controls so we used the means computed over two bacterial strains for analysis to consider the strain as a treatment. The effect of concentrations of BCA on ShB infection at different initial inocula of *R. solani* was investigated. ShB infection decreased as the concentration of biological control agents increased. In the study, densities of both BCA ranged from 10^4 to 10^{14} bacterial cells per mL and disease was related to the inoculum density of the pathogen (ranging from 2.5 to 15.0 g of RGH mixture per hill). The amount of disease reduction obtained by a BCA is influenced more by the inoculum density of the pathogen. At the highest density of the initial inoculum of pathogen (15.0 g of RGH mixture per hill), there was no significant effect of BCA introduction for all tested levels (Table 4). At the lower density of the pathogen introduced, the percentage of diseased area was significantly reduced on the plant protected with a BCA; however, this depended on the population density of the BCA applied. Significant disease suppression was observed by applying BCA at a density of 10^8 cfu mL^{-1} for the plants inoculated with 5.0 g of RGH inoculum of *R. solani.* However, at a double level of inoculum density of the pathogen (10 g of RGH mixture per hill) inoculated, a density of BCA of approximately 10^{12} cfu mL^{-1} is required for a significant suppression of ShB on rice (Fig. 3).

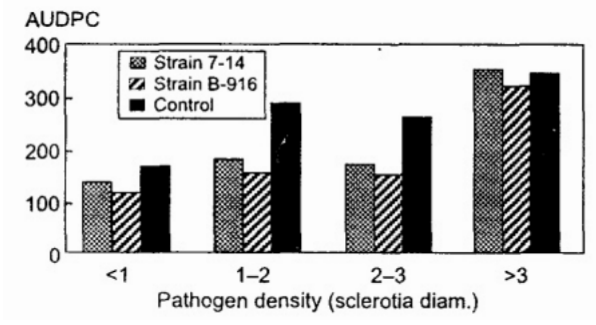

Fig. 2. Effect of initial inoculum density of pathogen on sheath blight suppression as influenced by biocontrol agent application in the plate test. AUDPC = area under disease progress curve.

Efficacy of antagonistic bacteria originated from the ShB lesion in suppressing ShB development

Out of 22 isolates used for evaluating, nine — nos. 4084,4274, 4298, 4346, 4363, 4364, 4760, 4761, and 4766—that were originated from the ShB lesion were effective in controlling rice ShB disease over time compared with the two strong antagonists from other sources in assay plates, with average lesion length ranging from 36.50 to 48.78 mm at 8 d after inoculation (DAI). Seven other isolates (4097, 4261, 4348,4351,4355, and 4358, and *P cepacia* strain 6858) were effective in the early stage, in which ShB lesion length with means lower than the check occurred at 2 DAI, but were ineffective against the pathogen later. A reduction in ShB infection was achieved with isolates 4760,4364, and 4079, *P. fluorescens* strain 7- 14, and *B. subtilis* strain B-916 at 8 DAI. However, a reduction in the incidence of ShB was not obtained in this test.

In greenhouse testing, among 18 isolates originated from the ShB lesion, 10 isolates—4097, 4 103, 4241, 4261,4274,4298,4358,4363,4760. and

Table 4. Sheath blight (ShB) development related to the initial density of the pathogen and density of biocontrol agent (BCA) application in the intact plant test.

Treatment		ShB development at 20 DAI[a]		
Pathogen (g of RGH hill^{-1})	BCA (cfu mL^{-1})	Infected area (%)	Severity (%)	Incidence (%)
2.5	0	33.9 a	34.6 a	100.0 a
	10^4	34.8 a	35.7 a	100.0 a
	10^6	33.8 a	35.2 a	100.0 a
	10^8	24.2 b	25.3 b	80.5 b
	10^{10}	25.3 b	26.4 b	77.0 b
	10^{12}	25.4 b	26.4 b	77.5 b
	10^{14}	24.1 b	24.4 b	77.7 b
5.0	0	37.9 a	39.2 a	100.0 a
	10^4	36.5 a	38.4 a	100.0 a
	10^6	36.7 a	37.7 a	100.0 a
	10^8	25.7 b	26.6 b	77.7 b
	10^{10}	24.3 b	25.2 b	77.7 b
	10^{12}	24.6 b	25.7 b	80.0 b
	10^{14}	25.4 b	27.4 b	83.3 b
10.0	0	39.4 a	38.0 ab	100.0 a
	10^4	40.0 a	40.8 a	100.0 a
	10^6	39.6 a	40.6 a	100.0 a
	10^8	38.7 a	39.7 ab	100.0 a
	10^{10}	37.0 a	36.8 b	98.7 a
	10^{12}	25.1 b	25.9 c	83.3 b
	10^{14}	25.0 b	25.5 c	83.3 b
15.0	0	41.5 a	43.9 a	100.0 a
	10^4	41.4 a	43.5 a	100.0 a
	10^6	40.8 a	42.3 ab	100.0 a
	10^8	41.3 a	41.6 ab	100.0 a
	10^{10}	39.7 a	40.8 ab	100.0 a
	10^{12}	39.9 a	41.4 ab	100.0 a
	10^{14}	38.5 a	39.6 b	100.0 a

[a] Numbers followed by the same letter within a column did not differ significantly according to Duncan's multiple range test (P = 0.01). DAI = days after inoculation, RGH = rice grain-hull mixture.

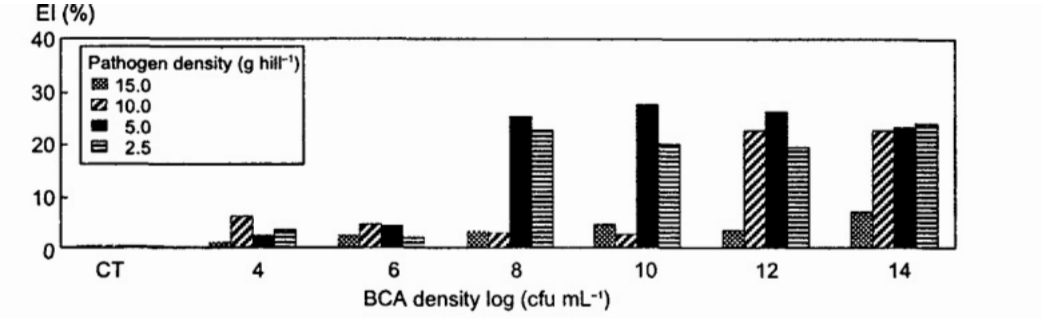

Fig. 3. Sheath blight suppression related to inoculum density of the pathogen and density of biocontrol agent (BCA) application. EI = effectiveness index (%), which was recorded at 20 d after inoculation.

4766—were significantly effective in controlling ShB of rice over time compared with the unsprayed control. Average lesion length increased from 3.30 to 35.78 mm and it was lower than that of the check, which increased from 4.51 to 52.81 mm at 5 and 20 DAI, respectively. At 20 DAI, the index of BCA efficacy was achieved with isolates 4097,4261, 4298, 4348, and 4351, *P. fluorescens* strain 7-14, and *B. subtilis* strain B-916 (Fig. 4).

Discussion

Development of good timing strategies for antagonists will be important as biocontrol advances toward commercial use. Previous reports indicated

that optimal timing of the initial treatment with antagonists differed from that of fungicides. According to Willocquet (1993, the bacterial spray applied just before inoculation led to the lowest ShB severity (30%). Recently, Chen and Mew (1998) found that the appropriate timing for spraying antagonistic bacteria was 1 d after inoculating *R. solani* when they used two strains, P. *cepacia* (P-6854) and *B. subtilis* (B-916), to control ShB of rice. In this study, results of both laboratory and greenhouse experiments indicated that suppression of ShB infection was observed in the bacterial spray applied at 1 d before inoculation and in simultaneous treatment. The most successful strategy to date involves protecting specific courts of infection on the plant by applying the antagonist before the inoculum of the pathogen arrives. Generally, the antagonist is expected to spread, or a signal induced in the plant by the antagonist is expected to be expressed locally or systemically as resistance to the pathogen. Since this approach may be too slow to protect against primary infection, it may be best suited to limiting disease development after primary infection or secondary infection. This might be the reason why the bacterial spray applied 2 DAI had no significant effect on ShB development and no reduction in disease incidence was observed in the plate tests. The results suggest that bacterial spray application should not be later than 2 d after inoculating *R. solani.* However, the best timing for introducing a BCA will depend on the particular interactions of the pathogen, introduced microorganism, and host plant.

Much of the current research on biological control focuses on understanding the mechanisms by which BCA reduce the impact of pathogen populations (e.g., antibiosis, competition, hyperparasitism, and induced resistance). Information is very limited on the complex interactions among the BCA, the pathogen, the plant, and the environment. Recently, Johnson (1994) defined various epidemiological parameters that may govern the efficacy of biological control of plant diseases. In the theoretical relationships he proposed, the degree of disease suppression by a BCA depends on the density of the agent, the density of the pathogen, how efficiently individual units of the agent render units of the pathogen ineffective, and the proportion of the pathogen population that potentially is affected by the agent. Chen and Mew (I 998) mentioned that the initial concentration of introduced antagonistic bacteria cannot be lower than 10^8 cfu mL^{-1} for controlling ShB by *B. subtilis* strain B-916 and P

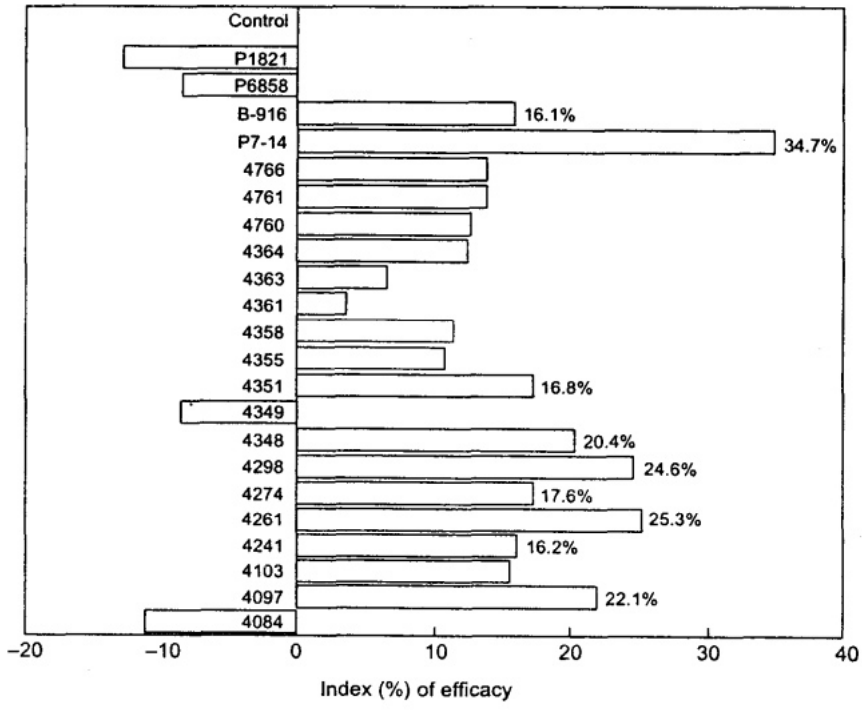

Fig. 4. Efficacy of biocontrol agents originated from sheath blight lesions on suppression in intact plant test at 20 d after inoculation.

cepacia strain P-6854. In our study, at 5 g of RGH per hill of pathogen inoculum, an inoculum dose of 10^8 cfu mL^{-1} of BCA was optimum because it suppressed disease to the same extent as a higher dose of antagonist and lower doses were less effective. When the plants were inoculated with a higher dose of inoculum of *R. solani,* the higher density of antagonist was needed to reduce disease development. However, suppression of *R. solani* by the antagonist was no longer effective at the highest dose of pathogen (15 g per hill) testing.

Results of the plate and greenhouse tests showed that there is no correlation between antagonism demonstrable in plate and greenhouse testing. Some bacterial isolates did not give good control in stem segment tests but showed a high antagonistic effect on ShB in the greenhouse, and vice versa. Mew and Rosales (1986) claimed that there is no correlation between antagonism demonstrable in culture and effectiveness in the field. In our experiments, effective antagonistic bacteria were isolated more from ShB lesions for control of ShB in the greenhouse. Two concepts need to be clearly understood: (1) lesion expansion or vertical development of the disease that may be related to primary infection (estimated by disease severity) and (2) focal point expansion or horizontal spread of the disease that may be related to secondary infection or spread of the disease epidemic (estimated by severity and incidence). So, BCA can prevent the spread of the pathogen. This might be why greenhouse tests gave better results than segment tests under optimum control conditions. In addition, there have been many reports of host interactions brought about by saprophytic microorganisms, leading to enhanced disease resistance in plants. Bacteria are well known to induce protective responses in host tissues. Since no direct effect of these treatments has been detected on the pathogen in vitro, it has been assumed that the metabolism of the host has been altered, thus enhancing its resistance to pathogen invasion (Blakeman and Fokkema 1982). The results showed that bacterial antagonists originated from ShB lesions were the most effective antagonists, providing significant and consistent disease control. This means that plant-associated microorganisms may make better biocontrol agents because they are already closely associated with and adapted to the plant or plant part as well as the particular environmental conditions in which they must function (Cook 1993).

The results of greenhouse and demonstration field trials in the Mekong Delta indicated that, to enable the introduction of a high enough number of bacterial cells to allow the expression of their beneficial activities in both greenhouse and field conditions, the BCA should be mixed with chemical fungicide at a low rate. These results strongly suggest that the combination of antagonistic bacteria with a very low rate of chemical fungicide compared with the recommended rate is needed for more efficient control effects, especially to prevent ShB expansion under field conditions (data not shown).

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Sustaining biological control in farmers' fields

N. Nilpanit, P. Arunyanart, and T.W. Mew

The efficiency and long-term effects of biological control agents (BCA) on sheath blight (ShB) disease development were demonstrated in the field in 10 successive cropping periods. Experiments were conducted in Lumlookka District, Pathum Thani Province (50 km north of Bangkok), from July 1995 to October 1999. Two bacterial isolates antagonistic to *Rhizoctonia solani* under field conditions were used in the field tests—*Bacillus subtilis* strain 90-562 and *Pseudomonas* sp. strain 90-321. An experimental field planted with seeds untreated with BCA was monitored during three continuous cropping periods from July 1995 to August 1996. At maximum tillering, sampling units (231 ShB-infected hills, 21 x 11 m) were marked with bamboo sticks at random. The sampling units with 10 ShB-infected hills were regarded as a disease focus to which the various BCA treatments were applied. Severity and incidence were measured after three BCA applications to the foci. In the 1996 wet season, two more adjacent fields were acquired, planted with BCA-treated seeds, assigned focal points, treated with BCA, and assessed for disease severity and incidence. The neighboring farmers' fields that did not receive any BCA treatments served as a check. The severity and incidence data obtained from January 1997 to October 1999 from these untreated fields did not differ significantly, although a very high variation among replications was noted. A decline in the number of disease foci was observed in the lst, 2nd, and 3rd experimental field plots after 10, 7, and 7 continuous cropping periods in each of the field plots, respectively. In the 1st field, the decline in percentage of ShB occurrence in 10 continuous cropping periods was 77.1%, 42.4%, 69.3%, 37.2%, 23.4%, 9.5%, 10.8%, 18.2%, 1.7%, and 7.4%. In an adjacent untreated field plot, disease foci declined from 100% to 4.1% in six cropping periods. This was attributed to BCA-contaminated water coming from the BCA-treated plots. A new field plot, which has not yet been treated with BCA, will be used in the 1999 dry season to confirm the observed carryover effects of BCA against ShB on BCA-treated plots.

In the rice ecosystem, many plant-associated microorganisms can be found and many of them show antagonistic activities to many fungal pathogens (Mew and Rosales 1986, Mew et al 1994). This was also demonstrated through the collaborative research network supported by the Asian Development Bank, in which the potential and prospects of these rice-associated microorganisms for disease management were assessed in both temperate and tropical environments (Mew et al 1994, Mew 1996). In Thailand, various antagonists were isolated and evaluated for their control ability against some rice fungal diseases. Considering the lack of host-plant resistance as a criterion for the target disease, sheath blight (ShB) caused by *Rhizoctonia solani* was chosen. Results from greenhouse tests showed the potential of some biological control agents (BCA) for suppressing ShB disease infection on rice plants by seed bacterization and foliar spraying. In 1994, a small-plot experiment was conducted at the Pathum Thani Rice Research Center to test the efficacy of two promising bacterial strains, *Bacillus subtilis* (no. 90-562) and *Pseudomonas* sp. (no. 90-321), against ShB disease. They were also tested as with individual bacterium, in combination with a fungicide, benomyl. The initial results indicated that the antagonists mixed individually with benomyl were very effective against sheath blight (Arunyanart et al 1995).

This experiment was carried out from July 1995 to December 1999 under the IRRI-DGIC (former BADC) project in farmers' fields in Lumlookka District, Pathum Thani Province, 50 km north of Bangkok. The objectives were (I) to test the efficacy of two promising BCA mentioned previously under a large field trial and (2) to test the long-term deployment of BCA for sustaining ShB disease management in farmers' fields.

Materials and methods

An area of 0.5 ha with naturally occurring inoculum for ShB infection was selected. In this area, because of a good irrigation system and the use of early rice varieties, farmers grow 2–3rice crops per year,

Effect of BCA on sheath blight disease

From July I995 to May 1997 (5 crop seasons), the study was first conducted on field 1 using normal rice seeds of varieties Supanburee 1 and Poung-ngeon for 2 and 3 crop seasons, respectively. At the tillering stage, 231 (21 × 11) sampling units for disease assessment were marked with bamboo sticks at random at 3 × 3-m spacing. Ten rice plants were labeled in each sampling unit for assessing ShB disease development. The sampling units in which ShB disease initially occurred were recorded as "disease foci." To test the efficacy of two BCA strains, *Bacillus subtilis* (no. 90-562) and *Pseudomonas* sp. (no. 90-321), the mixture of the two BCA as well as combinations of BCA and benomyl were applied on the 10 labeled rice plants of the disease foci. The treatments were (1) sprayed with mixed BCA, (2) sprayed with combinations of mixed BCA and benomyl, (3) sprayed with benomyl, and (4) sprayed with water as a control. They were laid out in a completely randomized design. Since all treatments were applied on disease foci, replications varied from crop to crop depending on the number of disease foci. Each treatment was applied 3 times at 2-wk intervals. Data on percentage incidence ([infected plants/total plants] × 100) and percentage of severity ([highest lesion height/plant height] × 100) were recorded. Data were analyzed using analysis of variance. Each crop season was analyzed separately.

Furthermore, during September 1996-May 1997 (2 crop seasons), the experimental fields were expanded into two more adjacent fields, fields 2 and 3. Some 121 (11 × 11) sampling units marked by bamboo sticks were sampled systematically on each field with 3 × 3-m spacing. Rice seeds of variety Poung-ngeon were soaked with mixed BCA 24 h before sowing in both fields. In addition to seed treatment, the same manner of four-treatment applications and data collection for ShB as described in field 1 was used in field 2, whereas field 3 was left as an observation field.

Deployment of BCA for controlling ShB disease

To determine the long-term effect on ShB disease control, the numbers of sampling units with ShB infection or disease foci were assessed as a parameter for disease incidence throughout the study.

Field 1. During the BCA efficacy trials (July 1995-May 1997), the number of disease foci in 5 consecutive crop seasons was recorded. The study was continued in the same field for 7 more crop seasons, using variety Poung-ngeon. However, mixed BCA were applied on disease foci of the two prior crop seasons (6th and 7th). In all crop seasons, 231 sampling units were taken randomly and the number of disease foci was then assessed. In field 1, rice was grown in 12 consecutive crop seasons and BCA were applied for 7 crop seasons before the rice fields were left without BCA applications until August 1999.

Field 2. A similar study was conducted in field 2. The experiment started with % disease foci achieved in 2 crop seasons of BCA efficacy trials (September 1996-May 1997). Then, from June 1997, data on ShB disease incidence were collected from 8 more crop seasons. In all crop seasons, 121 sampling units were systematically sampled. Only in two prior crop seasons in which rice was grown with bacterized seeds were normal rice seeds used in the following 6 crop seasons until August 1999.

Field 3. Starting in September 1996, data on the experiment were collected from 10 consecutive crop seasons. Seasonal changes in % disease foci were assessed in 121 sampling units of each crop season. Bacterized seeds were used in the 1st, 2nd, 3rd, and 4th crop seasons of field 3; after that, the seeds were not bacterized.

Field 4. Since January 1997, a farmer-practice field was observed as a control or check plot for the ShB disease situation without BCA application. Several disease foci were also collected on 12 1 sampling units of 8 consecutive crop seasons from January 1997 to August 1999.

Field 5. As a decline in % ShB disease incidence over 4 crop seasons in field 4 was noticeable, we suspected that this could be due to contamination of BCA coming from the BCA applied in the treated field plots. Therefore, a nearby farmer-practice field separated with a dike was used as another control plot. Some 258 sampling units were systematically sampled for ShB disease assessment. The data were collected from June 1998 to December 1999 for a total of 5 crop seasons.

Results and discussion

Effect of BCA on sheath blight disease

In both field 1 and field 2. although % ShB disease incidence and severity were mostly lower in the BCA treatments than in the control treatments. they were not statistically significant. With only two exceptions, the mixture of BCA (*Bacillus subtilis* + *Pseudomonas*

Table 1. Incidence (%) and severlty (%) of rice sheath blight recorded from disease foci treated by antagonistic bacteria and benomyl in field 1 in 5 consecutive cropd/seasons (July 1995-May 1997).

Treatment	1 st crop		2nd crop		3rd crop		4th crop		5th crop	
	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)I	Incidence (%)I	Severity (%)I	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
T1. *Bacillus* sp. + *Pseudomonas* sp.	28.2 ab	26.1 b	30.0 a	21.1 a	34.9 a	37.0 a	35.8 a	22.6 a	18.6 ab	21.3 a
T2. *Bacillus* sp. + *Pseudomonas* sp. + benomyl	21.4 ab	19.2 a	22.1 a	20.8 a	37.4 a	33.6 a	32.5 a	23.7 ab	18.6 ab	20.5 a
73. Benomyl	18.9 a	19.5 a	26.3 a	23.9 a	33.0 a	29.6a	27.5 a	21.8 a	14.3 a	21.0 a
14. Control	31.2 b	24.7 ab	31.6 a	28.6 a	55.2 b	52.5a	41.7 a	28.9 b	25.7 b	22.0 a
Mean	24.9	22.4	27.5	23.6	40.1	38.2	34.4	24.2	19.3	21 .o

Table 2. Incidence (%) and severity (%) of rice sheath blight recorded from disease foci treated by antagonistic bacteria and benomyl in field 2 in 2 consecutive crops/seasons (September 1996-May 1997).

Treatment	1 st crop		2nd crop	
	incidence (%)	Severity (%)	incidence (%)	Severity (%)
TI, Bacillus sp. + Pseudomonas sp.	34.4 a	28.8 a	25.0 a	35.3 a
T2. Bacillus sp. + Pseudomonas sp. + benomyl	34.4 a	28.1 a	22.5 a	25.4 a
T3. Benomyl	36.7 a	24.7 a	17.5 a	24.2 a
T4. Control	52.2 a	33.7 a	35.0 a	42.8 a
Mean	39.4	28.8	25.0	31.9

sp.) showed significant control efficacy by % disease incidence and severity in the 3rd and 4th crop seasons, respectively (Tables 1 and 2).

Unlike the results obtained from the previous small-plot experiments at the Pathum Thani Rice Research Center in 1994 (Arunyanart et al 1995), the good efficacy of BCA cannot be confirmed by these farmer field trials. Individual BCA and the combination of BCA and benomyl showed no signiticant difference from the control in almost every crop season in both field 1 and field 2. This was possibly because disease severity on the sampling units (disease foci) at the time of the first BCA application was not uniform, depending on naturally occurring BCA. BCA can be curative if we apply them at the right time. Therefore, in this study, BCA were applied on the disease foci. But in this study we used the sampling units or disease foci as the replications, so this certainly provided high variation among the replications. Another possibility is that heterogeneity factors affected the sampling units because of an improper experimental design, since the small-plot experiment that used a randomized complete block provided a significant difference among the treatments. Significant results were achieved by excluding experimental error by block layout. However, because BCA do not equate to fungicides (Mew et al 1995, Mew 1996), the immediate effects are difficult to see when BCA are applied and treated in the same manner as fungicides.

Deployment of BCA for controlling ShB disease

From July 1995 to January 1998, results obtained from field 1 during 7 consecutive crop seasons of BCA application showed disease foci as 73.6%. 48.5%, 84.4%, 36.4%, 22.5%. 13.0%, and 9.5%. Although from February 1997 to August 1999 BCA applications were terminated, the data on disease foci were continuously collected and resulted in 11.7%. 10.8%, 18.2%. 1.7%, and 7.4% (Table 3).

Results obtained from both field 2 and field 3 showed declining trends of ShB disease foci from 43.0% to 10.7% and 29.8% to 4.1 %, respectively (Table 3). A reduction in disease foci was also found in the untreated field 1 (field 4). Very high disease incidence (100% of sampling units) at first observation declined to 4.1 % in the 8th crop season of field 4 (Table 3).

In untreated field 2 (field 5), no declining trend of disease incidence was observed in 5 consecutive crop seasons. Disease foci were 54.0%, 73.396, 24.8%. 39.6%. and 45.6% (Table 3).

A decline in the percentage of disease foci that could be observed season by season in 12.9, and 9

Table 3. Last observation of sheath blight disease foci in 12 consecutive crop seasons in different experimental fields (%).

Number of field	1st crop 1995	2nd crop 1996	3rd crop 1996	4th crop 1996	5th crop 1997	6th crop 1997	7th crop 1997	8th crop 1998	9th crop 1998	10th crop 1998	11th crop 1999	12th crop 1999	13th crop 1999
1	73.6	48.5	84.4	36.4	22.5	13.0	9.5	11.7	10.8	18.2	1.7	7.4	–
2				43.0	47.9	20.7	5.0	5.8	4.1	18.2	0.8	10.7	–
3				29.8	11.6	10.7	8.3	2.5	6.6	9.9	0.8	4.1	–
Untreated field 1 (4th field)					100	71.1	41.3	1.7	8.3	5.0	3.3	4.1	–
Untreated field 2 (5th field)									54.0	73.3	24.8	39.9	45.6

consecutive crop seasons of fields 1, 2, and 3, respectively, indicated a long-term effect of BCA that were repeatedly applied in certain fields. Particularly, there was obviously a reduction in sheath blight incidence in field 1 from 73.6% to 7.4%. Interestingly, the number of disease foci increased in the 3rd crop season (from 48.5% to 84.4%) when rice variety Supanburee 1 was replaced by Poung-ngeon. This may be explained by the higher susceptibility of Poung-ngeon to ShB disease. However, in the following crop seasons, % disease incidence became lower again. Unfortunately, this also happened in the untreated field 1 (field 4): the number of disease foci in 8 crop seasons dropped from 100% to 4.1%. This may be due to BCA contaminated from experimental fields by flooding of irrigation water. Therefore, the data collected from another separate untreated field were needed. Observations made over 5 crop seasons of untreated field 2 (field 5), separated with a dike, showed nondeclining incidence of sheath blight. Results suggested that BCA *(Bacillus subtilis* 90-562 and *Pseudomonas* sp. 90-321) have potential to exist in the soil. The presence of a BCA residual effect in the experimental fields was clearly shown by the decline in % disease foci season by season, especially when no BCA were applied. This is supported by the work of Dhitikiattipong (1996), who showed that % disease incidence of rice plants sown in soil infested with ShB pathogen inoculum could be reduced by seed bacterization of BCA not only on the first planting but also on the second planting without seed bacterization. When rice plants were soaked with sterile distilled water and sown in the same infested soil in the seedbox of the first planting, % ShB disease incidence and number of sclerotia were lower than those of the control. There was a residual effect from antagonistic bacteria from previous seed bacterization to protect rice plants from infection by *R. solani.*

Conclusions

Tests on ShB control ability of two promising bacterial isolates, *Bacillus subtilis* (90-562) and *Pseudomonus* sp. (90-321), showed no significant difference compared with unsproyed treatments. Because of improper experimental design and high variation among the treatments in these farmer field trials, the results of good efficacy obtained from previous small-plot experiments cannot be confirmed. The study on the long-term effect of biological control on ShB disease resulted in a decline in disease incidence during the time that BCA were being applied or even in the following crop

seasons when BCA applications were terminated. This indicated the potential of BCA for sustainability of biological control in farmers' fields.

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Biological control of rice diseases

Chen Zhiyi, Xu Zhigang, Gao Taidong, Ni Shoukun, Yan Dafu, Lu Fan, and Liu Yongfeng

There are many antagonistic bacteria against rice pathogens in the soil. We have obtained 1,274 bacterial strains from rice soil, sclerotia of *Rhizoctonia solani,* sheath blight (ShB) lesions, and rice plants. Using the dual-culture test against *R. solani,* 61 strains (4.8%) exhibited a strong antagonistic effect, 727 strains (57%) had a moderate and weak effect, and 486 strains (38.1%) showed no effect against *R. solani.* Of the 24 selected strains tested for biocontrol of ShB at the rice-tillering stage in pots, six were chosen for field evaluation. B-916, a strain of *Bacillus subtilis,* was found to be the most effective in suppressing ShB development in the field. Strain B-916 was further evaluated in large field trials at five key rice production sites (Jiangyan, Wujiang, Jurong, Changshu, and Wujin City, Jiangsu Province, China) from 1994 to 1999. The fermentation product of B-916 used in these trials was applied first at maximum tillering, then at the booting stage. The biocontrol agent (BCA) was applied either singly or in combination with a mixture of fungicides and the fermentation product. When disease pressure at maximum tillering became very high, one application of the fungicide Jinggamycin was made following the first BCA application and before another BCA application was tested. As the field trials were organized at key rice production sites and as application was done by farmers (based on the BCA product and formulation provided by researchers through county crop protection specialists), farmers' assessments of the results were obtained. The initial trial in 1994 used only a few hectares of rice. Feedback from farmers was very favorable and the demand for BCA continued to increase. By 1999, the total area where B-916 was applied to control ShB (as part of the farmers' crop management strategy) had reached 30,000 ha and more than 20,000 liters of B-916 product were used annually. The results of the trials showed that B-916 can effectively control rice ShB in farmers' fields, with the control effect ranging from 50% to 81% using B-916 alone. The formulation, based on a mixture of B-916 and Jinggamycin, was comparable with fungicide action at a 50% reduced fungicide rate. The lesions obtained from the field trials of BCA in Jiangsu Province showed that, to scale up the BCA technology, rice farmers and county crop protection specialists should be involved in the process—from the very early stage of technology development, and from research to application. The use of BCA technology needs to be integrated into a local crop management and production system.

We have cooperated with the International Rice Research Institute (IRRI) in biological control of rice diseases since 1990. Microbial fungicide (antagonistic bacterium *Bacillus subtilis* B-916) partly replaced chemical fungicide in the rice ecosystem. The colonization and distribution of *Bacillus subtilis* B-916 in the rice ecosystem were investigated. By introducing microbial fungicide (*Bacillus subtilis* B-916), the microorganism population in the rice ecosystem will he manipulated and managed and a stable and biodiverse rice ecosystem will be formed. As a result, the prevalence of rice diseases will be effectively and sustainably controlled.

Biocontrol of rice sheath blight with antagonistic bacterium B-916

There are many antagonistic bacteria against rice pathogens in the soil. Some 1,274 bacterial isolates were obtained from paddy soil, sclerotia of *Rhizoctonia solani,* sheath blight (ShB) lesions, and rice plants. Sixty-one strains (4.8% of the total) had a strong antagonistic ability. Moderate and weak antagonistic strains (727) were 57% of the total and 486 strains (38.1% of the total) had no antagonistic ability. Twenty-four strains were tested for biocontrol of ShB at the rice-tillering stage in pots, 6 strains were screened by control effect on ShB at above

60%, and biocontrol was tested in field plots. The results indicated that strain B-916 had the best control effect on rice ShB in the field.

Performance tests of B-9 I6 for biocontrol of ShB were carried out continuously during 1994-99 at five sites (Jiangyan, Wujiang, Jurong, Changshu, and Wujin City, Jiangsu Province) and the area for the performance tests for different years at all five sites was 30,000 ha. The results showed that fermented product of B-916 could effectively control rice ShB infection under field conditions, and the fermented product of the control effect on ShB was from 50% to 81%.

Relationship among colonization, concentration, and spray timing of antagonistic bacterium B-916 and sheath blight of rice

The colonization of antagonistic bacterial strain B-916 on the rice plant surface was studied. B-9 16 was induced to be Rifampicin-resistant and used for monitoring. The antagonistic bacterial population on the rice plant surface was isolated and counted at 2 h and at 1, 3, 6, 10, and 15 d after inoculation of B-916. The results showed that the colonization of the antagonistic bacteria was related to the concentration of the introduced bacterial and sheath blight lesions on the rice plant.

Antagonistic bacteria introduced at low concentrations (106 cfu mL^{-1} of B-916) and sprayed on healthy plants could barely grow on the rice plant surface. When introduced at a high concentration (1,010 cfu mL^{-1} of B-916) and sprayed on the rice plant with sheath blight lesions, the population of the antagonistic bacteria could be maintained at more than 10^3 cfu mL^{-1} of B-916 on diseased plants at 15 d after introduction. The results indicated that the appropriate timing for spraying antagonistic bacteria is 1 d after inoculating *R. solani* and the initial concentration of introduced antagonistic bacteria cannot be lower than 10^8 cfu mL^{-1}.

Study on antagonism against *R. solani* and characteristics of antibiotic substance from the exudate of strain B-9 16

The function of the secondary metabolite of B-916 (antibiotic) against *R. solani* in biocontrol was studied. The antibiotic produced by B-916 possessed these abilities: the inhibition zone against *R. solani* was 6.7 mm, sclerotia formation was delayed 52 h, and the control effect on ShB was 58.1 %. So, the antibiotic of B-9 I6 played an important role in biocontrol of rice ShB.

The antibiotic of B-9 16 was precipitated with $(NH_4)_2SO_4$ for 24 h, the supernatant lost the ability to inhibit *R. solani,* and the precipitate enhanced the inhibiting ability. The antibiotic of B-916 was tested at different temperatures. The antibiotic treated at 100 °C for 20 min maintained the inhibiting ability; when treated at 120 °C for 20 min the ability decreased by 25%. The antibiotic of B-916 was hydrolized with 3 types of protease. The ability of inhibition against *R. solani* of the antibiotic decreased by 25% when treated with triprotease, decreased by 50% with protease E, and decreased by 75% with protease K. These results indicated that the main ingredient in the antibiotic of B-916 against *R. solani* was protein.

Inducing a resistance effect of antagonistic bacterial strain B-916 on the rice plant

Induced resistance of the rice plant mediated by B-9 16 was studied. Phenlalanine ammonia lynse (PAL), peroxidase (PO), polyphenoase (PPO), and superoxide dismutase (SOD) were screened as the target of rice resistance to diseases, and the activity level of four enzymes was compared after *R. sofani* was inoculated and B-916 introduced in three growth stages of rice. The results showed that PAL, PO, PPO, and SOD responded differently in time and level. PAL and SOD activity peaks arrived at 24 h after inoculation, the PO activity peak was at 48 h, and PPO had the highest value at 72 h. The time of the activity peak for the four enzymes was related to the time of their function in rice. There was no difference in activity levels of PAL and SOD after RH-2 was inoculated or B-916 introduced, but the activity peaks of PO and PPO for inoculated RH-2 were higher than that of introduced B-916. The reason inferred was that the rice plant did not cause disease lesions after B-916 was introduced. So, after spraying B-916 on leaves, rice plants were induced to increase the activity levels of four enzymes, and this enhanced the ability of resistance to rice pathogen infection.

The molecular marker of antagonistic genes of B-916 against rice sheath blight by RAPD-PCR

184 C+ bacterial isolates were identified by BOX-polymerase chain reaction fingerprints, 41 strains of which had relative genetic similarities up to 75% and belonged to *Bacillus amyloliquefaciens* They were

tested on their antagonism. Two isolates of *B. amyloliquefuciens* with antagonistic ability (G396+, G229+) and two without antagonistic ability (G433–, G434–) were selected to screen effective primers of random amplified polymorphic DNA (RAPD)-PCR. Some 124 random primers (AA, AB, AC, AD, AE, AM, AL) were tested for exploration of diagnostic DNA fragments related to antagonism using four isolates. Eight specific primers were found (AM- 18, AM-04, AA-01, AA-09, AC02, AD16, AE0l, AE06). and then reliability was checked using these primers with 20 isolates (10 isolates with antagonism, 10 isolates without antagonism of *B. amyloliqueficiens*) Comparing RAPD-PCR fingerprints generated *by* eight specific primers, AM-18 was the most effective in establishing the relationship between the diagnostic DNA fragment and antagonism of isolates of *B. amyloliquefaciens.* Another 21 isolates (8 isolates with antagonism, 13 isolates without antagonism) were tested with effective primer AM- 18 to prove the reliability of the diagnostic DNA fragment related to antagonism.

A diagnostic DNA fragment at 1,100 bp was suitable for 49 isolates but not for 2 isolates (G460 and G327). The relative probability between antagonism and the diagnostic DNA fragment generated by RAPD-PCR with primer AM-18 was up to 95% in *B. amyloliquefaciens,* B-916 (*Bacillus subtilis*), G329+, and G150- (*B. megaternurn*), and 5 isolates of *Bacillus* spp. from blast lesions with antagonism possessed a diagnostic DNA fragment. So, this diagnostic DNA fragment at 1,100 bp amplified by RAPD-PCR with primer AM- 18 was closely interrelated to *Bacillus* species of antagonistic genes.

Mechanism of antagonist bacterium Rh12542 for controlling rice sheath blight

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Biological control of soilborne pathogens by introducing microorganisms has been studied for more than 65 years. It can have an important role in agriculture in the future.

Sheath blight, a disease caused by an aerial form of the fungus *Thanatephorus cucumeris* (Frank) Donk (*Rhizoctonia solani* Kuhn), occurs in rice (*Oryza sativa* L.) under all types of cultivation throughout the world. Biological control of plant disease has been successful in rice. Recent work using antagonistic bacteria for biological control of rice sheath blight showed suppression of sheath blight development and protection of the plant from infection.

The antagonist bacterial isolate Rh12542 was isolated from rice seed and screened by the rapid method developed by Lin. It was selected from 288 antagonist isolates and its control effect on mungbean damping-off by seed bacterization was more than 50%. Antagonism to infection was tested by spraying the rice detached leaves with bacterial suspension and then placing mycelial discs of *R. solani* on each leaf. Inhibition of lesion expansion was tested by infecting the rice leaf with *R. solani* and spraying the leaf with bacterial suspension. Inoculated leaves were incubated in moist chambers at 28–30°C. Lesion length was measured 5 d after applying the bacteria. The lesion length of Rh12542 in the antagonism to infection and inhibition lesion expansion tests was 0 mm and 1.3 mm, respectively. That of the water control was 51.8 mm and 30.7 mm, respectively, in both tests. The data indicated that Rh12542 could protect the leaves from R. solani infection.

The inhibition percentage of mycelial growth of R. solani in bacterial suspension and heat-sterilized bacterial suspension after being treated for 48 h was 83.9% and 5.9% and that of Jinggamycin was 72.0%. This showed that the live bacteria and not the chemical produced by the isolate inhibited mycelial growth of the pathogen. The bacteria could also inhibit the production of sclerotia in the disc culture and reduce the pathogenicity of the mycelia and sclerotia. The inhibition percentage of pathogenicity of Rh12542 and Jinggamycin was 99.0% and 100%, respectively. Pathogenicity of germination of the sclerotia treated with Rh12542 was lost too, and that of germination of the sclerotia treated with Jinggamycin was not lost. The mechanism of the antagonist bacteria was much different from that of Jinggamycin.

To detect Rh12542 in rice after seed bacterization, the isolate was cultured in the rifupin medium to induce and obtain rifupin-tolerant isolates. The population of Rh12542 was detected every week by taking different parts of the rice plant (leaf, sheath, and root) and isolating the bacteria with rifupin-selective medium. The bacterial population could be detected about 3 mo after seed bacterization (from 7 July to 13 October). However, the bacterial population decreased from 1.80×10^8 to 8.93×10^4 about 3 mo after seed bacterization (from 7 July to 13 October). The data indicated that Rh12542 could grow and live in different parts of the rice plant and control rice sheath blight disease with live bacteria and not by inhibition chemicals.

On-Farm Impact of Seed Health Management

Seed quality and effect on rice yield: findings from farmer participatory experiments in Central Luzon, Philippines

C. Diaz, M. Hossain, S. Merca, and T.W. Mew

Farmer participatory research was conducted at two sites in Central Luzon, Philippines, to examine the impact of the best-quality seed on farm yield. For each site, 30 farmer-collaborators planted their own seed and IRRI supplied seed of the same variety in two subplots of a selected parcel. The farmers followed their own management practices and the researchers monitored them. The farmer-used seeds were analyzed. Results showed that a large portion of the seeds were not fully filled and were discolored, 34% were mixed with off-types, 5% had lethal seed infection, and the seeds had about a 96% purity level. The results of the experiment showed that plots planted with IRRI-supplied seeds had 7% higher yield than those planted with farmer-kept seeds at the site where yield was already high. At the site where yield was low, the difference between plots was 20%. A large part of the increase in yield was due to the lower weed and pest pressures achieved by the use of highquality seeds. A multivariate regression analysis of the determinants of rice yield showed that weeds and pests are important biotic constraints that reduce rice yield by nearly 25%.

Seeds are important in improving rice yields. They carry the genetic characteristics for successful crop production. It is important that clean and healthy seeds be used as planting materials in order to increase rice productivity. Contaminated seeds can often result in poor germination and poor seedling vigor, resulting in an unhealthy crop. The deterioration of seed vigor in the rice crop can account for 20% yield losses (Shenoy et al 1988).

One of the important determinants of seed quality is varietal purity. Varietal purity significantly influences crop yields besides affecting production practices (Seshu and Dadlani 1989). It is therefore important that farmers become aware of the presence of contaminants in their seeds. Weeds are a severe and widespread biological constraint to rice production, causing a conservative estimated loss of 10%, equivalent to 46 million tons per year (Moody 1991).

Studies on farmers' seed management practices reveal that in most cases farmers do not purchase certified seeds. Most farmers grow their own seeds or exchange seeds of available varieties with other farmers (Diaz et al 1994). They usually select fields that appear to have healthy plots with no off-types and that are relatively free of weeds as a source of seeds for the following season. Seeds obtained from the second cropping are considered to have better quality and are used for planting in the next season (Escalada et al 1996). Even with these practices, farmers often use seeds that have impurities and contaminants and seeds that are infected with pathogens (Fujisaka et al 1993). An important hypothesis in this context that needs testing through interdisciplinary research is that, if farmers' awareness of the importance of using clean and healthy seeds for growing is increased, rice productivity can be significantly raised without much additional cost.

This study uses the farmer participatory research approach to examine the impact of high-quality seeds on farm yield.

Two farmer participatory experiments were conducted in Central Luzon, Philippines, in 1996-97. The two sites selected were Tampac, Guimba, Nueva Ecija, and Gabaidon, Muñoz, Nueva Ecija. The first experiment was done in Tampac in the 1996 wet season and the second experiment was done in Gabaldon in the 1997 wet season. This chapter reports the findings of the experiments.

Data and methodology

The experimental sites

Tampac, Guimba, Nueva Ecija. The village is located in the southern part of the town of Guimba. It is located 7 km from the town proper. Transportation is underdeveloped due to bad roads, especially during the rainy season. Although deepwater pumps established by the National Irrigation Administration (NIA) and shallow well-pump systems installed by individual farmers are available, a large portion of the land remains fallow during the dry season. The most common variety planted is IR64. Farmers' preference for this variety is mainly based on high yield, good eating quality that helps command a high price in the market, and resistance to lodging as well as to major insect pests and diseases.

Gabaldon, Muñoz, Nueva Ecija. Gabaldon is a village in the northern part of the town of Muñoz. The dominant cropping pattern in the village is rice-fallow. As in any rainfed lowland area, the lack of an irrigation facility limits rice production. The NIA had surveyed the village for the installation of irrigation pumps but, until now, they have not started an irrigation project. Some farmers (25%) own private irrigation pumps that could be used to grow two crops of rice. But those who have experience using pumps for growing dry-season rice complain of severe water scarcity during the late season because of lowering of the water table and the consequent failure of irrigation pumps. Farmers who have available capital and land with light soils locally known as "galas" grow onions, garlic, and watermelon during the dry season with irrigation from the pumps. The type of soil and water availability appear to play a critical role in determining whether to plant other crops instead of rice during the dry season. IR64 is the most common rice variety, covering nearly 89% of the rice land during the wet season.

Materials and methods

Thirty selected farmers were mobilized to conduct an experiment in a parcel using clean and healthy seeds provided by the International Rice Research Institute (IRRI). The farmer-participants were given 500 g of clean and healthy seeds of IR64 from IRRI's Plant Breeding, Genetics, and Biochemistry Division. These seeds are high-quality seeds with 98% normal seedlings and are classified as foundation seeds. Farmer-participants in the experiment planted the clean and healthy seeds in a portion of the selected plot (intervention). In the remaining portion of the same plot (check), they planted their own IR64 seeds. The same parcel was used for the experiment as well as for the check to dissociate the effect of other factors such as toposequence, soil type and quality, and access to irrigation. This is to effectively measure with the paired test the effect of using clean and healthy seeds. By making a group of farmers plant both the high-quality seeds and their own seeds of the same variety (IR64), biophysical characteristics were held constant and only one variable, seed quality, differed. Farmer-participants used their own management practices, which the researchers monitored. Crop management practices were monitored for another 30 parcels, one each from a sample of 30 fanners (control). The set of control parcels was used to study farmers' current practices. No intervention was made in the control parcels.

Yield data were measured using the crop cut of 2 m × 3 m from the three plots: two subparcels from the farmer-participant (intervention and check) and the control plot. A total of 90 plots were considered in the analysis of the results for every season.

To test the difference in the quality of farmers' seeds, seed samples harvested from the 6-m^2 plots were brought to the Seed Health Unit Laboratory. The seed samples were dried to about 14% moisture content and subjected to routine seed health analysis in which the samples are tested on the basis of the capacity of seeds to germinate in relation to seed infection and vigor.

To test the effect of seed quality on crop yield, two yield estimates were used: yield estimated based on the fresh weight at harvesting time and yield adjusted to 14% moisture content.

Weed incidence and pest infestation (insects and diseases) were assessed during harvesting time to effectively evaluate the cumulative damage of weeds, insects, and diseases. Weed incidence was measured by counting the number of weeds in the 6-m^2 harvest area. Plots with the number of weeds greater than 10 within the harvest area were regarded as having been severely damaged by weeds. Pest incidence, on the other hand, was assessed by the presence of insects and diseases such as whiteheads (WH), narrow brown leaf spot (NBLS), rice tungro virus (RTV), and sheath rot (ShR) in the harvest area. The IRRI standard evaluation system (SES) was used to determine the severity of insect and disease damage. Whiteheads were assessed by counting the number of panicles infected. If more than 10 panicles in the harvest area exhibited infestation, then the damage caused by whiteheads was considered. For the severity of damage caused by diseases, the following measures were used: (a) NBLS, a score of 7 and above, (b) RTV, 2% and above, and (c) sheath rot, 5%

and above. The plot is considered as pest-infested when any or a combination of the four variables (NBLS, RTV, ShR. and WH) reached the threshold.

Socioeconomic and biophysical data were gathered using a structured questionnaire. Informal interviews were also conducted to capture other relevant information on farmers' perceptions and practices on seed health management.

Testing sampling bias

The randomness of the selection of farmers as participants and the controls in the experiment is critical in the successful analysis of the results of the experiments. To test whether the farmer-participants are representative of the general farmers of the village, the differences in the socioeconomic characteristics of the farmer-participants and the control farmers were tested for statistical significance.

Table I shows the results of the tests for Guimba during the 1996 wet season. The variables are exogenous variables, which are not affected by the experiment. Analysis of the differences in socioeconomic characteristics of the two groups shows that, in terms of age, education, farm size, percentage of farmers with a pump, participation of women in rice fanning, value of machinery owned, and major source of income, the differences between the project and the control groups were not statistically significant. The two groups of farmers differed significantly, however, in household size and tenure status. Farmer-participants have larger household sizes. This implies that they could use more labor in rice production. A larger portion of farmer-participants were tenant farmers, which indicates they could be poorer farmers with limited capacity to buy modern agricultural inputs, such as chemical fertilizers and irrigation water.

Table 2 shows the differences in characteristics of the two groups of farmers for Gabaldon during the 1997 wet season. Farmer-participants have a smaller farm size and have more participation of women compared with the control group. These variables may affect the use of labor in rice farming. A mulitvariate regression could be used to dissociate the effect of these factors that are statistically

Table I. Differences in socioeconomic characteristics of farmer-participants and control farmers in the 1996 wet-season experiment, Guimba, Nueva Ecija, Philippines.

Variables	Mean values		Difference	Level of statistical significance
	Participant farmers (n = 30)	Control farmers (n = 30)		
Age (y),	42.0	40.0	2.0	0.702
Education (y)	8.0	8.5	0.5	0.381
Household size (no.)	5.9	4.7	1.2	0.065
Farm size (ha)	1.3	1.3	0.0	0.990
Tenant farmers (%)	87.0	67.0	20.0	0.069
Farmers with pump (%)	60.0	53.0	7.0	0.610
Farm machinery owned (000 Philippine pesos)	38.6	45.6	7.0	0.717
Major source of income (nonagricultural)	11.0	9.0	2.0	0.591
Women's participation (high)	20.0	16.0	4.0	0.441

Table 2. Differences in socioeconomic characteristics of farmer-participants and control farmers in the 1997 wet-season experiment, Gabaldon, Nueva Ecija, Philippines.

Variables	Mean values		Difference	Level of statistical significance
	Participant farmers (n = 30)	Control farmers (n = 30)		
Age (y)	40.9	43.4	2.5	0.479
Education (y)	7.7	7.8	0.1	0.844
Household size (no.)	4.5	4.7	0.2	0.759
Farm size (ha)	1.1	1.8	0.7	0.010
Tenant farmers (%)	67.0	83.0	16.0	0.141
Farmers with pump (%)	60.0	53.0	7.0	0.610
Farm machinery owned (000 Philippine pesos)	36.9	66.0	29.1	0.119
Major source of income (nonagricultural)	15.0	17.0	2.0	0.612
Women's participation (high)	23.0	14.0	9.0	0.017

different between the farmer-participants and the control in assessing the impact of seed quality and yield.

Results and discussion

Seed management practices

About 60% of the farmers in Guimba obtained seeds from their own harvest and 37% exchanged seeds with other farmers. Very few, only about 3%, used certified seeds. In Gabaldon, 50% of the farmers used seeds harvested from their own fields and 38% exchanged seeds with friends and relatives. Only 12% of the farmers bought certified seeds.

In both areas, farmers usually select seeds for the next season from a portion of the field with a good crop stand, with less weeds and with uniform maturity of grains. Farmers usually consider fully filled grains, locally known in the dialect as "busog" as good seeds.

Farmers usually clean a selected portion of the field for seeds. While in the field, they take special care of the selected portion of the field by cleaning or removing the off-types (roguing) and weeds. Farmers claim that it is easier to see the off-types and weeds while in the field rather than after harvest.

A majority of the farmers clean their seeds by winnowing. Women do winnowing by placing the seeds in a winnowing basket against the direction of the wind and slowly tilting it until all the seeds drop. The idea in cleaning the seeds by winnowing is to separate the light (unfilled) grains from the heavy (filled) grains. Farmers believe that winnowing is the most effective and efficient method for removing impurities. Farmers do not clean their seeds by physical sorting since this is time-consuming and hence costly. Farmers do not use chemical treatment because of the perception that chemicals are unsafe and expensive.

Some farmers use the flotation method in cleaning seeds. They place the seeds on a container with water and let the unfilled seeds float. Farmers adopt the flotation method prior to sowing.

Interviews with farmers revealed that they use different combinations of seed-cleaning techniques to obtain good-quality seeds. Farmers claimed that flotation and roguing were effective methods of cleaning seeds aside from winnowing and special care in drying and selecting fields. In Guimba, 18% of the farmers involved in the experiment used flotation in cleaning seeds. Thirteen percent used roguing, whereas 47% used both flotation and roguing. About 22% indicated not practicing either of the two methods.

Meanwhile, in Gabaldon, more farmers involved in the experiment practiced flotation (25%). About 22% practiced roguing and only about 15% adopted both methods. A higher percentage of farmers (38%) indicated that they use neither flotation nor roguing.

Assessment of seed health conditions

Seed-cleaning techniques can affect seed quality. Seed health analysis provides an efficient and reliable method for assessing seed quality. Seed health conditions can be assessed in terms of the proportion of normal seedlings, lethal seed infection, rice mixtures, purity, fully filled seeds, 100-grain weight, and discoloration. Table 3 shows the differences in

Table 3. Differences in seed health conditions by seed source, Central Luzon, Philippines.

Variables	High-quality seeds[a]	Farmer seeds	Difference	t-value
Guimba (wet season 1996)				
Germination of normal seedlings (%)	98.8	30.8	8.0	10.99
Lethal seed infection (%)	1.1	5.7	-4.6	–13.74
Mixture with off-types (%)	0.0	4.3	-4.3	–6.91
Purity (%)	100.0	95.6	4.4	6.99
Best seeds (fully tilled) (%)	90.5	39.6	50.9	34.22
100-grain weight (g)	2.6	2.4	0.2	10.78
Discoloration (%)	0.0	23.9	–23.9	–14.27
Gabaldon (wet season 1997)				
Germination of normal seedlings (%)	98.0	90.2	7.6	4.60
Lethal seed infection (%)	1.2	4.8	–3.6	-4.86
Mixture with off-types (%)	0.0	3.5	–3.5	–8.45
Purity (%)	100.0	96.6	3.4	8.44
Best seeds (fully filled) (%)	89.4	38.0	51.4	37.65
100-grain weight (g)	2.7	2.4	0.3	9.67
Discoloration (%)	0.0	23.3	–23.3	–14.27

[a]IRRI foundation seeds. Note: The differences are statistically significant at the 1% level.

seed health conditions for the collected seeds and the seeds considered as high-quality (IRRI foundation seeds) used by farmer-participants in the experiment in Guimba and Gabaldon. The results of the analysis indicate that there is a substantial difference in the seed health conditions of farmer seeds compared with the foundation seeds for both areas.

The major difference was observed in four seed factors: the lethal seed infection, rice mixtures, fully filled seeds, and discoloration. Seeds collected from Guimba had a higher lethal seed infection (5.7%) than the seed samples from IRRI (1.1%). IRRI seeds had no mixtures, whereas seeds collected from Guimba showed an average of 4.3% mixtures with off-types. Only 40% of the farmer seeds were fully filled compared with 90% of the IRRI seeds. None of the IRRI seeds was discolored, whereas 24% of the seeds from farmers were discolored. Seed samples collected from Gabaldon were also inferior to the IRRI samples for the four seed factors. The differences are all statistically significant. Both areas, however, had minor differences between farmer seeds and IRRI samples for normal seedlings, purity, and grain weight. Farmer seeds from Gabaldon were of better quality than those from Guimba for lethal seed infection and mixtures with off-types. For Gabaldon, 96.6% of the farmer seeds were judged as pure compared with 95.6% for Guimba.

Effect of seed quality on rice yield

To assess the effect of seed quality on rice yield, we carried out a farmer participatory experiment (explained earlier in the section on methodology). We observed that the moisture content of the fresh yield differed widely among farmers because of the timing of harvest. Some farmers harvest their crop early before the ripening period, whereas others harvest late because of the unavailability of labor. To eliminate the effect of differences in moisture content, we adjusted yield to the standard moisture content of 14%.

The t-test for paired samples (the intervention and check subplots from the same parcel for the same farmer) was used in testing the hypothesis on differences or equality in mean yield. Table 4 reports the results. Yield was higher in Gabaldon than in Guimba, indicating favorable biophysical factors (larger proportion of parcels located in low-lying areas and having clayey soils) and better crop management practiced by farmers (early establishment of the crop, more tillage, and better weed control) in Gabaldon than in Guimba. The moisture-adjusted crop yield in the check subplot was 5.0 t ha^{-1} in Gabaldon versus 3.5 t ha^{-1} in Guimba. In the plot of the control farmers (farmer practice), the difference in yield between the two sites was also substantial, 4.85 versus 3.74 t ha^{-1}, respectively. The difference in rice yield between the intervention and the check subplot for the farmer-participants was 0.8 t ha^{-1}, or about 23% of the yield for the check subplot. The higher yield due to the high-quality seed for Gabaldon was 0.4 t ha^{-1}, or about 8% of the yield for the check subplot. For both areas, the increase in yield due to the difference in seed quality was judged statistically highly significant by the paired t-test.

The difference in yield between the high-quality seeds and the control shows how much production can still be increased if the farmers had used good-quality seeds.

To assess the contribution of different seed quality-related factors that lead to higher crop yields, we conducted the t-test for the difference between the intervention and check subplots for factors such as weed incidence, pest incidence, panicle number, and grain weight after harvest. The effect of seed vigor would be reflected in the difference in panicle number and grain weight. But as good-quality seeds have few off-types, weed incidence should be lower and healthy seeds free of lethal seed infection and discoloration would contribute to less incidence of seedborne diseases and insect infestation.

Table 4 shows the results on the differences in those factors measured during the experiment. For weed incidence for both areas, plots where farmers used high-quality seeds had significantly less incidence of weeds and insects. For example, in Guimba, 27% of the subplots with farmer seeds had significantly high weed incidence, compared with only 7% for subplots with IRRI seeds. In Gabaldon, these numbers were 50% and 10%, respectively. Low weed pressure was a significant factor contributing to higher yields on the intervention subplot.

We also found a significant difference for pest incidence between the check and the intervention subplots for Guimba but not for Gabaldon. Low pest pressure could be due to less incidence of seedborne diseases when farmers use high-quality seeds. Presumably, Gabaldon farmers are more knowledgeable about pest management practices so they were able to control pest pressure in spite of the use of low-quality seeds.

In Guimba, the intervention plots had 11% higher panicle number and 4% higher grain weights than the check plots and the differences in both variables were statistically highly significant. For Gabaldon, however, the difference was significant

Table 4. Effect of seed quality on rice yield, Central Luzon, Philippines.

Variables	Farmer-participants		Difference (%)	t-value	Level of significance
	High-quality seeds[a] (intervention plot)	Farmer seeds (check plot)			
Guimba (wet season 1996)					
Yield (adjusted)[b] (t ha^{-1})	4.3	3.5	22.8	5.91	0.000
With high weed incidence (%)	7.0	27.0	–20.0	–2.26	0.031
With high pest incidence (%)	27.0	53.0	–26.0	–2.80	0.009
Number of panicles	318	286	11.0	2.68	0.012
100-grain weight after harvest (g)	2.4	2.3	3.9	2.74	0.010
Gabaldon (wet season 1997)					
Yield (adjusted)[b] (t ha^{-1})	5.4	5.0	8.0	2.45	0.010
With high weed incidence (%)	10.0	50.0	-40.0	–3.89	0.001
With high pest incidence (%)	0.5	0.5	0.0	0.00	1.000
Number of panicles	267	249	7.3	2.79	0.009
100-grain weight after harvest (g)	2.6	2.6	–1.0	-0.82	0.416

[a] Clean and healthy seeds from IRRI with 98% normal seedlings and classified as foundation seeds. yield adjusted at 14% moisture content.

only for the number of panicles, which was 7% higher for the intervention subplot than for the check subplot.

Determinants of rice yields

To assess how much increase in rice yield is possible with farmers using the best-quality seeds, we ran a multiple regression model with the experimental data in which we incorporated the biophysical and socioeconomic factors that would affect rice yield besides the seed quality factor. Table 5 shows the factors included in the model, along with their mean values and standard deviations for both areas. The model was estimated with the values of the variables for both the farmer-participants and the control farmers.

For socioeconomic variables, farm size and the extent of women's participation in rice farming were included as these would affect the extent of use of family labor for crop care. Smaller farms would have more labor in their households relative to land than larger farms. Similarly, households in which women participate in rice farming would do a better job for operations such as transplanting and weeding that are done mostly by women. Tenant farmers may have fewer incentives to use purchased inputs as they have to share the additional output with the landowners. Educated farmers could be better entrepreneurs with the knowledge of how to use the information-intensive technologies (integrated pest management, timing and balanced use of fertilizer, etc.). Households with a major source of income originating from nonfarm sources would have better capacity to mobilize resources to finance agricultural inputs, but would be allocating less family labor for crop care because of the higher opportunity cost of such labor. Table 2 shows that, in both areas, the average farm size is small (1.28 and 1.36 ha), farmers are well-educated (8 years of schooling), and on a majority of the farms women participate in rice farming. The incidence of tenancy is low because of the implementation of land reforms. In Gabaldon, almost half of the households earn a major portion of their income from nonfarm sources versus one-third in Guimba.

The justification for including biophysical and crop management factors need not be explained. The mean values of the variables show that Gabaldon has a superior biophysical environment for rice farming than Guimba. Nearly 43% of the parcels in Gabaldon are at medium elevation compared with 20% in Guimba; hence, the rice land is less prone to submergence or drought in Gabaldon than in Guimba. Nearly two-thirds of the land in Gabaldon has clayey soils good for rice farming (with better water-holding capacity) compared with one-third in Guimba. However, Gabaldon has higher weed and pest pressures than Guimba.

Table 6 reports the results of the regression model. Equation 1 reports the estimated parameters of the full model. Since seed quality affects yield partly by reducing weed and pest pressure. a second variant of the model is estimated by excluding these two variables. which is reported as equation 2. The value of R^2 for equation 1 shows that 74% of the variation in yield for the participant and control farmers in Guimba and 65% in Gabaldon are explained by the model.

Table 5. Variables in the model for explaining variations in rice yield.

Variables	Unit of measurement	Gabaldon		Guimba	
		Mean	SD[a]	Mean	SD
A. Socioeconomic factors					
Farm size	ha	1.36	0.93	1.28	0.89
Women's participation	1 for participation, 0 otherwise	0.67	0.47	0.60	0.49
Land tenure	1 for tenant farm, 0 otherwise	0.28	0.45	0.20	0.40
Education of household head	Years of schooling for household head	7.71	2.52	8.18	2.25
Major source of income for household	1 for nonfarm, 0 otherwise	.52	0.50	0.34	0.48
B. Biophysical factors (parcel characteristics)					
Toposequence	1 for medium elevation, 0 otherwise	0.43	0.50	0.20	0.40
Soil type	1 for clay, 0 otherwise	0.66	1.21	0.34	0.48
Access to pump irrigation	1 for pump, 0 otherwise	0.58	0.50	0.58	0.50
Access to NIA projects	1 for NIA. 0 otherwise	0	0	0.14	0.35
Weed pressure	1 for parcels with critical weed levels, 0 otherwise	0.38	0.49	0.11	0.32
Pest pressure	1 for parcels with critical pest pressure, 0 otherwise	0.51	0.50	0.43	0.50
C. Crop management factors					
Timing of planting	1 for early planting, 0 otherwise	0.63	0.49	0.42	0.50
Number of tillage	Number	6.5	1.1	4.2	0.89
Application of insecticides	L ha^{-1}	0.36	0.37	0.52	0.93
Application of herbicides	L ha^{-1}	0.72	0.38	0.57	0.71

[a]SD = standard deviation.

Table 6. Contribution of biophysical and socioeconomic factors to rice yield: estimates of a multivariate model.

Variables	Gabaldon				Guimba			
	Equation 1		Equation 2		Equation 1		Equation 2	
	Regression coefficient	t-value[a]	Regression coefficient	t-value	Regression coefficient	t-value	Regression coefficient	t-value
Socioeconomic								
Farm size	−0.129	2.73**	−0.21 8	−2.29**	−0.041	-0.52	−0.038	-0.45
Women's participation	0.321	1.93*	0.528	2.78**	1.012	6.65**	1.166	7.44**
Education	0.016	0.55	0.013	0.39	0.001	0.01	−0.037	1.05
Land tenure	0.046	0.28	0.123	0.63	−0.322	−1.65	−0.352	−1.69*
Source of income	−0.021	−0.16	−0.118	−0.73	−0.466	−2.81**	−0.467	−2.64**
Biophysical								
Elevation of parcel	0.264	1.90*	0.351	2.13**	0.263	1.24	0.360	1.60
Type of soil	−0.013	−0.24	−0.063	-0.96	−0.066	-0.34	-0.228	−1.14
Pump irrigation	0.326	2.20**	0.519	3.02**	0.847	4.71**	0.999	5.51**
NIA irrigation	-	-	-	-	0.487	2.10**	0.482	1.96*
Weed pressure	−0.801	−5.21**			-0.395	−2.51**		
Pest pressure	−0.331	−2.46**			−0.597	−2.60*		
Management								
Planting date	0.623	4.03**	0.502	2.78**	0.564	3.67**	0.649	4.02**
Tillage number	−0.050	−0.66	0.024	0.27	0.113	1.29	0.145	1.56
insecticide use	0.134	0.63	0.501	2.07**	−0.287	−3.37**	−0.347	−3.91**
Herbicide use	0.438	2.15**	0.119	0.51	0.246	2.35**	0.300	2.70**
Fertilizer use	0.002	1.13	0.002	0.79	−0.002	−0.43	-0.002	−0.30
Seed quality	0.091	0.63	0.359	2.25**	0.591	3.91**	0.717	4.86**
Constant	4.44	7.97**	3.43	5.41	2.33	4.60	2.01	3.77**
R^2	0.65		0.49		0.74		0.69	
F-statistics	8.54		5.08		11.93		11.08	

[a]**denotes that the value of the regression coefficient is significantly different from zero at 5% probability ana * denotes statistical significance at 10% probability. Note: The dependent variable is the yield adjusted to 14% moisture content, measured in t ha^{-1} For the definition and measurement of the explanatory variables, please see Table 5.

The results show that biophysical and crop management factors are more important determinants of yield than socioeconomic factors. For both areas, smaller farms and households where women participate in economic activities have higher yields, indicating that farm size and gender roles influence farmers' decisions on labor use in rice farming. Households whose major source of income originates from nonfarm activities had lower yields than those with farming as a major source. This result suggests that the predominantly nonfarm households do not take as good care of the crop as the farm households, and the negative effect of this factor outweighs the positive effect of higher capacity of nonfarm households to mobilize finances for purchasing inputs.

Among biophysical factors, source of irrigation and weed and pest pressure are significant determinants of rice yield. The values of the parameters for the irrigation variables in Guimba indicate that parcels irrigated by the National Irrigation Authority system had higher rice yields than rainfed parcels by 0.5 t ha^{-1}. whereas parcels irrigated by privately owned pumps had higher yields by 0.8 t ha^{-1}. This suggests that the quality of irrigation is better under the private-sector operation than under the NIA system. Weed pressure reduced rice yield by 0.8 t ha^{-1} for Gabaldon and 0.4 t ha^{-1} for Guimba, which were 16% and 10% of the yield, respectively, for those areas. Pest pressure reduced yield by 0.3 t ha^{-1} for Gabaldon and 0.6 t ha^{-1} for Guimba, 7% and 16% of the yield, respectively, for the areas. Thus, weeds and pests combined reduced yield by 23–26%. The use of herbicides compensated for the yield loss from weeds to some extent for both areas, whereas the use of insecticides to control pests was ineffective for Gabaldon and further reduced yield for Guimba, as indicated by the statistically significant negative coefficient of this variable. Planting date was found to be the most important crop management factor determining yield. Parcels where the crop was planted early had higher yields by 0.62 t ha^{-1} in Gabaldon and 0.56 t ha'' in Guimba, 14% and 12% of the yield, respectively.

The value of the coefficient of the seed quality factor shows the effect of best-quality seed on rice yield after controlling for the effects of the differences in biophysical environments, socioeconomic factors, and farmers' crop management practices. The value of the coefficient indicates that, all other factors held constant, parcels in which IRRI foundation seed was used had higher yield by 0.59 t ha^{-1} in Guimba, which is a low-yield site, and only 0.91 t ha^{-1} in Gabaldon, which is a high-yield area. If we exclude weed and pest pressure from the model (as these are partly influenced by the use of seed and hence the effect of seed quality is included in their parameters), the use of IRRI seed increased rice yield by 0.75 t ha^{-1} (19.6% of the yield) for Guimba and 0.36 t ha^{-1} (7%) for Gabaldon.

Conclusions

Few Filipino farmers purchase certified seeds. Although a majority of the farmers select seeds from the portion of the field with a good crop stand, and practice roguing and flotation, the quality of the seeds that farmers keep from the harvest for use in the next season is not of a high standard. An analysis of the seed health of farmer-kept seeds at two experimental sites in Central Luzon showed a large proportion of seeds not fully filled and discolored, with 3% to 4% of the seeds having mixtures with off-types and 5% of the seeds having lethal seed infection. These seeds had about a 96% purity level. A farmer participatory experiment with the best-quality seeds as opposed to farmers' own seeds of the same variety showed an increase in rice yield by nearly 20% at the site with a low-yield environment and 7% at the site with a high-yield environment.

A large part of the increase in yield came from lower weed and pest pressures achieved by the use of high-quality seeds. Weeds and pests are important biotic constraints that reduce rice yields by nearly 25% at both sites, which induces farmers to use harmful agrochemicals. Thus, the use of high-quality seed could be a part of the strategy for preventive care against pests and diseases that may contribute to environmental protection, besides being a low-cost intervention for increasing rice yield. The challenge to the rice research and extension community is how to communicate this important knowledge to millions of rice farmers in Asia.

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On-farm impact of seed health management: experience from a collaborative network

Somsak Thongdeethae

In Thailand, farmers use two types of seed for rice cultivation: certified seed and noncertified seed. Certified seeds are produced by the Department of Agricultural Extension (DOAE) and sold to farmers directly or through a farmers' cooperative. Noncertified seeds are produced by farmers themselves or their neighbors. Several reasons were given for using noncertified seed. The common responses were no cost involved, easily accessible, and insufficient supply of certified seed. The criteria used to select seeds were crop of the same height, uniform ripening, weed-free, no off-types, and discolored grains.

In 1995, 270 rice seed samples were collected from farmers in nine provinces: Chiang Mai and Chiang Rai, representative of the north; Nakhon Ratchasima and Udon Thani of the northeast; Chacheongsao, Suphan Buri, and Chai Nat of the central region; and Nakhon Si Thammarat and Phatthalung of the south. To assess current on-farm seed management practices in rice production, farmers and seed owners were individually interviewed during seed collection. A questionnaire was used consisting of 22 principal questions concerning current on-farm pest and seed management practices in rice production.

The interviews indicate that most farmers prefer to change their rice varieties every 2–3 years because they cannot ensure uniformity in height, ripening, and quality after five seasons of continuous use. Farmers mainly use government-recommended varieties (79.0%) combined with local varieties (11.l%) and other varieties (10.0%).

In response to questions about why farmers do not change to new seed/varieties, about 72% prefer to use old varieties because they can still produce very high yield. About 67% process their own seed stock lor the next cropping season by winnowing, sun-drying and packing seeds in sacks and then storing them in a warehouse (common traditional practice). Most farmers select clean seed by removing inert matter, empty seed, and weed seeds by winnowing, 20% select their clean seed by the flotation method, and 6% choose clean and good seed from high-yielding plots.

About 66% of the farmers relied on chemicals for their rice pest control. The reasons for chemical seed treatment were to control seedborne pathogens (78%), to control storage pests (11 %), and to control rice blast (11%). Chemical seed treatment was done by mixing seeds and fungicides in a plastic bag and shaking to incorporate (73%), soaking seed in fungicide solution (20%), and using fungicide dry seed treatment and storage for 1 mo (7%).

For those who do not use clean seed, the following reasons were given: waste of time (26%), they use government-recommended seeds (23%), their own seeds are good enough (18%), and other reasons (21%). Reasons for not treating seed were know of no chemicals (35%), do not know how to treat seed (15%), have not done so before (14%), use government-certified seeds (14%), have no pest problem, therefore no need (11 %), not necessary and waste of time and money (5%), chemicals are hazardous (2%), and chemicals cause poor seed germination (1%).

The 270 seed samples were analyzed and contaminants were assessed. The results showed that the average percentage of contaminants (empty grains, plant debris, straw, husk, dung, stones, etc.) ranged between 4.4% and 6.5%, which is higher than the standard (2% by weight), which indicated that all seeds collected from farmers from different regions were below the national standard. However, seeds from the north, northeast, and central regions showed better germination (more than 80%) and lower moisture content (under 14%) than those from the south, which had a low germination rate of 59.0% and high moisture content of 16.45%.

Weed seeds of *Ischaemum rugosum* were found in all regions, with the highest percentage (55%) in the northeast, followed by central (45.1%), north (22.7%), and south (11.7%). *Echinochloa colona* and *E. crus-galli* were the major problems in the central

region, whereas *Oryza* spp., the so-called "red rice" considered as a noxious weed, show heavy contamination in every region.

Eleven genera of fungi were found. Six genera, *Alternaria padwickii. Curvularia lunata, Drechslera oryzae, Fusarium semitectum, F. moniliforme, and Rhizoctonia* sp., are causal agents of major rice diseases and the rest are saprophytes. Seeds from the south carried the most fungi (average of 3.83%), followed by those from the central (2.95%), northeast (0.58%). and north (0.24%) regions. *Alternaria padwickii* and *Curvularia lunata* were the major pathogenic contaminants in the central part of the country. Saprophytic *Penicillium* sp. was mostly found on samples from the south, where rainfall and humidity are favorable for fungal growth, and was higher there than in other regions. Moreover, two genera of bacteria (*Xanthomonas* and *Pseudomonas*) were also occasionally found in every location.

The rice seed samples from each region were analyzed and compared with the foundation seed standard. The results indicated that only 1.7% from the north and 3.3% from the central regions passed the standard level.

The following are constraints to clean seed: lack of farmers' knowledge/information on the importance of clean seed, lack of funding to educate farmers, and a lack of facilities for proper seed storage. Possible solutions are demonstration plots using clean seed versus farmers' seed and demonstration plots using farmers' seed with and without chemical seed treatment.

Status of rice seed health in Bangladesh and farmers' seed production and management scenario

M.A. Taher Mia and M.A. Nahar

In Bangladesh, rice is grown in four overlapping seasons on about 75% of the total cultivable land and the crop contributes 95% of the total food production. The average annual food shortage is about 2 million t Of the 31 diseases of rice occurring in Bangladesh, 16 are seedborne.

Research on different aspects of seed health of rice generated much information. Seedborne *Bipolaris oryzae* is more prevalent in the northern districts but *Pyricularia grisea* is rare. T. aman seeds are more vulnerable to different pathogens. *Trichoconis padwickii* and *Curvularia lunata* are also predominant. After 10 mo of storage, the internal inoculum of *B. oryzae* did not change much, similar to Microdochiurn oryzae after 18 mo of storage. Both fungi survived well at or below 82.9% relative humidity in storage. Under farmers' storage conditions, storage fungi were more abundant in *motka* (an earthen container) but *B. oryzae* were more abundant in sacks.

Seed infection with *B. oryzae* reduced seedling emergence significantly. However, no relationship between seed infection and disease development in the plant or subsequent seed infection could be established. The rate of transmission of *B. oryzae* from seed to seedling was up to 80% and that for *M. oryzae* was 59.2%. *Sarocladium oryzae* causes seed discoloration, resulting in poor grain filling, and reduces seed germination by 76%. Seed infection with Bipolaris oryzae and *T. padwickii* increased progressively with seed maturity.

In Bangladesh, seed supplied by the public sector never exceeds 5% of the total seed requirement. Farmers seldom take extra care for seed production. They generally keep seed from a portion of their harvest. The seed rate of modern rice varieties used by farmers ranged from 65.8 to 89.3 kg ha^{-1}. The mean mixture of farmers' seed ranged from 12.8% to 19.3% and germination of farmers' seed at three locations ranged from 17.6% to 71.3%.

In Bangladesh, rice covers about 75% of the total cultivable land of 13.52 million ha. It contributes about 95% of the total food production in Bangladesh, 76% of the average calories, and 66% of the protein intake (BBS 1996). Annual rice production in 1997-98 was 18.86 million t (BBS 1998). Presently, modern varieties (MV) of rice cover almost 56% of the total rice area. To feed the nation, the country needs to import around 2 million t of food grains every year. Biotic and abiotic stresses, excluding severe flood and drought, reduce rice production by nearly 21% (Mustafi et al 1999). Through proper management of biotic stresses, production could be increased considerably. Rice is grown in Bangladesh in four overlapping seasons, providing pathogens ample chances to perpetuate and multiply. The humid environment and cultivation of high-nitrogen-responsive varieties aggravate the situation. A total of 31 diseases of rice are known to occur in Bangladesh. Among these, 16 are seedborne, including five major diseases: brown spot, blast, sheath rot, leaf scald, and bakanae. Besides causing diseases on different plant parts, seedborne pathogens cause grain spotting and discoloration (Shahjahan et al 1988). Research on seed health or seedborne diseases of rice has been carried out on a limited scale due to several limitations. This chapter reviews current information related to rice seed health in Bangladesh and farmers' seed production and management practices.

Survey of seedborne pathogens

Fungi associated with freshly harvested seeds collected from selected parts of the country were studied (Hossain and Fakir 1976, Mia and Mathur 1983, Basak and Mridha 1985). The incidence of seedborne pathogens varied by locality and season of seed production. *Bipolaris oryzae,* the pathogen of brown spot disease of rice, was predominant, whereas the incidence of *Pyricularia grisea* was rare in seeds from the northern districts of the country (Hossain et

al 1976, Islam et al 1994, Table 1). Survey reports revealed that B. oryzae is the most predominant among the seedborne pathogens. It is seedborne both internally and externally (Shahjahan et al 1988).

Pyricularia grisea, the causal agent of blast disease, was not generally found in seed samples unless they were collected from infected fields. In general, the rate of seed infection was comparatively low, the highest being 18%. The blast pathogen was present in 16% of the 173 seed samples. Incidence was higher in unfilled grains than in filled ones (Bhuiyan et al 1994).

The incidence of seedborne pathogens such as *B. oryzae, Microdochiurn oryzae, Fusarium moniliforme,* and *Trichoconis padwickii* was higher in T. aman seeds (Mia and Mathur 1983, Table 2). Another important seedborne pathogen is Sarocladium oryzae, the causal agent of sheath rot disease. Its incidence in seed was moderate.

Besides these pathogens, others with minor status might have a large role in the planting value of the seed. Among this group of pathogens, Trichoconis [Alternaria] *padwickii* and *Curvularia lunata* are the most predominant fungi. Other pathogens associated with seeds are *Nigrospora oryzae, Tilletia barclayana, Pyrenochaeta oryzae, Cercospora janseana, Ustilaginoidea virens,* and *Epicoccum purpurescens.* Besides the pathogenic fungi, a dozen other fungi are associated with seeds. The role of these fungi is yet to be explored.

Limited research on the fungi associated with farmers' stored rice seeds was conducted. It was observed that B. oryzae was more prevalent in gunny bags than in motka, an earthen container. On the other hand, the storage fungal species *Aspergillus* and *Penicillium* were more prevalent in motka (Table 3). Seed germination was reduced significantly due to storage fungi (Mian and Fakir 1989).

Table 1. Incidence (%) of seedborne fungi in freshly harvested T. aman rice seeds.

Fungal pathogen	Mean occurrence in			
	Southern districts[a]		Northern districts[b]	
Bipolaris oryzae	10.6	(3.3–22.6)	32.7	(12.5–75.3)
Fusarium moniliforme	0.9	(0.0–3.1)	0.4	(0.0–1.3)
Microdochiurn oryzae	3.8	(0.0-8.0)	3.2	(0.2–11.3)
Pyricularia grisea	0.2	(0.0–1.2)	0.0	
Sarocladium oryzae	4.0	(1.1–9.1)	1.4	(0.0–3.9)
Tilletia barclayana	0.6	(0.0–2.9)	0.9	(0.0–3.0)
Trichoconis padwickii	43.0	(31.0-70.6)	52.1	(39.9-65.5)

[a]Mean of 31 samples from eight districts. [b]Mean of 30 samples from seven districts. Source: Islam et al (1994).

Table 2. Seasonal variation in incidence (%) of seedborne fungi.

Fungi	Boro	Aus	T. aman
Bipolaris oryzae	1.5	4.0	39.5
Fusarium moniliforme	0.5	1.0	6.0
Microdochium oryzae	0.0	0.0	2.5
Trichoconis padwickii	4.0	3.0	36.5

Source: Mia and Mathur (1983).

Table 3. Fungal flora associated with farmers' stored seed, T. aman season, 1996.

Associated fungi	Mean incidence (%)		Range of infection in	
	Gunny bag	Earthen container	Gunny bag	Earthen container
Bipolaris oryzae	14.03	5.47	1.0-41.0	0.0–18.0
Trichoconis padwickii	18.23	20.97	1.0–51.0	1.5-45.0
Aspergillus spp.	1.17	5.30	0.0-4.5	0.0–24.5
Penicillium spp.	2.93	8.50	1.0-10.0	0.0-46.0
Fusarium spp.	0.97	1.02	0.0–4.0	0.0–3.0
Microdochiurn oryzae	0.33	0.10	0.0–1.5	0.5–1.0
Curvularia lunata	3.00	1.30	0.0–18.0	0.0–4.5

Studies on the association of seedborne bacteria have begun recently. *Burkholderia, Pseudomonas,* and *Erwinia* species were found associated with freshly harvested as well as stored seeds.

Survival of seedborne pathogens in storage

Successful transmission of a pathogen to the next crop depends on its ability to survive in storage. The longevity of internally borne inoculum is more than that of external inoculum. It has been observed that, even after 10 mo of storage under laboratory conditions, the incidence of internal inoculum of *B. oryzae* did not change much. Similarly, 81.4% of the internal inoculum of *M. oryzae* remained viable even after I8 mo of storage. Both fungi survived best at or below 82.9% relative humidity in storage (Fig. 1, Mia et a1 1987, Mia and Safeeulla 1998). This indicates the potential of the pathogens to carry over to the next season.

Impact of seedborne pathogens on seed germination and transmission

Seeds infected by *Bipolaris oryzae,* when directly sown under upland conditions, reduced seedling emergence significantly. They also caused seedling blight. However, no relationship between seed infection and disease development in the plant or subsequent seed infection could be established. For a nonsystemic disease such as brown spot, infection on the aerial parts of the plant is governed by environmental factors. Seed samples with a similar infection category gave a different scenario although the experiments were conducted in the same place. In one year, there was practically no disease and seed infection showed only traces, whereas, in another year, considerable leaf and seed infection was noted (Mia, unpublished). The rate of transmission of *B. oryzae* and *M. oryzae* from seed to seedling was as high as 80% and 59.2%, respectively. *Sarocladium oryzae* causes seed discoloration and affects grain filling. Mia et al (1986) observed that, with the increase in disease severity from 3 to 7, discoloration of filled and unfilled grains increased from 4.8% to 11.7% and from 8.4% to 3I%, respectively, and discoloration reduced seed germination by 76% (Table 4).

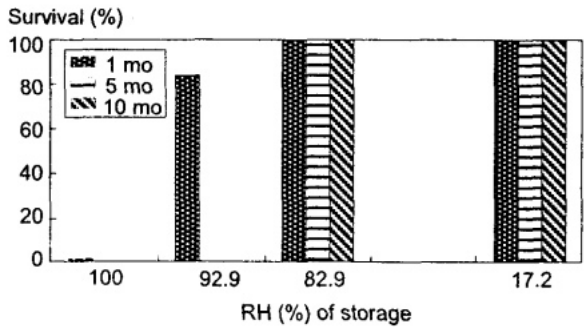

Fig. 1. Survival of internal inoculum of Bipolaris oryzae at different levels of relative humidity (RH).

An attempt was made to relate leaf infection to seed infection. Data from a single-season experiment indicated that the correlation coefficient (r) values for flag leaf, 2nd, 3rd, and 4th leaf (from the top) infection with those of seed infection were 0.72, 0.82, 0.80, and 0.72, respectively.

The incidence of *B. oryzae* increased progressively with seed maturity. The mean incidence in six varieties at the flowering, milky, dough, and ripening stages was 4.22%, 6.44%, 11.83%, and 25.56%, respectively. The average incidence of *Trichoconis padwickii* also increased with seed maturity. The average incidence of *Curvularia lunata* decreased at the milky stage and increased at the dough and ripening stages. With the advancement of seed developmental stage, the incidence of *Verticillium* sp. decreased. The overall incidence of *Surocladium oryzae* was comparatively very low (Fig. 2).

Table 4. Effect of sheath rot disease severity on the extent of discoloration and seed germination.

Disease severity	Discolored grain (%)		Apparently healthy grain (%)	
	Filled	Unfilled	Filled	Unfilled
3	4.8	8.4	76.9	9.9
5	11.0	11.5	68.0	9.6
7	11.7	31.0	40.7	16.6
Germination (%)	17.0		72.3	
1,000-grain wt. (g)	16.4		21.3	

Source: Mia et al (1986).

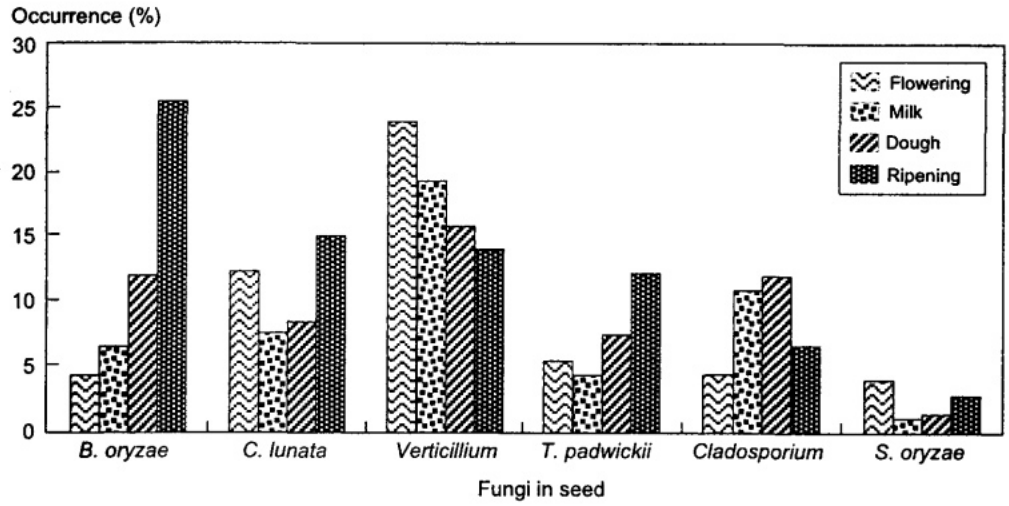

Fig. 2. Population dynamics of mycoflora at different seed maturity stages (mean of six varieties).

Scenario of farmers' seed production and management

Farmers in Bangladesh generally use their own seeds, as the seed supplied by the public sector never exceeds 5% of the total seed requirement. Besides this 5% certified seed, the quality of farmers' seed is unknown. Until now, farmers in Bangladesh have not been aware of seed health practices. Even the Seed Certification Agency is not yet in a position to include seed health as one of the components of seed quality control. At present, this agency only follows seed standards enforced by the National Seed Board (NSB, Table 5). The NSB also approved standards for seedborne pathogens and field diseases (Tables 6 and 7); however, these have not yet been implemented in the true sense. During the 1994 boro season, a survey was conducted at six locations in Bangladesh. It showed that 75-88% of the farmers used their own seed, the lowest in Rangpur and the highest in Rajshahi. Only 1–2% of the farmers obtained seeds from the Bangladesh Agriculture Development Council (BADC); the rest came from different sources, mostly from the market and neighbors (Table

Table 5. Seed standards for rice as fixed by the National Seed Board.

Components	Breeders' seed	Foundation seed	Certified seed
Pure seed (min. %)	99	96	94
Other varieties (max. no. kg^{-1})	0	1	2
Obnoxious weed seed (max no. kg^{-1})	0	8	10
Inert matter (max. g kg^{-1})	1	3	4
Germination %	8	80	80
Moisture % (max.)	12	12	12

Source: Report on National Seed Board Activities (Vol. 2), 1993.

Table 6. Seed standards for seedborne pathogens fixed by the National Seed Board.

Pathogen	Max. % of seed infection		
Bipolaris oryzae	0.5	1.0	5.0
Pyricularia grisea	0.0	0.1	0.5
Fusarium moniliforme	0.0	0.5	1.0
Microdochim oryzae	0.0	1.0	2.0
Aphelenchoides besseyi	0.0	0.1	0.5

Source: Report on National Seed Board Activities (Vol. 2). 1993.

Table 7. Field standards for seedborne diseases fixed by the National Seed Board.

Disease	Max. % of infected plants		
Brown spot	5.0	10.0	20.0
Blast	0.5	1.0	2.0
Bakanae	0.1	0.5	1.0
Leaf scald	5.0	10.0	20.0
White tip	0.5	1.0	2.0

Source: Report on National Seed Board Activities (Vol. 2). 1993.

8). Farmers seldom take extra care for seed production (Table 9). They generally keep seed from a portion of their harvest. The rate of MV rice seeds used by small farmers ranged from 65.8 to 83.2 kg ha^{-1}, and by medium and large farmers from 68.8 to 84.2 and from 69.2 to 89.3 kg ha^{-1}, respectively (Table 10), which is much higher than the recommended rate (40 kg ha^{-1}) (Alam and Mustafi 1995). In general, farmers' seeds are of poor quality for purity and germination. A survey in three districts revealed seed mixture from 12.8% to 19.3% and in individual samples it was as high as 34% (Table 11). It was surprising to note that the germination percentage of seed at the three locations was 17.6, 7 1.3, and 64.0 in contrast to 90.7 for BADC seed (Bashar and Nashiruddin 1995). The reason for using such a high rate by the farmers might be low-quality seed.

Table 8. Farmers' seed source at different locations in Bangladesh (boro season, 1994).

Source	Percent of farmers in					
	Gazipur	Comilla	Habiganj	Barisal	Rangpur	Rajshahi
Own	87	88	86	88	75	81
Market	4	3	5	6	7	15
BADC[a]	1	1	2	1	1	1
Neighbor	4	2	4	2	11	2
Other	4	6	3	3	6	1

[a]BADC = Bangladesh Agriculture Development Council. Source: Alam and Mustafi (1995).

Table 9. Farmers' practices in rice seed selection, aus season, 1994.

Selection method	Percent of farmers at different locations				
	Gazipur	Faridpur	Barisal	Rajshahi	Feni
From general lot after harvest	41	48	56	39	51
General crop cut after separating off-types	49	40	34	41	39
From specially selected area	8	12	9	17	10
Farmer did not respond	2	0	1	3	0

Source: Alam and Muslafi (1995).

Table 10. Rate of modern variety rice seed used by farm size category, boro season, 1994.

Farm size category	Seed use (kg ha**'') at different locations					
	Gazipur	Comilla	Habiganj	Barisal	Rangpur	Rajshahi
Small	71.1	65.8	72.3	70.0	68.2	83.2
Medium	72.0	70.4	71.6	68.8	70.0	84.2
Large	79.3	71.0	78.3	69.2	70.8	89.3

Source: Alam and Mustafi (1995).

Table 11. Comparison of farmers'seed quality with that of Bangladesh Agriculture Development Council (BADC) certified seeds in aus season, 1994 (var. BR14).

Location	Varietal mixture[a] (%)	Moisture (%)	Germination (%)
Dinajpud/Rangpur	14.4 (9-2 8)	14.5 (13.6-15.7)	17.6 (2-41)
0 Faridpur	19.3 (1 6-24)	15.1 (14.8-15.4)	71.3 (64-82)
1 Feni	12.8 (0-34)	14.4 (14.1-15.0)	64.0 (55-73)
2 BADC (Chudanga and Faridpur)	3.7 (0-9)	13.2 (13.1-13.6)	90.7 (87-95)

[a]Numbers in parentheses are the ranges. Source: Bashar and Nashiruddin (1995).

From this discussion, it could he concluded that there is scope for increasing rice yield by improving farmers' knowledge on healthy and quality seed production and management practices. For this, development of appropriate technological options and interventions is the supreme need.

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Analysis of farmers' rice seed storage in South Vietnam

Ho Van Chien, Vo Van Le, Le Van Thiet, and Vo Thi Thu Suong

Most pathogen species have been, and still are, inadvertently disseminated between and within provinces and regions as contaminants in traded seed in Vietnam. Rice seed contaminated with fungi has become widespread through trading of high-yielding rice varieties, Fungi are also being disseminated in the rice seed saved for the next season without seed treatment by chemical, physical, or quarantine programs. One of the objectives of this testing is to determine the identity of the various species of pathogens that live on rice seed. This important information relates to common seedborne rice diseases and rice seed contaminants. We hope that our approach to seed health will help farmers assess rice seed health properly. We have also had good results from this testing and we will send our suggestions and comments to farmers in the form of a comic book "How to make future rice seed storage methods more complete and more useful to avoid seedborne diseases."

Method

- Technicians of the provincial Plant Protection Departments prepared questionnaires for farmer interviews and distributed them to 20 farmers in each province. We collected 2 kg of rice seed samples from every household to which we provided the questionnaire for the interview.
- All samplings of rice seed recorded the percentage of seed germination, seed moisture content, and empty grains and identified diseases and nematodes.

Results

Testing was carried out in 18 provinces. A total of 35 I farmers (households) were interviewed. There were 35 1 rice seed samples and 73 rice varieties. For rice seed resources, 8 1.4% of the fanners sorted the rice seeds themselves for the next season and 18.6% of the farmers changed or bought rice seed from other farmers or rice variety companies. Average yields were 5.75 t ha^{-1} for the winter-spring crop, 4.46 t ha^{-1} for the summer crop, and 3.89 t ha" for the main crop (with local, long-duration varieties).

Results in Table 1 showed that there were 35 1 rice seed samples in 18 provinces. Seed germination averaged 80% but seven provinces (Binh Phuoc, Long An, Ben Tre, Vinh Long, Tra Vinh, Bac Lieu, and Ca Mau) had seed germination of less than 80% with 129 rice seed samples out of 351 (nearly 36.8% of the total samplings). Grain humidity was mostly higher than 14% (72%) except in five provinces: Binh Duong, Tien Giang, Dong Thap, Can Tho, and An Giang with 99 rice seed samples out of 35 1 (28%). The percentage of unfilled grains was higher than 5% in ten provinces, with 185 (52%) out of 35 1. These results did not match Vietnamese rice variety standards for release to farmers.

Results on nematodes living in rice seed samples (Table 2) showed that there were three kinds (*Ditylenchus angustus, Tylenchus* sp., and

Table 1. Percentage of seed germination (SG), grain humidity (GH), and unfilled grains (UG) for 351 seed samples.

Province	Samples (no.)	SG (%)	GH (%)	UG (%)
Binh Thuan	23	94	14.3	2.8
Dong Nai	20	84	14.7	6.7
Binh Phuoc	19	61	14.3	4.7
Binh Duong	9	86	14.0	3.0
Tay Ninh	12	88	14.1	8.3
Ho Chi Minh City	25	83	14.4	4.4
Long An	18	55	14.7	7.4
Tien Giang	20	87	13.9	5.0
Ben Tre	19	65	14.9	5.3
Dong Thap	28	81	13.8	6.9
Vinh Long	16	79	15.0	4.7
Tra Vinh	23	77	15.1	5.4
Can Tho	20	80	13.8	4.9
Soc Trang	24	85	14.9	6.6
An Giang	22	87	13.8	6.2
Kien Giang	19	91	15.1	5.1
Bac Lieu	14	73	15.2	3.4
Ca Mau	20	72	14.6	2.7
Average		80	14.5	5.2

Table 2. identification of nematodes on rice seed samples (total 351).

Province	Samplings (no.)	Occurrence frequency (%)	
		Ditylenchus angustus and *Tylenchus* sp.	*Aphelep choides besseyi*
Binh Thuan	23	-	-
Dong Nai	20	5.0	5.0
Binh Phuoc	19	21.1	-
Binh Duong	9	22.2	-
Tay Ninh	12	50.0	8.3
Ho Chi Minh City	25	8.0	-
Long An	18	5.6	-
Tien Giang	20	5.0	-
Ben Tre	19	31.6	10.5
Dong Thap	28	3.6	3.6
Vinh Long	16	6.3	-
Tra Vinh	23	26.1	4.3
Can Tho	20	5.0	5.0
Soc Trang	24	4.2	4.2
An Giang	22	4.5	-
Kien Giang	19	26.3	10.5
Bac Lieu	14	7.1	-
Ca Mau ,	20	5.0	-
Average		13.1	2.9

Aphelenchoides besseyi) and that occurrence frequency was 16%, whereas 84% of the samples had no incidence of nematode. Tay Ninh Province had the highest occurrence frequency of nematodes on total rice seed samples collected in that province.

Results in Table 3 indicated that I I species of fungi were living on rice seed samples in 18 provinces. Three species had a higher average of occurrence frequency than the rest. *Alternaria* sp., *Curvularia lunata,* and *Aspergillus* spp. had 31%, 55%, and 52% frequency, respectively. Most provinces with a large area for maize growing showed that *Ustilago* sp. infected rice seed samples.

Conclusions

Most rice seed samples were collected and identified by the Subdepartment of Plant Protection at the provincial level and the Regional Plant Protection Center of South Vietnam but did not match Vietnamese rice variety standards for release to rice farmers.

Seed germination was less than 80% in 36.8% of the samples and grain humidity was higher than 14% in 72% of the samples. Some 16% of the rice seed samples were infected by nematodes, whereas 64% of the samples were infected by some or all of the 11 fungal diseases.

Nematodes were responsible for seed infestation and seedling health problems for the following crop and they led to decreased rice yield and production.

Table. 3. Appearance frequency (%) of fungi on rice seed samples (total 351).

Province	Samples (no.)	*Alternaria* sp.	*Curvularia lunata*	*Helminthosporium oryzae*	*Fusarium moniliforme*	*Pyricularia oryzae*	*Tilletia barclayana*	*Ustilago* sp.	*Aspergillus* spp.	*Penicillium* sp.	*Mucor* sp.	*Rhizopus*
Binh Thuan	23	28	52	19	14	24	43	14	38	19	14	24
Dong Nai	20	25	40	-	-	-	35	20	50	20	25	40
Binh Phuoc	19	26	32	11	-	37	26	-	47	16	21	32
Binh Duong	9	33	56	-	11	-	44	22	56	22	33	44
Tay Ninh	12	42	50	17	-	25	33	25	42	17	25	33
Ho Chi Minh City	25	42	83	-	4	-	-	-	58	-	-	-
Long An	18	11	33	-	-	6	-	-	39	-	-	-
Tien Giang	20	30	30	-	-	30	-	-	65	-	30	30
Ben Tre	19	32	32	-	-	-	-	-	63	-	5	-
Dong Thap	28	-	75	25	-	-	-	-	50	-	25	-
Vinh Long	16	56	44	-	19	-	31	-	31	19	19	25
Tra Vinh	23	50	92	-	17	-	-	-	46	4	-	-
Can Tho	20	-	67	33	-	-	-	-	67	-	33	-
Soc Trang	24	46	50	-	8	-	-	-	25	-	17	-
An Giang	22	71	92	-	8	-	-	13	83	13	25	-
Kien Giang	19	64	58	16	16	-	32	-	68	16	11	21
Bac Lieu	14	29	71	29	-	-	-	-	64	29	-	-
Ca Mau	20	79	42	-	4	-	-	-	42	-	-	-
Average		37	55	8	6	7	15	5	52	10	16	14

Collaborative Networks: Organization of Impact

Dissemination of scientific results. New challenges for scientists and science information officers. Importance for the Third World to strengthen its collaborative research among national programs, international agricultural research centers, and advanced research institutes.

Marcel De Cleene

Scientific results can be disseminated through a wide array of channels: professional journals, the media (press agencies, newspapers, magazines, television, radio, and others), exhibitions, museums, science centers, books, films, videos, lectures, and new communication tools such as expert data banks and Internet facilities.

The last decade saw new challenges and implications for the field of scientific publication. As international publishers monopolize the scientific journals and publication fees keep on increasing, the expected quality of research often demands application of expensive technology that is not always affordable or available in developing countries. Collaboration with advanced research institutes can ensure that scientists from developing countries are not prevented from enjoying the advances in information technology. In addition, collaborative networks enhance the dissemination of scientific results through different levels: advanced research centers, international agricultural research centers (such as those of the Consultative Group on International Agricultural Research), and national agricultural research systems (NARS). Practical applications of scientific work done within these institutes are disseminated through local networks of NARS to the farm level.

A general overview will be given of the dissemination of scientific results. What happens with scientific results when they come out of the laboratories? A lot of possibilities are shown and discussed, as well as the effect (of some of these channels) on scientists' careers and activities: professional magazines and journals, the media (press agencies, newspapers, magazines, television, radio, and others), exhibitions, museums, science centers, books, films, videos, lectures, and new developments such as expert data banks and Internet facilities for journalists and scientists.

Professional scientific journals

The most direct and professional way is publication in scientific journals. In the last decade, science and technology publishing companies became very interested in taking over journals with high standards. On 12 April 1995, the giant German publishing company Verlagsgruppe Georg von Holtzbrinck GmbH of Stuttgart bought a majority (70%) share in Nature's parent company, Macmillan Limited of London. Nature, which is often referred to in the United States as "the British journal," is now in German hands, Holtzbrinck, which is privately owned. It began as a humble *Leserkreis* (i.e., a "reading circle") company that circulated scarce books and magazines by mail in post-war Germany. In 1995, the company had an annual turnover of $1.6 billion and made a solid profit, in large part from regional German newspapers, a German weekly business magazine, and a German daily business newspaper. The company also owns several American publishing houses, including W.H. Freeman and Worth Publishers, which publish science textbooks.

Why this economic interest in scientific and technological high-quality journals and magazines? Because it's big business. Top journals are indeed playing a bigger role, not only because of their content but also because of their impact on scientists'

careers. Therefore, they become economically valuable. The development of huge data banks with information on scientific articles creates an objective tool not only to show the journals in which the articles have been published but also to indicate the number of citations and their sources. Many research institutes and universities are paying more and more attention to the list of journals in which their scientists publish. Those journals are ranked by the American ISI (Institute for Scientific Information) and are available on CD-ROM for social sciences, arts and humanities, and sciences in general.

For the plant sciences, more than 10,000 scientific journals are grouped in 207 scientific fields, and classified according to their impact factor, half-lifetime, and number of citations in publications. At the University of Gent, Belgium, articles published in the top 10% of those ranking lists are published in an internal communication magazine (*Onderzoeks-beleid*). When we look at the list for plant sciences, 129 journals are mentioned. The top 10% means the first 13 journals. For phytopathology, only one journal is mentioned: *The Annual Review of Phytpathology,* situated in fifth place (in 1995). *Phytopathology* ranks 16th, *Phytoparasitica* 63rd, *Plant Diseuse* 67th, *Zeitschrift fur Pflanzen-krankheiten* 93rd, and *Phytoprotection* 94th.

In addition, the Web of science, another ISI publication, provides another strong tool for evaluating the impact of scientific papers or publications published or cited after 1988. Both instruments — the ranking of journals and citation reports — are playing an important role. So far, there is no problem in dealing with achieving the highest standards in science communication. However, publishing in scientific journals depends not only on the quality of the submitted papers; it can also cost a lot of money and a lot of people cannot afford those high fees.

The fact that one has to pay high amounts of money to get a publication in such journals is a doubtful and dangerous evolution, especially because those journals are fully commercialized. Collaboration with advanced research institutes (ARI) can prevent scientists from developing countries from being excluded.

Press agencies

Besides communication through scientific journals, a lot of other possibilities exist for disseminating scientific results: international press agencies (Associated Press, AP; United Press International, UPI; Reuters; Agence française de la Presse, AFP; and International Press Service, IPS, especially focused on the Third World) and national press agencies such as the important Deutsche Presse Agentur (DPA).

Newspapers

Newspapers are informed by press agencies, congresses, symposium results, high-level scientific magazines (which send their articles under embargo to the international press one week before publication!), university information services (press releases, conferences, brochures, etc.), industrial press releases, informal contacts with researchers, and institutes for public communication on science and technology, such as American Media Resource Service, British Media Resource Service, Novartis, Dutch PWT-Science Line, and French Science Contact.

According to an inquiry of the European Union of Science Journalists Association (EUSJA), the resources of European science magazines are, in order of importance, news agencies; news on TV and radio and in printed media; scientific magazines, especially *Science, Nature,* and *New Scientist;* press releases and reports; congresses; personal contacts; and books.

Television

The importance of science on television was studied extensively in 1991 in Belgium, The Netherlands, Great Britain, and Germany. This comparative study revealed that in all of those countries about 2% of the total broadcasting time was spent on science or technology.

Mainly environmental and medical topics were treated. Of course, a lot of scientific news crept in in a variety of nonscientific programs, varying from 80% (The Netherlands) to 55% (Great Britain).

Clearly recognizable scientific and technological programs appeared to be most common in Great Britain (46%), whereas in The Netherlands they are much less common (10%).

The length of the scientific programs also varied considerably by country studied: programs of 30 min covered 54% of the science broadcasting time in Great Britain. 16% in Belgium, 9% in Germany. and only 6% in The Netherlands. Very short scientific programs were most popular in Germany (47%), followed by The Netherlands (44%), Belgium (21 %), and Great Britain (O%!).

Family magazines

The presence of science in Dutch/Flemish family magazines has also been studied. During the first three months of 1991, between 1.8% and 6.6% of the volume was dedicated to scientific items. Medical topics are usually very popular.

Between 2.5% and 5% of the Dutch newspaper volume dealt with science or technology. Human sciences and natural sciences (and technology) scored similarly, about 33%, medical sciences 25%, and environmental sciences 10%.

There are more recent comparable studies for radio, TV, and printed media, but, in our daily experience, we don't believe that these numbers will vary considerably now.

Although the presence of science and technology seemed to be limited in the media, these subjects are of nonneglectable importance for managers of science centers or universities because a positive correlation between an institute and scientific discoveries or news is more than welcome publicity. At a lower level, scientists can also profit from their presence in the media, especially when it comes to institutional and national grants. The Public Relations Service of the University of Gent has been analyzing since the beginning of 1999 the presence of scientists in newspapers and local magazines. This gives an idea of the "mediability" of certain scientific fields and scientists.

New developments

Besides all these variations in the dissemination of scientific results, several initiatives have been undertaken to facilitate the life of the science journalist.

The American Media Resource Service (MRS) started a similar service in London in 1984. The aim is to develop data banks with the names and locations of scientific experts and to use them as a tool in communication between journalists and experts. In 1987, The Netherlands created a PWT-Science Line, in 1988 Germany had its own Information Umwelt, France in I99 I had its Science Contact, and Belgium in 1994 had its Science Line.

Last but not least, we want to focus on recent developments in Internet facilities for science journalists. Profnet started in 1992 in the United States. It is a link between science journalists and public relations and information officers. Its access is free for journalists. However, public relations and information services have to pay to become a member. In Germany, a similar network was worked out for the German-speaking universities in Europe (65 Swiss. German, and Austrian universities are connected to that network): Elster (1995), later IDW (1998). In the United States, the American Association for the Advancement of Science launched Eurekalert (1997). A governmental public relations office has to pay a fee of $100 per submitted press news or an annual fee of $1,000.

The British Association for the Advancement of Science launched in 1998 Alpha Galileo, an Internet-based press center for European science, engineering, and technology. It provides journalists with 24-hour-a-day access to press releases, event details, an address book of researchers and press officers, and background press information. Only registered contributors can post releases and event information. They cannot see embargoed material unless they have posted it themselves. Alpha Galileo grew out of concerns shared by many researchers across Europe, particularly in the United Kingdom, that their newspapers and television carried a preponderance of news of American science achievements and that they rarely covered European developments as extensively. This project is managed by the British Association for the Advancement of Science in close collaboration with the supporting and funding organizations: United Kingdom's Office of Science and Technology, the French Government, research councils in the UK and France, Euroscience, The Wellcome Trust, the Novartis Foundation, and the European Science Foundation. The objective is to always provide free access to contributors and journalists. But is there any guarantee that it will continue like that? There are three types of users: contributors, journalists, and nonspecialists. They only accept research public relations staff and senior researchers from European research organizations.

Conclusions

Many new challenges and implications for the field of publication of scientific results have taken place in the last decade. As international publishers are monopolizing the scientific journals, and their price is going up, the expected quality of research often demands application of expensive technology, which is not always affordable or available in developing countries. Public relations and information offices are facing new trends and attitudes in the way they are spreading scientific news. To become a member of a news network, journalists don't have to pay, but public relations and science information offices do (the European Alpha Galileo Internet news service, however, is still a free service, but it is available only

for European public relations offices). Collaboration with advanced research institutes can prevent scientists from developing countries from being excluded.

In addition, collaborative networks enhance the dissemination of scientific results through different levels: advanced research institutes, international agricultural research centers (such as those of the CGIAR), and national agricultural research systems. The final result should be that the practical applications of the scientific work done within those institutes would reach local farmers in their own language.

Therefore, the International Rice Research Institute (IRRI), as the link between ARIs and NARS. seeks the knowledge and infrastructure of the laboratory of microbiology and the LMG bacterial collection of the University of Gent (RUG) for analyzing the bacterial strains collected in the collaborative RUG-IRRI project to make identifications in accordance with the present taxonomy and to reach the quality level for publication of project outputs in international journals. Research outputs with practical implications for seed health management are in turn disseminated through local networks of NARS to the farm level.

In addition, collaboration between academic and agricultural institutes from developing countries can be very beneficial for basic science. The collaborative research done between the Entomology and Plant Pathology Division of IRRI (Philippines) and the University of Gent (Belgium) resulted in the isolation and identification of 1,384 bacterial isolates from rice, including *P. syringae* pv. *panici, A. avenae* subsp. *avenae, Erwinia* sp., *B. glumae, P. plantarii, X. oryzae* pv. *oryzae, X. oryzae* pv. *oryzicola, P. syringae* pv. *syringae,* and *P. fuscovaginae.* The results from this common research have been published in 9 peer-reviewed journals, together with 4 abstracts and 28 related publications on *Xanthomonas.*

Six RUG scientists from the laboratory of microbiology of the University of Gent were sent to IRRI to work from a few weeks to several years. Five scientists from NARS in developing countries were sent by IRRI to learn sophisticated techniques in taxonomy at the laboratory of microbiology of the University of Gent.

As a co-project leader (together with Dr. T.W. Mew, IRRI, and Prof. Dr. J. Swings, University of Gent) of the joint project "Managing Rice Disease Through Seed Health and Rice Seed-Associated Antagonistic Bacteria: A Key Component of IPM (1995-99)", I want to express my gratitude to all the people who helped realize the scientific and social goals of this international project.

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