Suppression of Rice Blast by Preinoculation with Avirulent *Pyricularia oryzae* and the Nonrice Pathogen *Bipolaris sorokiniana*

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ABSTRACT

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Avirulent isolates of *Pyricularia oryzae* and isolates of *Bipolaris sorokiniana*, a nonrice pathogen, were used to suppress rice blast caused by *P. oryzae*. In greenhouse experiments, both fungi substantially reduced leaf blast when applied 24 h or more before the pathogen. *B. sorokiniana*,

but not avirulent isolates of *P. oryzae*, systemically reduced disease in leaf 5 when applied to whole plants at the four-leaf stage. In field experiments, both fungi were able to reduce neck blast significantly. No increase in grain yield was obtained by using avirulent isolates of *P. oryzae*, whereas five sprays with *B. sorokiniana* from seedling to heading stages increased the grain yield in two of three experiments conducted at two locations in Nepal. The significant increase in yield was observed under high inoculum pressure of *P. oryzae*. Induced resistance is suggested to be involved in the suppression of disease.

Rice blast, caused by *Pyricularia oryzae* Cavara (synonym *P. grisea* Sacc.; teleomorph *Magnaporthe grisea* (Hebert) Barr), is generally considered to be the most important fungal disease of rice (*Oryza sativa* L.) because of its widespread distribution and destructiveness (21). A continuous effort has been made to control the disease, especially by using fungicides and breeding cultivars resistant to the disease. Biological control, including induction of host resistance against the disease, also has been attempted since the early 1950s. Both biotic and abiotic agents have been suggested to induce resistance in rice against *P. oryzae*. The biotic agents studied in this respect include avirulent isolates of *P. oryzae* (3,7,9,13,20,22,27, 34) and elicitors derived from *P. oryzae* (10,26,35). *Pseudomonas syringae* pv. *syringae* van Hall has also been reported to induce local (23) and systemic resistance in rice against *P. oryzae* (32).

Although several reports have claimed to have found induced resistance in rice under laboratory conditions, demonstrations in the field are very limited. The objective of this study was to test avirulent isolates of *P. oryzae* and isolates of the nonrice pathogen *Bipolaris sorokiniana* (Sacc.) Shoemaker under greenhouse and field conditions for ability to suppress rice blast.

MATERIALS AND METHODS

Fungal isolates and inoculum. *P. oryzae* isolates CP 2071 and CP 2069 were used as biocontrol agents on the rice cultivars Masuli and Taichung-176, respectively. As pathogens, *P. oryzae* isolates CP 2069 and CP 2070 were used on 'Masuli' and 'Taichung-176', respectively. All *P. oryzae* isolates were isolated from rice seeds from Nepal. Similarly, *B. sorokiniana* isolates CP 2072, a Nepalese isolate from wheat, and CP 1623, a Danish isolate from barley, were used as biocontrol agents. Isolates of *P. oryzae* were grown

on oatmeal agar (OMA) (Difco Laboratories, Detroit) under 12-h cycles of darkness and near-UV light (Philips TLD 36W/08; Philips Lighting B.V., Rosendaal, the Netherlands) (11) at 25°C for 10 days. *B. sorokiniana* was grown on potato dextrose agar (PDA) (Difco Laboratories) under the same conditions as *P. oryzae*. Inoculum of all isolates was harvested in glass-distilled water by gently scraping the surface of the agar. In greenhouse experiments, the concentrations of *P. oryzae* as biocontrol agent and as pathogen were adjusted to 75,000 and 50,000 conidia per ml of water, respectively, whereas, in field experiments, the concentrations were adjusted to 100,000 and 75,000 conidia per ml of water, respectively. The concentrations of *B. sorokiniana* used in greenhouse and field experiments were 30,000 and 40,000 conidia per ml, respectively.

Greenhouse experiments. Greenhouse experiments were conducted at the Royal Veterinary and Agricultural University, Copenhagen, Denmark, during 1992 to 1993 at 25 to 30°C under natural daylight conditions. Two rice cultivars, Taichung-176 (Tsai-Yuan-Chung × Dee-geo-woo-gen, from Taiwan) and Masuli (Mayang Ebos 80*2 × Taichung 65, from Malaysia) were used. Both are susceptible to *P. oryzae* under field conditions in Nepal (16). 'Masuli' was released in Nepal in 1973 and is widely grown in terai (plains) and warm valleys of Nepal. 'Taichung-176' was released in Nepal in 1966 and is one of the most popular rice cultivars in the Kathmandu Valley and surroundings.

About 30 seeds were sown in a row in a 10- by 12-cm plastic pot containing approximately 1 liter of the soil mix Weibulls Enhetsjord (K jord, Svalöf Weibull AB, Hammenhög, Sweden) (11). The pots were arranged in a randomized complete block design with three replications. Ammonium sulfate (1 g) containing 21% N (Svovlsur ammoniak 21; Kemira Danmark A/S, Vedbæk, Denmark) and 0.1 g of 13% iron chelate (Pioner Jernchelat 13%; P. Brøste A/S, Lyngby, Denmark) were added to the soil mix in each pot as a basal dose of fertilizer through watering when the rice was sown. Iron was added because the iron content of the soil mix was insufficient for the rice plants, which otherwise developed typical iron deficiency symptoms. The plants were fertilized 15 days after seeding with approx-

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imately 1 ml of a complete fertilizer with micronutrients added (i.e., Hornum Næring; P. Brøste Industri A/S, Lyngby, Denmark) and approximately 1 g of ammonium sulfate per pot. The soil was kept moist throughout the growing period, but not flooded.

The fifth developed leaf of each of 10 rice plants (about 5 weeks old) per pot was fixed with the abaxial side up in a horizontal position on bent plastic plates (6) using unbleached cotton strings. Avirulent *P. oryzae* (isolate CP 2071 on 'Masuli' and CP 2069 on 'Taichung-176') and *B. sorokiniana* (CP 1623) were used as biocontrol agents. The leaves were inoculated with the biocontrol agent 12, 24, and 48 h prior to inoculation with the pathogen. A 1:1 mixture of biocontrol agent and pathogen represented the 0-h control. Inoculum of the biocontrol agent was removed (except for 0 h) by gently wiping the inoculated leaves towards the tip with wet cotton and then rinsing them with water before inoculation with the pathogen. This technique effectively remove fungal spores (6). To test for a possible systemic protection against disease, whole plants were inoculated with the biocontrol agents at the four-leaf stage (before the fifth leaf started to emerge) and with the pathogen at the five-leaf stage (before the sixth leaf started to emerge). All types of inoculum were sprayed onto the fixed leaves or whole plants until runoff, using a plastic hand sprayer. Inoculated plants were incubated at 25 to 28°C under 100% humidity in plastic bags for 72 h in darkness. The plants were then exposed to open air in the greenhouse and grown there until assessment of disease. The experiments were repeated three times.

Field experiments. Field experiments were conducted in Nepal at the National Agricultural Research Institute, Khumaltar, Lalitpur, and at the Agricultural Research Station, Rampur, Chitwan, during the summer of 1994. Both are part of the Nepal Agricultural Research Council. Khumaltar is situated in the Kathmandu Valley (midhill, 1,350 m above sea level) and Rampur is located in the Chitwan Valley (inner terai, 228 m above sea level). The soil type (33) at Khumaltar is Ustocrept (31% clay, 55% silt, and 14% sand) and Dystrocrept at Rampur (12% clay, 28% silt, and 60% sand). Two field experiments using 'Taichung-176' were conducted at Khumaltar, and one at Rampur using 'Masuli'. Four-week-old rice seedlings, raised in dry bed, were transplanted (two to three seedlings per hill) in plots of 10 m² (2.5 by 4 m) at a plant distance of 15 by 15 cm. The plots were arranged in a randomized complete block design with four replications. Transplanting was done on 29 June at Khumaltar (experiment 1), 10 August at Rampur (experiment 2), and 3 July at Khumaltar (experiment 3). One day before transplanting, nitrogen was applied as urea (60 kg of N per ha), phosphorus was applied as triple super phosphate (40 kg of P per ha), and potassium was applied as muriate of potash (30 kg of K per ha). Furthermore, urea was top-dressed at the rate of 30 kg

of N per ha at the tillering and heading stages after hand-weeding. All fertilizers were broadcast by hand. The experiment at Rampur was irrigated by flooding three times. The first irrigation took place just before transplanting (9 August) and the following two during the tillering and booting stages, respectively. The experiments at Khumaltar were only irrigated during transplanting.

In experiment 1 at Khumaltar and experiment 2 at Rampur, *B. sorokiniana* (isolate CP 2072) and avirulent *P. oryzae* (i.e., isolate CP 2069 at Khumaltar and isolate CP 2071 at Rampur, respectively) were used. In addition, both experiments included the fungicide edifenphos (Hinosan 50 EC, 2 ml/liter of water; Bayer (India) Limited, Bombay, India), commonly used in Nepal for control of rice blast (19). Each plot received five sprays during the growing season with the biocontrol agents or fungicide. Thus, the treatments were carried out at seedling (growth stage [GS] 2) (2), tillering (GS 3), stem elongation (GS 4), booting (GS 5), and heading (GS 6) stages. The dates of applications were 21 June, 5 August, 21 August, 7 September, and 24 September at Khumaltar, respectively; and 1 August, 13 September, 2 October, 18 October, and 5 November at Rampur, respectively. In experiment 3 at Khumaltar, the biocontrol agents *B. sorokiniana* (isolate CP 2072) and avirulent *P. oryzae* (isolate CP 2069) were applied in various combinations of growth stages: seedling stage; seedling plus tillering plus stem elongation stages; seedling plus tillering plus stem elongation plus booting plus heading stages; seedling plus booting plus heading stages; tillering plus stem elongation stages; tillering plus stem elongation plus booting plus heading stages; and booting plus heading stages.

The biocontrol agents were inoculated after sunset so that the inoculated plants remained wet until next morning. In experiment 1 at Khumaltar, the rice was inoculated with virulent *P. oryzae* (isolate CP 2070) 3 to 4 days after each application of the biocontrol agents or fungicide. In the two other experiments, disease resulted from natural inoculum.

In all experiments, about 1 liter of inoculum of either biocontrol agent or pathogen and about 0.6 liter of fungicide was applied per plot, using a hand-compression sprayer (Maruti, 9-liter capacity; American Spring & Pressing Works Pvt. Ltd., Malad, Bombay, India).

Assessment of disease and yield. In the greenhouse experiments, leaf blast was scored 6 to 7 days after inoculation with the pathogen. Each seedling was examined and rated on a 0 to 9 scale according to the international specification for seedling blast in nurseries (2). Systemic disease suppression caused by inoculation of whole plants with biocontrol agents at the four-leaf stage was assessed by comparing the number of susceptible-type blast lesions per leaf (broad, spindle-shaped lesions with yellow, brown, or purple margins) (2) on the fifth leaves of plants with and without prior application of biocontrol agents.

In the field experiments, neck blast was recorded at maturity (GS 9). The neck blast incidence was computed according to the method adopted by the Agriculture Production Bureau, Japan (1).

$$\% \text{ neck blast incidence} = \frac{4A + 3B + 2C + D + 0E}{4 \times \text{number of hills examined}}$$

in which A = hills for which 61% or more panicles are found to be infected, B = hills for which 31 to 60% of panicles are found to be in-

TABLE 1. Severity of leaf blast caused by *Pyricularia oryzae* on the fifth developed leaves after preinoculation of the same leaves of rice 'Taichung-176' and 'Masuli' with avirulent isolates of *P. oryzae* or *Bipolaris sorokiniana* in the greenhouse

Time (h) ^a	Mean leaf blast score (0 to 9 scale) ^b			
	Avirulent <i>P. oryzae</i>		<i>B. sorokiniana</i>	
	'Taichung 176'	'Masuli'	'Taichung 176'	'Masuli'
0 ^c	6.9	6.9	6.5	6.9
12	6.1	6.4	5.9	6.4
24	4.0	4.3	3.2	3.5
48	3.5	3.8	2.8	3.0
Nontreated control	7.4	7.5	7.3	7.7
P value	0.0001	0.0001	0.0001	0.0001
LSD _{0.95}	0.4	0.3	0.3	0.2

^a Interval of time between inoculation with avirulent *P. oryzae* (isolate CP 2071 on 'Masuli' and isolate CP 2069 on 'Taichung-176') or *B. sorokiniana* (isolate CP 1623) and inoculation with virulent *P. oryzae* (isolate CP 2069 on 'Masuli' and isolate CP 2070 on 'Taichung-176').

^b Data are means of three replications (10 leaves per replication).

^c 1:1 mixture of both fungi.

TABLE 2. Number of blast lesions on the fifth leaf of 'Taichung-176' in the greenhouse after preinoculation of the whole plant at the four-leaf stage with an avirulent isolate of *Pyricularia oryzae* or *Bipolaris sorokiniana*

Treatment	Lesions per leaf ^a	Decrease (%) ^b
<i>P. oryzae</i> (CP 2069)	11.1	3.5
<i>B. sorokiniana</i> (CP 1623)	6.3	45.2
Nontreated control (CP 2070)	11.5	...
P value	0.0005	
LSD _{0.95}	1.2	

^a Average number of blast lesions per leaf counted 5 days after challenge inoculation. Data are means of three replications (10 leaves per replication).

^b Percent decrease in lesion number compared with control.

fectured, C = hills for which 11 to 30% of panicles are found to be infected, D = hills for which 10% or less panicles are found to be infected, and E = hills for which 0% panicles are found to be infected.

The experiments were harvested on 7 to 10 November at Khumaltar and on 10 to 11 December at Rampur, and grain yield and thousand-grain weight were measured. From each plot, 7.5 m² was harvested by hand using a sickle, leaving border rows in each side. The grain was threshed using a manual paddy thresher, winnowed by hand winnower, and sun-dried for 2 days. The percentage of moisture was determined using a moisture meter (Osaw Agro moisture meter; The Oriental Science Apparatus Workshop, Haryana, India) and the grain weight calculated at 14% moisture level.

Disease ratings, grain yield, and thousand-grain weight were analyzed using the GLM procedure in PC-SAS (release 6.11; SAS Institute, Cary, NC).

Dual culture and germination fluid tests. To examine possible antagonistic activity between the biocontrol agents and the pathogens, isolates CP 1623 and CP 2072 of *B. sorokiniana* and isolates CP 2069 and CP 2070 of *P. oryzae* were grown in dual cultures on PDA plates in all combinations. Dual cultures were also made reciprocally between the *P. oryzae* isolates. Discs (5-mm diameter) from actively growing cultures of *B. sorokiniana* and *P. oryzae* were placed alternately 2.5 cm apart in a circle (a total of six discs per plate) and replicated in six plates. Each of the test organisms were plated alone as controls. The plates were incubated for 10 days under the same conditions as for inoculum production.

The effect of germination fluid of the biocontrol agents on growth of *P. oryzae* isolates was also tested. Suspensions (100,000 conidia per ml of water) of *B. sorokiniana* or *P. oryzae* were incubated in darkness at 25°C for 12 h, filtered through filter paper, and finally through a sterile millipore filter with 0.45-µm pore size (Minisart NML, 165 55K; Sartorius AG, Göttingen, Germany). The fluid (100 µl) was spread on the agar surface of plates containing 15 ml of OMA. Six discs of *P. oryzae* isolates were placed on each plate. There were six replications. As controls, each *P. oryzae* isolate was transferred to OMA plates without germination fluid. The plates were incubated for 10 days as described before.

RESULTS

In incompatible interactions between avirulent isolates of *P. oryzae* and rice, the fungus produced small necrotic resistant-type blast lesions, less than 0.5 mm in diameter, 60 to 72 h after inoculation. The two isolates of *B. sorokiniana* caused small necrotic lesions less than 1 mm in diameter on both 'Taichung-176' and 'Masuli' within 24 h after inoculation. At high temperature (28 to 30°C) and high humidity, the lesions enlarged up to about 1.5 mm in diameter, but sporulation never occurred. *B. sorokiniana* produced more lesions than did avirulent isolates of *P. oryzae* (data not shown).

TABLE 3. Suppression of blast in the field at Khumaltar on 'Taichung-176' using foliar sprays of an avirulent isolate of *Pyricularia oryzae*, an isolate of *Bipolaris sorokiniana*, and the fungicide Hinosan

Treatment ^a	Incidence (%) ^b	Reduction (%) ^c	Yield (kg/ha)	Increase (%) ^d
<i>P. oryzae</i> (CP 2069)	41.7	20.3	3496	5.6
<i>B. sorokiniana</i> (CP 2072)	36.5	30.2	3661	10.6
Hinosan	19.5	62.7	4084	23.4
Nontreated control	52.3	...	3310	...
<i>P</i> value	0.0001		0.0001	
LSD _{0.95}	6.9		201	

^a The biocontrol agents and the fungicide were applied five times, i.e., at seedling, tillering, stem elongation, booting, and heading stages.

^b Percent neck blast incidence. All plants were inoculated with virulent *P. oryzae* 3 to 4 days after each application of biocontrol agent or fungicide.

^c Percent reduction of disease compared with nontreated control.

^d Percent increase in grain yield compared with nontreated control.

In the greenhouse, preinoculation of rice with avirulent *P. oryzae* or *B. sorokiniana* prior to inoculation with virulent *P. oryzae* significantly reduced the leaf blast severity compared with the nontreated control at all treatment times (Table 1). The disease reduction increased with increasing time between application of the biocontrol agents and the pathogen. Hence, at an interval of 48 h between inoculations, avirulent *P. oryzae* and *B. sorokiniana* reduced the leaf blast scores from 7.4 to 3.5 and from 7.3 to 2.7, respectively, on 'Taichung-176' compared with the nontreated control. On 'Masuli', the corresponding reductions were from 7.5 to 3.8 and from 7.7 to 3.0 for avirulent *P. oryzae* and *B. sorokiniana*, respectively.

Disease was suppressed in the fifth leaves of 'Taichung-176' when the whole plant was inoculated at the four-leaf stage with *B. sorokiniana*, 15 days before inoculation with *P. oryzae* (Table 2). Hence, the number of blast lesions was reduced by 45% on fifth leaves compared with the nontreated control. Disease was not reduced significantly when using avirulent *P. oryzae* as the biocontrol agent.

Neck blast incidence in field experiment 1 at Khumaltar (Table 3), where the plants were inoculated with virulent *P. oryzae*, averaged 52% in nontreated control plots. In experiment 2 at Rampur (Table 4) and experiment 3 at Khumaltar (Table 5), where plants were not inoculated, the neck blast incidence in the nontreated control plots was about 80 and 21 to 22%, respectively.

Avirulent isolates of *P. oryzae* reduced neck blast incidence significantly in all three experiments (Tables 3 to 5). Disease reductions were 20% in experiment 1 at Khumaltar (Table 3) and 26% in the corresponding experiment at Rampur (Table 4). In experiment 3 at Khumaltar (Table 5), disease incidence was reduced significantly when avirulent *P. oryzae* isolates were applied at both booting and heading stages (Table 5), but grain yield (Tables 3 to 5) and thousand-grain weight (data not shown) were not significantly increased in any of the experiments.

B. sorokiniana significantly reduced neck blast incidence and increased the grain yield both in experiment 1 at Khumaltar (Table 3) and in experiment 2 at Rampur (Table 4). The percent reduction in disease was 30% at Khumaltar and 42% at Rampur compared with the nontreated controls. Similarly, grain yield was significantly increased by 11% at Khumaltar (Table 3) and 16% at Rampur (Table 4). In experiment 3 at Khumaltar (Table 5), with the exception of treatment 4, significant control of the disease occurred only in treatments involving application of *B. sorokiniana* at both booting and heading stages. There was, however, no effect on grain yield. Thousand-grain weight was not increased in any of the three experiments (data not shown).

At both Khumaltar and Rampur (Tables 3 and 4), the effect of edifenphos was greater than that of avirulent isolates of *P. oryzae* and *B. sorokiniana* in reducing disease and increasing grain yield.

In dual culture experiments, no zones of inhibition were formed in any of the combinations between *B. sorokiniana* and *P. oryzae* or between different *P. oryzae* isolates. After 10 days, *B. sorokiniana*

TABLE 4. Suppression of blast in the field at Rampur on 'Masuli' using foliar sprays of an avirulent isolate of *Pyricularia oryzae*, an isolate of *Bipolaris sorokiniana*, and the fungicide Hinosan

Treatment ^a	Incidence (%) ^b	Reduction (%) ^c	Yield (kg/ha)	Increase (%) ^d
<i>P. oryzae</i> (CP 2071)	58.9	26.1	2909	6.7
<i>B. sorokiniana</i> (CP 2072)	46.1	42.2	3150	15.6
Hinosan	33.6	57.8	3412	25.2
Nontreated control	79.7	...	2726	...
<i>P</i> value	0.0003		0.0036	
LSD _{0.95}	14.4		307	

^a The biocontrol agents and the fungicide were applied five times, i.e., at seedling, tillering, stem elongation, booting, and heading stages.

^b Percent neck blast incidence. Disease resulted from natural inoculum.

^c Percent reduction of disease compared with nontreated control.

^d Percent increase in grain yield compared with nontreated control.

ana completely covered the agar surface except for the area occupied by *P. oryzae*, but did not overgrow *P. oryzae* even when the plates were incubated under the same conditions for 1 month. The colony size of *P. oryzae* in dual cultures with *B. sorokiniana* was smaller (2.1 to 2.2 cm) than in control plates (2.7 to 2.8 cm), whereas, in dual cultures between isolates of *P. oryzae*, colony size of one isolate (2.6 to 2.8 cm) was not affected by the other. Germination fluids of *B. sorokiniana* and *P. oryzae* did not affect colony size or appearance of *P. oryzae*; the colony size ranged between 2.7 and 2.9 cm.

DISCUSSION

Both avirulent isolates of *P. oryzae* and the nonrice pathogen *B. sorokiniana* reduced blast significantly under greenhouse conditions when sprayed onto the rice plants prior to inoculation with virulent isolates of *P. oryzae*. Avirulent isolates of *P. oryzae* were previously reported to be able to suppress infection of virulent isolates of the same fungus (3,7,9,13,20,22,27,34). In some of these reports (3,9,22,27,34), induced resistance or induced inaccessibility was suggested to be involved. To our knowledge, *B. sorokiniana* has not previously been shown to suppress rice blast except in a preliminary report (18). However, *Bipolaris* spp. have been used as biocontrol agents. Thus, *B. sorokiniana* and *B. oryzae* were used to control spot blotch caused by *B. sorokiniana* in wheat (5) and in barley (24,25). Similarly, the severity of brown spot of rice (*B. oryzae*) was reduced by preinoculation with a conidial suspension or conidial extracts of the same fungus (8,28–30,36). Induced or acquired resistance was suggested to be responsible for the disease reductions observed. Recently, Jørgensen et al. (11) reported the control of *Drechslera teres* and other pathogens of barley by preinoculation with *B. maydis*. In this investigation, induced resistance was also suggested to be involved.

We found that the effect of the biocontrol agents was greater as the time interval between application of the agents and the pathogen increased. Similar results were reported previously in rice

(3,9) and oilseed rape (15). However, we also found that periods shorter than 24 h, and even coinoculation of biocontrol agents and pathogen, resulted in significant disease reductions. This was previously demonstrated (9,13,20,34). Observations of such inhibition might suggest that antibiosis or nutrient competition was involved in disease suppression. However, dual-culture experiments with *B. sorokiniana* and *P. oryzae* indicated that this was not the case, because the two organisms did not suppress each others' growth on PDA, as indicated by the lack of inhibition zones. The colonies of *P. oryzae* in dual cultures with *B. sorokiniana* were smaller than in control plates, but this was because *B. sorokiniana* grew faster than *P. oryzae* and thus occupied all free space, limiting the growth possibilities of *P. oryzae*. These results were further supported by the observation that growth of *P. oryzae* was not affected by germination fluids of *B. sorokiniana* or other isolates of *P. oryzae*. Even when conidia of *P. oryzae* and *B. sorokiniana* were incubated together in water, germination of the former was not inhibited by the latter (data not shown). For *P. oryzae*, mixing of virulent and avirulent inoculum did not affect germination of the virulent race (20). According to Bell et al. (4), if two organisms each colonizes approximately one-half of the medium surface in dual-culture experiments and neither appears to dominate the other, they are considered nonantagonistic to each other.

On the other hand, the involvement of induced resistance, as defined by Kloepper et al. (14), is strongly suggested by Northern blot analyses showing the accumulation of several defense-related transcripts in the rice leaves following inoculation either with avirulent *P. oryzae* or with *B. sorokiniana* alone (17), indicating that the defense response in the rice is activated. These observations are consistent with findings by Ohata and Kozaka (20), who reported the production of two fluorescent compounds with antifungal properties in rice leaves even when avirulent and virulent *P. oryzae* were inoculated onto the plants at the same time. The compounds were not detected in healthy plants or in *P. oryzae*. Arase and Fujita (3) observed that, in preinoculated leaf sheaths, penetration and hyphal growth of virulent *P. oryzae* in the host cells were inhibited, coinciding with a hyaline-to-pale yellow color previously reported to be related to resistance. In oilseed rape, Mahuku et al. (15) reported systemic induced resistance against a highly virulent isolate of *Leptosphaeria maculans* when coinoculating it with a weakly virulent isolate of the same fungus.

Although *B. sorokiniana* protected the rice both locally and systemically, the local effect was stronger than the systemic effect. The systemic protection was not the result of migration of *B. sorokiniana* spores. Light microscopy studies (data not shown) revealed that *B. sorokiniana* was never found on the fifth leaves when inoculated only on the fourth leaves. No systemic protective effect was found in response to an avirulent isolate of *P. oryzae*, and this is in accordance with results from other investigations (9,13). Park and Kim (22), however, found reduced size of lesions produced by virulent *P. oryzae* up to 3 cm above the site of inoculation with avirulent *P. oryzae* on the same leaf. To our knowledge, systemic protection of rice against *P. oryzae* using a biological agent previously has been reported only by Smith and Métraux (32), who used *P. syringae* pv. *syringae*; however, Reimann et al. (23) were unable to duplicate these results.

Having found biocontrol agents with substantial disease-reducing abilities under controlled conditions, the next important step in examination of their effect and potential future applicability is to perform field experiments. In the field, avirulent isolates of *P. oryzae* reduced the neck blast incidence on 'Taichung-176' and 'Masuli' when the plants were sprayed at both booting and heading stages, but the effect on grain yield and thousand-grain weight was not significant in any of the three experiments. Iwano (9) also reported that preinoculation with an avirulent isolate of *P. oryzae* at the tillering or heading stages under field conditions reduced the leaf blast and panicle blast, respectively.

TABLE 5. Suppression of blast in the field at Khumaltar on 'Taichung 176' using foliar sprays of an avirulent isolate of *Pyricularia oryzae* and an isolate of *Bipolaris sorokiniana*

Treatment ^a	Incidence (%) ^b	Reduction (%) ^c	Yield (kg/ha)	Increase (%) ^d
<i>P. oryzae</i> (CP 2069)				
S	17.7	21.0	3,495	-1.7
S+T+SE	18.5	17.4	3,661	3.0
S+T+SE+B+H	15.4	31.3	3,676	3.4
S+B+H	14.3	36.2	3,561	0.2
T+SE	21.6	3.6	3,658	2.9
T+SE+B+H	14.3	36.2	3,688	3.8
B+H	14.8	33.9	3,589	1.0
Nontreated control	22.4	...	3,554	...
<i>P</i> value	0.0475		0.9967	
LSD _{0.95}	6.2		ns ^e	
<i>B. sorokiniana</i> (CP 2072)				
S	21.6	-4.9	3,525	0.5
S+T+SE	18.5	10.2	3,620	3.3
S+T+SE+B+H	13.3	35.4	3,686	5.1
S+B+H	15.9	22.8	3,648	4.1
T+SE	17.2	16.5	3,602	2.7
T+SE+B+H	11.5	44.2	3,672	4.7
B+H	15.1	26.7	3,639	3.8
Nontreated control	20.6	...	3,506	...
<i>P</i> value	0.0083		0.9944	
LSD _{0.95}	5.3		ns	

^a Spraying at stages S = seedling, T = tillering, SE = stem elongation, B = booting, and H = heading.

^b Percent neck blast incidence. Disease resulted from natural inoculum.

^c Percent reduction of disease compared with nontreated control.

^d Percent increase in grain yield compared with nontreated control.

^e ns = nonsignificant.

B. sorokiniana showed greater protection against blast in the field than avirulent isolates of *P. oryzae*. Because there were differences in the disease pressure between the experiments, it is important to consider whether the disease suppression by the biocontrol agents is greater under high (Tables 3 and 4) than under low disease pressure (Table 5). If this is the case, it is an important observation, because under low disease pressure or in the absence of disease, a biocontrol agent based on or involving induced resistance may itself have negative effects on the rice (i.e., host defense reactions are energy consuming and thus yield may be reduced) (31).

The biocontrol agents used in the current experiments could be potential candidates for future development of biological protectants involving induced resistance to be used commercially in rice. A crop like rice is particularly suited for protection by induced resistance, because the plants can be treated with the inducer in several ways before transplanting to the field. However, use of living biocontrol agents may be problematic under field conditions because of high cost and sensitivity to environmental conditions. Problems in development of a biological control agent based on induced resistance have been described previously (12). There are at least two points in favor of using *B. sorokiniana* as a biocontrol agent. The fungus is cosmopolitan, and it should be possible to find strains endemic to various geographic regions rather than introducing new strains to a region. However, the protective ability of isolates may vary and should be determined before use. Furthermore, the fungus causes necrosis on rice leaves, but does not sporulate on them. This means that there is very little risk for multiplication of the fungus during the growth season and a subsequent transfer of inoculum to the following crops such as wheat and barley. However, because *B. sorokiniana* is a cosmopolite, such transfer of a little extra inoculum of this organism should not pose any greatly increased risk for the environment.

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