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A Fragment of the *Xanthomonas oryzae* pv. *oryzicola* Harpin $HpaG_{Xooc}$ Reduces Disease and Increases Yield of Rice in Extensive Grower Plantings

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ABSTRACT

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Harpins of phytopathogenic bacteria stimulate defense and plant growth in many types of plants, conferring disease resistance and enhanced yield. In a previous study, we characterized nine fragments of the harpin protein $HpaG_{Xooc}$ from *Xanthomonas oryzae* pv. *oryzicola* for plant defense elicitation and plant growth stimulation activity relative to the intact protein. In plants grown under controlled conditions, the fragment $HpaG_{10-42}$ was more active in both regards than $HpaG_{Xooc}$. Here, we demonstrate that the activity of $HpaG_{10-42}$ in rice under field conditions significantly exceeds that of $HpaG_{Xooc}$, stimulating resistance to three important diseases and increasing grain yield. We carried out tests in 672 experimental plots with nine cultivars of rice planted at three locations. Application protocols were optimized by testing variations in application rate, frequency, and timing with respect to rice growth stage. Of the concentrations (24, 24, 12, and 6 μg/ml), and number and timing of applications (at one to four different stages of growth) tested, $HpaG_{10-42}$ at 6 μg/ml applied to plants once at nursery seedling stage and three times in the field was most effective. Bacterial blight, rice blast, and sheath blight were reduced 61.6 and 56.4, 93.6 and 76.0, and 93.2 and 55.0% in *indica* and *japonica* cultivars, respectively, relative to controls. Grain yields were 22 to 27% greater. These results are similar to results obtained with typical local management practices, including use of chemicals, to decrease disease severities and increase yield in rice. Our results demonstrate that the $HpaG_{10-42}$ protein fragment can be used effectively to control diseases and increase yield of this staple food crop.

Additional keywords: disease severity index, secure crop production.

With public concern about chemical use in crops, great attention has been paid to agricultural application of alternative, bioactive products from various sources, including plant pathogens (9,36,47,54). Harpins produced by plant-pathogenic bacteria are multifunctional proteins (1,2,4,23,51,55) showing great potential for practical use in crops. In several plant species, applying harpin can promote plant growth (11,20) and induce defensive responses against pathogens (10,14,15,42,46,50), insects (11), and drought stress (12). These effects have been noted in rice (5,43) and other plants (22,24,29,31) treated with $HpaG_{Xooc}$, a harpin from the rice bacterial streak pathogen *Xanthomonas oryzae* pv. *oryzicola* (25). Nine fragments of $HpaG_{Xooc}$ created using recombinant DNA expression technology showed greater bioactivity than the intact protein (5). The fragment $HpaG_{10-42}$, consisting of amino acids 10 to 42 of the protein, is most active in promoting plant growth and defense (5).

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In assays with rice and other plants growing under controlled conditions, $HpaG_{10-42}$ is 1.5- and 7.5-fold better than $HpaG_{Xooc}$ in eliciting plant growth and disease resistance, respectively (5). In response to $HpaG_{10-42}$, signaling pathways that regulate plant growth and defense are activated (5) similarly as in response to $HpaG_{Xooc}$ (31) and other harpins (24,25,39–41). Applying $HpaG_{Xooc}$, $HpaG_{1-94}$, or $HpaG_{10-42}$ to *Camellia sinensis* expedites growth of the germinal leaves, which are harvested for tea (53). The expression of several genes related to plant growth is induced in expanding leaves of tea plants treated with the proteins. In germinal leaves, the expression of genes involved in biosynthesis of catechols, which have potential in prevention and treatment of cancers and cardiovascular disorders (16,17,48), also is induced (53). Contents of catechols are elevated in processed tea leaves harvested from treated plants. In stimulating these responses in *Camellia* spp., $HpaG_{10-42}$ is more active than $HpaG_{Xooc}$ but, in contrast to rice, less active than $HpaG_{1-94}$ (53). Thus, for treatment in new candidate crops, testing of multiple fragments is necessary to determine the best choice.

Testing under field conditions is also essential. Here, we present the results of application of $HpaG_{10-42}$ to rice grown under nursery and field conditions. Rice is a staple food crop, supporting half the world population (21). Diseases are major impediments in rice production. Rice blast caused by *Magnaporthe grisea* is devastating worldwide (35,38); sheath blight caused by *Thanatephorus cucumeris* and bacterial blight caused by *X. oryzae* pv. *oryzae* severely constrain rice production in Asia and many other rice-growing regions (29,33). China has ≈31,800,000 ha in use to grow rice, ≈21% of the world total (19). In China, rice blast, bacterial blight, and sheath blight are responsible for the greatest loss of rice yield due to diseases (28,37,45). Yunnan Province is an important rice-producing region in China and is unique in its plant biodiversity and prevalence of plant diseases (55). Landforms and climatic patterns are also diverse and favor epidemic development of various plant diseases (55,57). This is true especially in Yuxi District, a major area of rice production in Yunnan which, despite its location (24.35°N, 102.52°E), has subtropical characteristics. Wild species of rice are maintained (8), multiple cultivars of both *indica* and *japonica* rice are grown, three cycles of rice planting per year are feasible, and rice diseases occur successively across seasons (3). Therefore, the location has been used in many agricultural and plant biology studies.

Here, we report results of field experiments conducted at three locations (Fig. 1) in Yuxi District to compare effects of $HpaG_{10\text{-}42}$ and $HpaG_{Xooc}$ on diseases and yield of both *indica* and *japonica* rice cultivars in grower plantings. Five tests important for judging whether $HpaG_{10\text{-}42}$ is worthy of practical agricultural use were carried out (Table 1). We present evidence that, under production agricultural conditions, $HpaG_{10\text{-}42}$ is effective and better than $HpaG_{Xooc}$ at decreasing the severity of the three top diseases and at increasing grain yield of rice, to an extent comparable with local agronomic management measures.

MATERIALS AND METHODS

Protein preparation. The $HpaG_{Xooc}$ protein and the $HpaG_{10\text{-}42}$ protein fragment (hereafter referred to as a protein, for con-

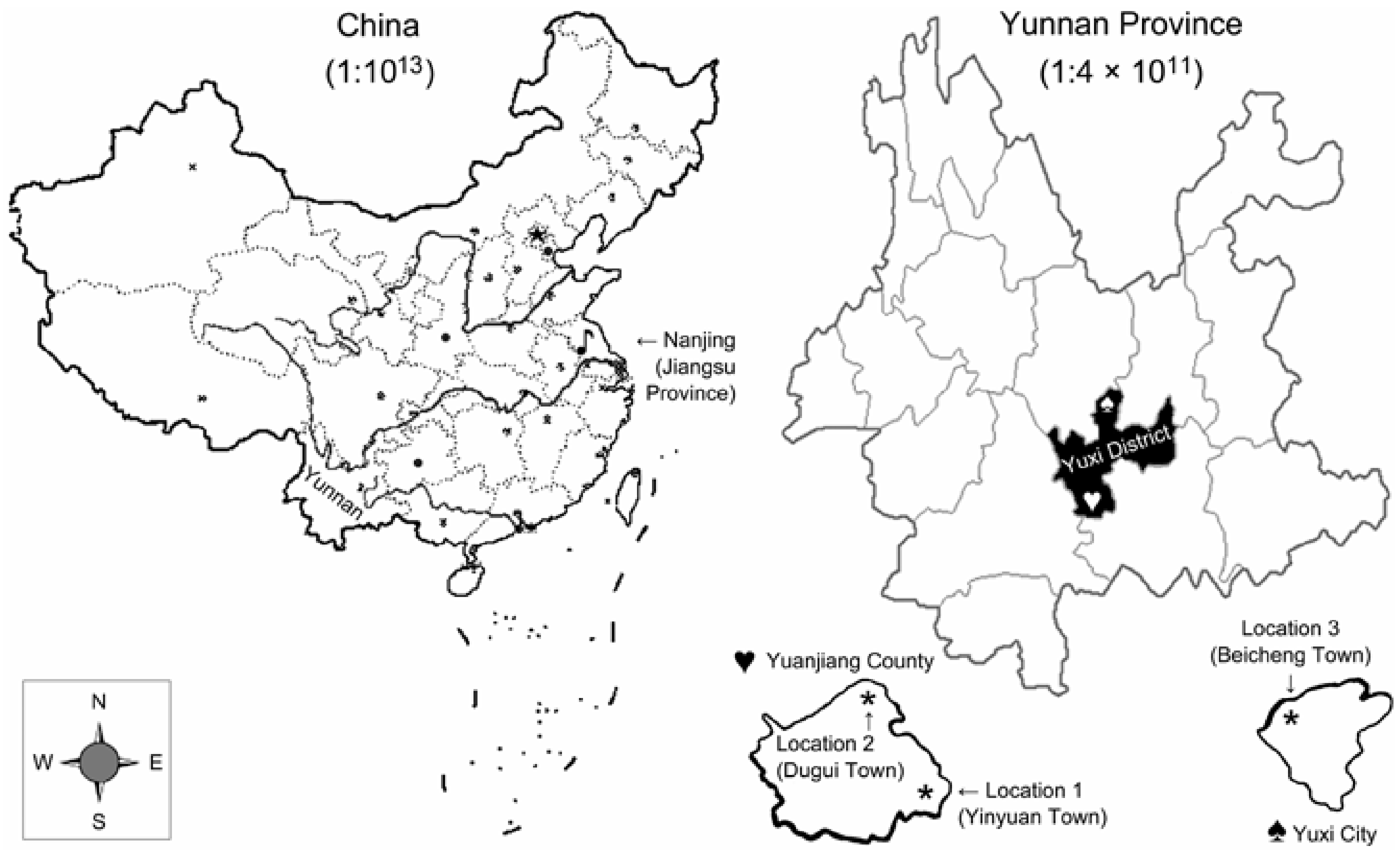

Fig. 1. Location of experiments. Experiments were carried out in Yunnan Province, in southwest China (map at left). The magnified map of Yunnan Province (top right) highlights Yuxi District, which is composed of Yuxi City and several counties. The playing card club and heart symbols indicate experimental sites. Experimental sites 1 and 2 are situated at two townships (YinYuan and Duigui; bottom left) in Yuanjiang County (23.50°N, 101.90°E). Experimental site 3 is situated at a township (Beicheng) in the northernmost portion of Yuxi City (24.35°N, 102.52°E). Tests carried out at the three locations are stated in Table 1. Maps were downloaded from http://www.ivwo.com; scales are approximate.

TABLE 1. Field experiments

Test	Year	Locations[a]	Numbers of: Rice cvs.	Treatments[b]	Repeats	Plots	Results shown in
Application rate and timing combinations	2003	1 to 3	2	20	3	360	Tables 2 to 4
Effects of single application of proteins	2003	1 and 3	2	4	3	48	Text
	2004	1 and 3	1	9	3	54	Table 5
Efficacy of protein application relative to local management practices	2004	1 and 3	2	4	3	48	Figure 2, Table 6
Protein effects on different rice cultivars	2005	1 and 3	9	2	3	108	Table 7
Large-scale application trials	2005	1	2	3	3	18	…
	…	1 to 3	2	2	3	36	Figures 3 to 6
Total	…	3	9	51	3	672	…

[a] Locations described in Figure 1.
[b] "Untreated" was regarded as a treatment.

venience), were expressed in recombinant *Escherichia coli* cells and purified as described (5,44). These preparations were diluted to 200 μg/ml in water amended with 0.3% of the surfactant Silwet-77 and the protease inhibitor phenylmethylsulfonyl fluoride at 50 μg/ml (18,50). The resulting formulas were maintained under 4°C and diluted with tap water immediately before use in rice. Reagents used were from Momentive Performance Materials, Inc. (China Branch, Beijing).

Study sites and rice cultivars. Experiments were done in three townships of Yuxi District, Yunnan Province, China (Fig. 1). Nine cultivars of rice (*Oryzae sativa*) planted by local growers were evaluated. The *indica* rice cv. HaiLuHong 2 and the *japonica* rice cv. ChuJingXiang 1 were tested in all experiments. They are the predominant cultivars grown in Yuxi and are susceptible to rice blast and bacterial blight. Five Other *indica* rice cultivars (DaLiXiang, MaZhaGu, XiangYu 1, 98Yu10, and 93E3) and two other *japonica* cultivars (HeXi24 and YuYiu 1) also were tested.

Field tests and plant treatments. Field studies were conducted in 3 years to carry out five tests (Table 1). Test 1 was devised to optimize application rate and timing combinations by the orthogonal (incomplete plot) experiment. This type of experiment is used to ensure accuracy of tests at reduced numbers of numerous treatment and influencing factors by selecting representative and normalized factors with the aid of an orthogonal table (32). $HpaG_{Xooc}$ and $HpaG_{10-42}$ were used in nine combinations of three concentrations, 6, 12, and 24 μg/ml, which equate to 0.395, 0.790, and 1.580 μM $HpaG_{Xooc}$ or 1.653, 3.306, and 6.612 μM $HpaG_{10-42}$ (5). Plants were sprayed once in the nursery and three times in the field at vegetative growth stages V6 and V11 (collar formation on leaves 6 and 11, respectively, on the main stem) and the reproductive stage R2 (collar formation on flag leaf) (7). The application rate and timing combinations were selected based on orthogonal L_9 (3^4) arrays (32). These arrays were tested in comparison with no spray (CK) in 2003.

Tests 2 and 3 were intended to evaluate the practicality of protein application in rice. Test 2 was devised to determine whether a single application of the proteins affects diseases and yields. The use of proteins was compared with CK in an independent plot experiment conducted in 2003 and a confirmative plot experiment in 2004. Plants were treated once in either the nursery or transplanting fields at V6, V11, or R2. $HpaG_{Xooc}$ and $HpaG_{10-42}$ were applied at 6 and 12 μg/ml, respectively. Test 3 was conducted in 2004 to determine the efficacy of protein application relative to local agronomic management measures for disease control and yield enhancement of rice. $HpaG_{Xooc}$ and $HpaG_{10-42}$ were applied at the optimum array of rate and times determined by the orthogonal experiments in 2003. Agronomic management measures were carried out by growers and mainly included one-leaf fertilization before V6 and two to six applications of chemicals for disease control when required (Table 2). These measures were regarded as a single test unit. The types of chemicals used and rates of application were chosen by farmers and varied with study locations, rice cultivars, and local forecasting of disease epidemic development and, thus, are not specified, except for the chemicals used most frequently at three locations, which are listed in Table 2.

Incomplete plot experiments for test 1 and complete plot experiments for tests 2 and 3 all were composed of three repeats distributed as three plots for each treatment (including CK). Each plot occupied 5 m^2 in a seedling nursery and 22 m^2 in a field.

Tests 4 and 5 were designed to evaluate the extensiveness of $HpaG_{10-42}$ utility in rice. Test 4 was devised to test effects of $HpaG_{10-42}$ on nine different rice cultivars. Test 5 was intended to confirm $HpaG_{10-42}$ effects by large-scale experimentation. For both tests, $HpaG_{10-42}$ was compared with CK and applied at the optimized array of rate and times. Treatments were assigned randomly to three plots. Each plot occupied 20 to 40 m^2 in a nursery and areas of various sizes in a field.

For all tests, the solution of $HpaG_{Xooc}$ or $HpaG_{10-42}$ was applied 10 days prior to transplant by spraying nursery seedlings from above using a handheld sprayer (SX-5073; Shixia Sprayer, Ltd., Zhejiang, China) or by spraying plants in the field using an overhead mechanical sprayer (SX-16; Shixia Sprayer, Ltd.). Except for designated plots for test 3, no chemicals were used other than fertilizer, which was applied in accordance with growers' usual practices.

Surveys of rice growth, yield, and diseases. Rice growth in the nursery and in the field was monitored. In fields, plant weight was determined using nine plants per plot. Grain yield was surveyed as previously described (57). Severities of rice bacterial blight, rice blast, and sheath blight were assessed based on sampling at five sites per plot (13,26). Numbers of plants surveyed are noted in figures and tables. Depending on diseases, symptoms were classified by treating a plant as an assessment unit (Table 3). A disease severity index (Table 3) was used because it can reflect disease severity variations in large plant

TABLE 2. Major chemicals used in disease control at the study locations

Chemicals (formula, dose)[a]	Diseases	Application methods and timing
Prochloraz (25% emulsion, 1/2,500)	Rice blast, sheath blight	Soaking seeds for 24–48 h
Tricycloazole (20% wettable powder, 4 g/liter)	Rice blast	Spraying plant tops at R2 and 10–20 days later
Validamycin (5% aqueous solution, 40–60 ml/liter)	Sheath blight	Spraying plant tops 2 to 3 times starting before V6 and at 10- to 25-day intervals
Sodium hypochlorite (85% aquatic solution 1/400)	Bacterial rice blight	Soaking seed for 24–48 h
Bismerthlazol (20% wettable powder, 1/400)	Bacterial rice blight	Spraying over plants 4 to 6 times since V6 and at 7-day interval

[a] Manufacturers: Prochloraz, Yancheng Luye Chemical Co., Ltd., Shandong, China; Tricycloazole and validamycin, Guoying Kunshan Biochemical Co., Ltd., Najing, China; Sodium hypochlorite, Shanghai Tongya Chemical Technology Co., Ltd., Shanghai, China; and Bismerthlazol, Zhejiang Dongfeng Chemical Co., Ltd., Hangzhou, China.

TABLE 3. Disease severity indexes used

	Part of plant that shows symptoms per class[a]				
Disease	1	2	3	4	5
Bacterial blight of rice[b]	No disease	<10% leaf area	10–20% leaves	20–40% leaves	>40% leaves
Rice blast[c]	No disease	<10% of leaves or panicles	10.1–20% of leaves or panicles	20.1–40% of leaves or panicles	>40% of leaves or panicles
Rice sheath blight	No disease	<5% of plant	5–10% of plant	10.1–20% of plant	>20% of plant

[a] Each class was arbitrarily given a representative number (RN): Class 1, RN = 1; class 2, RN = 2; class 3, RN = 3; class 4, RN = 4; and class 5, RN = 5. Number (N) of rice plants in each class were scored. Disease severity index was calculated using the formula disease severity index = 100 × Σ(RN of a class × N in the class)/(RN of the greatest class × total N surveyed).

[b] Leaf blight was mainly assessed.

[c] Mixture of leaf blast and panicle blast was scored with similar criteria.

populations and is determined based on dividing symptoms into measurable or describable classes (13,30) (Table 3).

Data treatment. Data were analyzed separately by year and study site owing to differences in rice cultivar and disease pressure. Each survey plot was regarded as a statistical unit ($n = 3$ plots). Distribution patterns of observed values were plotted with Microsoft Excel graph tools. One-way F tests were used to judge whether the use of $\mathrm{HpaG}_{10\text{-}42}$ or HpaG_{Xooc} caused significant effects on disease severities and grain yields.

RESULTS

$\mathrm{HpaG}_{10\text{-}42}$ is more active than HpaG_{Xooc} in decreasing disease severities and increasing grain yields in rice. Orthogonal arrays were used to optimize nine combinations of rate and timing of application of HpaG_{Xooc} or $\mathrm{HpaG}_{10\text{-}42}$ (Tables 4 to 6) for effects on diseases and yields of rice (Table 1, test 1). In 2003, experiments were done with the *indica* rice cv. HaiLuHong 2 and the *japonica* rice cv. ChuJingXiang 1. Plants were surveyed at three study sites and data were analyzed by one-way F tests ($n = 3$ plots). F tests were the same in all analyses and only the P value is noted below. Difference and significance regarding a treatment were specified in contrast to CK unless otherwise noted.

There were consistent differences between treatments in severities of bacterial blight on leaves (leaf blight) and rice blast on panicle (panicle blast) in the *indica* (Table 4) and *japonica* (Table 5) rice cultivars. Leaf blight and panicle blast declined significantly ($P < 0.05$) in both cultivars treated with HpaG_{Xooc} or $\mathrm{HpaG}_{10\text{-}42}$ in 13 of 18 rate and timing arrays. Greater extents of disease decrease were observed in the *indica* rice cultivar than the *japonica* rice cultivar. In both cultivars, panicle blast was alleviated more than leaf blight. $\mathrm{HpaG}_{10\text{-}42}$ was more active than HpaG_{Xooc} in reducing severities of both diseases. HpaG_{Xooc} rates of 12, 24, 6, and 12 µg/ml applied in turn at the four stages of rice growth caused significant reductions in disease severities ($P < 0.05$); however, but the greatest and most significant effects ($P < 0.01$) were observed when $\mathrm{HpaG}_{10\text{-}42}$ at 6 µg/ml was used for each application. With this optimal array of rate and timing, HpaG_{Xooc} caused 21.9 and 86.0% decreases while $\mathrm{HpaG}_{10\text{-}42}$ induced 61.6 and 93.6% decreases in severities of leaf blight and panicle blast, respectively, in the *indica* rice cultivar (Table 4). Effects were similar in the *japonica* rice cultivar (Table 5).

HpaG_{Xooc} and $\mathrm{HpaG}_{10\text{-}42}$ also differed from one another in affecting rice yield. Eight of nine HpaG_{Xooc} rate and timing arrays and all arrays for $\mathrm{HpaG}_{10\text{-}42}$ increased grain yields (Table 6). The extent of increase was greater for the *japonica* rice cultivar than the *indica* rice cultivar. Two HpaG_{Xooc} arrays (12, 24, 6, and 12 µg/ml; and 24, 12, 6, and 24 µg/ml) significantly increased grain yields in both cultivars and two other HpaG_{Xooc} arrays significantly increased yields of the *indica* rice cultivar ($P < 0.05$). Noticeably, all $\mathrm{HpaG}_{10\text{-}42}$ arrays elevated yield in both the *indica* and the *japonica* rice cultivar. Two $\mathrm{HpaG}_{10\text{-}42}$ arrays, 6 µg/ml all four times and 6, 12, 12, and 12 µg/ml, increased yields of both cultivars in a particular robust fashion ($P < 0.01$).

Single application of the proteins is less effective. We tested whether a single application of HpaG_{Xooc} and $\mathrm{HpaG}_{10\text{-}42}$ caused significant effects on rice (Table 1, test 2). Plant surveys in plot experiments conducted in 2003 indicated that effects of a single application on diseases and yields of rice varied markedly with the time of the application (Table 7). The maximum effect on yield was observed when HpaG_{Xooc} or $\mathrm{HpaG}_{10\text{-}42}$ were applied at the V6 stage (collar formation on leaf 6 on main stem), resulting in 15.5 and 20.0% increases, respectively, in the *japonica* rice cv. ChuJingXiang 1. Leaf blight was reduced the most with application at this stage as well, with HpaG_{Xooc} and $\mathrm{HpaG}_{10\text{-}42}$ causing 18.5 and 55.0% reductions, respectively, in disease severity. Decreases in panicle blast, however, were greatest when plants were treated at the R2 heading stage (collar formation on flag leaf): 73.4 and 82.4% ($P < 0.01$) in plots treated with HpaG_{Xooc} and $\mathrm{HpaG}_{10\text{-}42}$, respectively. When the proteins were applied at this stage, leaf blight was 30% more severe than it was when plants were treated at V6. In the *japonica* rice cultivar, the magni-

TABLE 4. Effects of HpaG_{Xooc} and $\mathrm{HpaG}_{10\text{-}42}$ on leaf blight and panicle blast of the *indica* rice cv. HaiLuHong 2 according to different arrays of rate and timing of application optimized by orthogonal design[a]

	Leaf blight severity index		Panicle blast severity index	
Protein, concentrations (µg/ml)[b]	Observed[c]	Decrease (%)	Observed[c]	Decrease (%)
HpaG_{Xooc}				
6, 6, 6, 6	80.0 ± 7.8	13.3 ± 2.3	18.1 ± 1.8	31.4 ± 5.7
6, 12, 12, 12	83.5 ± 7.5	9.5 ± 2.0	11.8 ± 1.4	55.3 ± 6.4
6, 24, 24, 24	80.1 ± 8.4	13.2 ± 2.9	9.5 ± 0.8	64.0 ± 9.5
12, 6, 12, 24	75.6 ± 6.3	18.1 ± 0.7	10.2 ± 1.0	61.4 ± 8.7
12, 12, 24, 6	75.1 ± 6.6	18.6 ± 1.0	7.4 ± 0.8	72.0 ± 9.5
12, 24, 6, 12	72.1 ± 6.9	21.9 ± 1.3	3.7 ± 0.8	86.0 ± 9.5
24, 6, 24, 12	75.1 ± 8.0	18.6 ± 2.5	12.8 ± 0.6	51.5 ± 10.2
24, 12, 6, 24	78.7 ± 8.4	14.7 ± 2.9	8.9 ± 1.0	66.3 ± 8.7
24, 24, 12, 6	82.0 ± 8.5	11.2 ± 3.0	6.8 ± 1.2	74.2 ± 8.0
$\mathrm{HpaG}_{10\text{-}42}$				
6, 6, 6, 6	35.4 ± 3.5	61.6 ± 2.4	1.7 ± 0.3	93.6 ± 11.4
6, 12, 12, 12	52.5 ± 3.5	43.1 ± 2.4	1.7 ± 0.2	93.6 ± 11.7
6, 24, 24, 24	51.2 ± 3.2	44.5 ± 2.7	1.9 ± 0.1	92.8 ± 12.1
6, 24, 24, 24	69.1 ± 5.6	25.1 ± 0.1	2.2 ± 0.2	91.7 ± 11.7
12, 12, 24, 6	48.6 ± 4.2	47.3 ± 1.6	2.3 ± 0.2	91.3 ± 11.7
12, 24, 6, 12	49.0 ± 4.5	46.9 ± 1.3	2.0 ± 0.2	92.4 ± 11.7
24, 6, 24, 12	46.1 ± 3.9	50.1 ± 2.0	2.2 ± 0.3	91.7 ± 11.4
24, 12, 6, 24	57.6 ± 3.4	37.6 ± 2.5	2.1 ± 0.2	92.0 ± 11.7
24, 24, 12, 6	56.8 ± 5.6	38.5 ± 0.1	2.1 ± 0.2	92.0 ± 11.7
CK				
0, 0, 0, 0	92.3 ± 5.7	…	26.4 ± 3.3	…

[a] Diseases were surveyed with 60 plants per plot. Leaf blight and panicle blast were investigated prior to heading stage and during seed saturation, respectively. Disease severity index is given as mean ± standard deviation of results from three plots (repeats). Percent reduction was relative to untreated control (CK). Trends in differences between treatments were similar at the three study sites. Results from location 2 are shown.

[b] Concentrations are listed in the order they were used, at 10 days before transplant, at vegetative stages V6 and V11, and at reproductive stage R2 (7).

[c] One-way F tests ($n = 3$ pots; $P < 0.05$ and 0.01) were done based on the observed values to test significance in differences between CK and each array of rate and timing of application. Significance at $P < 0.05$ is not shown if applied to all compared combinations; other cases are noted with tables or in text; only P value is indicated below.

tude of the effects was roughly half of that observed in the *indica* rice cultivar under the same experimental conditions; however, relative effects of treating at different times were similar.

A confirmatory experiment was conducted in 2004. Diseases were not investigated until the V11 stage (collar formation on leaf 11 on main stem) based on our experience the previous year. Greater effects of $HpaG_{10-42}$ than $HpaG_{Xooc}$ and the importance of timing and multiple applications were corroborated (Table 6). In CK plots, both bacterial blight and rice blast progressed with time, leaf blight occurred cyclically, and blast appeared on leaves earlier and panicles later and scored as a mixture after R2. The application of $HpaG_{Xooc}$ and $HpaG_{10-42}$ impeded progression and severities of both diseases as observed at three intervals after the last treatment. Against both diseases, protection following application at any of the three stages of rice growth in fields was robust in 10 and 20 days, and did not begin to wane until 30 days. Treating plants at V6 or V11 provided the best protection against leaf blight ($P < 0.05$). However, leaf blast was most significantly decreased following application at V11 and panicle blast at R2. When plants were treated with either of the proteins late, at V6, or surprisingly, early, at the nursery seedling stage, increases in grain yield were significant ($P < 0.01$ for $HpaG_{10-42}$ and $P < 0.05$ for $HpaG_{Xooc}$). In contrast, $HpaG_{10-42}$ treatment at R2 and treatment with $HpaG_{Xooc}$ at V11 conferred smaller and insignificant increases in grain yields. Thus, early effects of the proteins on rice productivity seem to contribute to grain yield. In contrast to effects on yield, treating nursery seedlings caused little reduction in subsequent disease severities in the field.

$HpaG_{10-42}$ is comparable with standard agronomic management practices in benefiting rice production. To determine the efficacy of protein application relative to local agronomic management measures for disease control and yield enhancement of rice (Table 1, test 3), $HpaG_{Xooc}$ and $HpaG_{10-42}$ were compared with CK and local practices in effects on diseases and yields of the *indica* rice cv. HaiLuHong 2 and the *japonica* rice cv. ChuJingXiang 1. Plants growing in experimental plots were surveyed together with plants growing in production fields adjacent to the experimental plots; plants in production fields were managed according to local practices, including the application of chemicals. Leaf blight and panicle blast were alleviated significantly ($P < 0.01$) under all three conditions but to the greatest extent (62.4 and 90.7%, respectively) by $HpaG_{10-42}$ and to a lesser extent by standard local practices (11.7 and 82.7%, respectively) and $HpaG_{Xooc}$ (22.9 and 79.8%, respectively) (Fig. 2A). With respect to yield, $HpaG_{10-42}$ caused 24.7 and 23.6% increases in the *indica* and *japonica* rice cultivars, respectively, in contrast to 17.4 and 22.0% increases in the case of standard local practices and 12.3 and 15.8% increases caused by $HpaG_{Xooc}$ (Fig. 2B).

$HpaG_{10-42}$ is effective at different levels in different cultivars of rice. Six *indica* rice cultivars and three *japonica* rice cultivars planted in locations 1 and 3 (Fig. 1), respectively, were tested to determine whether $HpaG_{Xooc}$ and $HpaG_{10-42}$ differentially affected these cultivars (Table 1, test 4). Significant decreases in severities of leaf blight and panicle blast were observed in all cultivars treated with $HpaG_{Xooc}$ or $HpaG_{10-42}$ (Table 8). Increases in grain yield of all rice cultivars also were significant following application of either protein relative to CK ($P < 0.01$). Nevertheless, great variation was found among cultivars which did not correlate with the subspecies *indica* or *japonica*. For example, in treatment with $HpaG_{Xooc}$, the optimal increase in grain yield (52.7%) was in the *indica* rice cv. 98Yu10, in contrast to only a 5.2% increase in the cv. HaiLuHong 2 of the same rice type. Ranges of 20.0 to 97.4% decreases in leaf blight severities and 15.5 to 97.5% decreases in panicle blast severities were observed in the nine cultivars of rice. As with yield, there was no definite relationship between rice subspecies and effect of protein application on disease severities.

TABLE 5. Effects of $HpaG_{Xooc}$ and $HpaG_{10-42}$ on leaf blight and panicle blast of the *japonica* rice cv. ChuJingXiang 1 according to different arrays of rate and timing of application[a]

Protein, concentrations (μg/ml)[b]	Leaf blight severity index		Panicle blast severity index	
	Observed[c]	Decrease (%)	Observed[c]	Decrease (%)
$HpaG_{Xooc}$				
6, 6, 6, 6	53.3 ± 8.0*	3.1 ± 0.9	46.2 ± 4.8	20.6 ± 6.0
6, 12, 12, 12	55.7 ± 6.5*	–1.3 ± 3.6	40.2 ± 4.0	30.9 ± 7.4
6, 24, 24, 24	53.4 ± 7.4*	2.9 ± 2.0	41.4 ± 4.5	28.9 ± 6.5
12, 6, 12, 24	50.4 ± 4.3	8.4 ± 7.6	44.9 ± 5.5	22.9 ± 4.8
12, 12, 24, 6	50.1 ± 5.4	8.9 ± 5.6	43.3 ± 5.5	25.6 ± 4.8
12, 24, 6, 12	46.7 ± 3.3	15.1 ± 9.5	38.2 ± 6.0	34.4 ± 4.0
24, 6, 24, 12	50.1 ± 6.3	8.9 ± 4.0	42.5 ± 4.5	27.0 ± 6.5
24, 12, 6, 24	52.5 ± 8.5*	4.5 ± 0.0	40.5 ± 5.0	30.4 ± 5.7
24, 24, 12, 6	54.7 ± 8.3*	0.5 ± 0.4	43.3 ± 4.2	25.6 ± 7.0
$HpaG_{10-42}$				
6, 6, 6, 6	24.0 ± 4.0	56.4 ± 8.2	14.0 ± 2.5	76.0 ± 10.0
6, 12, 12, 12	35.0 ± 3.6	36.4 ± 8.9	17.2 ± 2.8	70.4 ± 9.5
6, 24, 24, 24	34.1 ± 3.9	38.0 ± 8.4	16.8 ± 2.2	71.1 ± 10.5
12, 6, 12, 24	36.1 ± 4.2	34.4 ± 7.8	16.6 ± 2.8	71.5 ± 9.5
12, 12, 24, 6	32.4 ± 3.3	41.1 ± 9.5	16.2 ± 2.3	72.2 ± 10.3
12, 24, 6, 12	32.7 ± 3.6	40.5 ± 8.9	15.2 ± 2.5	73.9 ± 10.0
24, 6, 24, 12	37.7 ± 4.3	31.5 ± 7.6	16.3 ± 3.3	72.0 ± 8.6
24, 12, 6, 24	38.4 ± 4.0	30.2 ± 8.2	16.8 ± 2.5	71.1 ± 10.0
24, 24, 12, 6	37.9 ± 3.6	31.1 ± 8.9	16.5 ± 2.5	71.6 ± 10.0
CK				
0, 0, 0, 0	55.0 ± 8.5	…	58.2 ± 8.3	…

[a] Experimentation was designed for test 1 (Table 1). Diseases were surveyed with 60 plants per plot. Leaf blight and panicle blast were investigated prior to heading stage and during seed saturation, respectively. Disease severity index is given as mean ± standard deviation of results from three plots (repeats). Percent reduction was relative to untreated control (CK). Trends in differences between treatments were similar at the three study sites. Results from location 2 are shown. Tendency in differences between CK and the rate and timing arrays was similar at the three study sites (Fig. 1). Results obtained from location 3 are presented. An asterisk (*) refers to insignificant difference between CK and the corresponding rate and timing array ($P < 0.05$); significant differences are not indicated.

[b] Concentrations are listed in the order they were used, at 10 days before transplant, at vegetative stages V6 and V11, and at reproductive stage R2 (7).

[c] One-way F tests ($n = 3$ pots; $P < 0.05$ and 0.01) were done based on the observed values to test significance in differences between CK and each array of rate and timing of application. Significance at $P < 0.05$ is not shown if applied to all compared combinations; other cases are noted with tables or in text; only P value is indicated below.

Application of $HpaG_{10-42}$ on a large scale consistently promotes growth, decreases diseases, and increases grain yield of rice. To test $HpaG_{10-42}$ effects on plant growth, disease, and yield over a large area (Table 1, test 5), field trails were done in 2005 on a total of 40-ha distributed across the three townships. At location 1 (Fig. 1), $HpaG_{10-42}$ was compared with $HpaG_{Xooc}$ and CK on a 10-ha area for effects on rice growth and grain yield. Effects of treating nursery seedlings were apparent subsequently in the field, contributing to growth of the *indica* rice cv. HaiLuHong 2 and the *japonica* rice cv. ChuJingXiang 1 (Fig. 3). $HpaG_{10-42}$-treated nursery seedlings were larger and more uniform than those in CK plots, as observed 7 and 20 days after transplanting (Fig. 3A and data not shown). $HpaG_{10-42}$-treated seedlings recovered from transplanting 4 to 5 days earlier, as judged by reinitiation of growth (Fig. 3B). Plant height was 36 and 46% greater (Fig. 3C) and plant weight was 45 and 48% greater (Fig. 3D and data not shown) in HaiLuHong 2 and ChuJingXiang 1, respectively, at 20 days after transplant. $HpaG_{Xooc}$ also was effective, but ≈10% less so ($P < 0.05$). Similar results were obtained when plant growth vitality was investigated at the V11 stage (Fig. 3D and E). In the *indica* and *japonica* rice cultivars, $HpaG_{10-42}$ resulted in 25.6 and 17.7% increases, respectively, in plant weight relative to CK and significantly exceeded $HpaG_{Xooc}$ in this effect ($P < 0.01$).

TABLE 6. Effects of $HpaG_{Xooc}$ and $HpaG_{10-42}$ on grain yield of the *indica* rice cv. HaiLuHong 2 (HLH) and the *japonica* rice cv. ChuJingXiang 1 (CJX)[a]

	HLH grain yield (kg/22 m^2)		CJX grain yield (kg/22 m^2)	
Protein, concentrations (µg/ml)[b]	Observed[c]	Decrease (%)	Observed[c]	Decrease (%)
$HpaG_{Xooc}$				
6, 6, 6, 6	15.3 ± 1.3	–0.6 ± 9.7	12.0 ± 0.8	–6.3 ± 6.3
6, 12, 12, 12	16.0 ± 0.8	3.9 ± 13.0	14.7 ± 1.3*	14.8 ± 3.9
6, 24, 24, 24	16.4 ± 1.6	6.5 ± 7.8	14.6 ± 1.0*	14.1 ± 1.6
12, 6, 12, 24	15.6 ± 1.2	1.3 ± 10.4	12.4 ± 0.6	–3.1 ± 4.7
12, 12, 24, 6	15.6 ± 1.4	1.3 ± 9.1	12.6 ± 1.5	–1.6 ± 2.3
12, 24, 6, 12	16.8 ± 0.8*	9.1 ± 13.0	15.4 ± 0.5*	20.3 ± 5.5
24, 6, 24, 12	15.7 ± 1.5	1.9 ± 8.4	13.4 ± 0.6	4.7 ± 4.7
24, 12, 6, 24	16.7 ± 0.8*	8.4 ± 13.0	14.1 ± 1.1*	10.2 ± 0.8
24, 24, 12, 6	16.1 ± 1.5	4.5 ± 8.4	12.6 ± 1.8	–1.6 ± 4.7
$HpaG_{10-42}$				
6, 6, 6, 6	19.6 ± 1.4*	27.3 ± 9.1	16.3 ± 1.2*	27.3 ± 4.7
6, 12, 12, 12	18.3 ± 1.5*	18.8 ± 8.4	16.6 ± 0.9*	29.7 ± 2.3
6, 24, 24, 24	18.8 ± 1.5*	22.1 ± 8.4	15.7 ± 0.8*	22.7 ± 3.1
12, 6, 12, 24	18.5 ± 2.2*	20.1 ± 3.9	15.2 ± 1.3*	18.8 ± 0.8
12, 12, 24, 6	18.8 ± 1.3*	22.1 ± 9.7	15.3 ± 1.2*	19.5 ± 0.0
12, 24, 6, 12	19.0 ± 1.5*	23.4 ± 8.4	13.2 ± 2.0*	3.1 ± 6.3
24, 6, 24, 12	18.4 ± 1.6*	19.5 ± 7.8	14.9 ± 2.1*	16.4 ± 7.0
24, 12, 6, 24	18.6 ± 0.8*	20.8 ± 13.0	15.6 ± 0.8*	21.9 ± 3.1
24, 24, 12, 6	18.1 ± 1.3*	17.5 ± 9.7	14.4 ± 1.4*	12.5 ± 1.6
CK				
0, 0, 0, 0	15.4 ± 2.8	…	12.8 ± 1.2	…

[a] Diseases were surveyed with 60 plants per plot. Leaf blight and panicle blast were investigated prior to heading stage and during seed saturation, respectively. Disease severity index is given as mean ± standard deviation (SD) of results from three plots (repeats). Percent reduction was relative to untreated control (CK). Trends in differences between treatments were similar at the three study sites. Results from location 2 are shown. Actual grain yields were determined for all plants growing at three study locations and are presented as mean ± SD of results from three plots. Percent increases in yield are relative to CK. Trends in grain yield differences between treatments were similar across the three locations. Results are from location 1 for HaiLuHong 2 and location 3 for ChuJingXiang 1. Asterisks indicate significant differences in the observed variants between CK and the indicated arrays ($P < 0.05$); insignificant differences are not annotated.

[b] Concentrations are listed in the order they were used, at 10 days before transplant, at vegetative stages V6 and V11, and at reproductive stage R2 (7).

[c] One-way F tests ($n = 3$ pots; $P < 0.05$ and 0.01) were done based on the observed values to test significance in differences between CK and each array of rate and timing of application. Significance at $P < 0.05$ is not shown if applied to all compared combinations; other cases are noted with tables or in text; only P value is indicated below.

TABLE 7. Effects of a single application of the proteins on bacterial blight and rice blast severities and grain yields of rice[a]

	Blight severity index (decrease [%])			Blast severity index (decrease [%])			
Protein, stage[b]	10 days	20 days	30 days	10 days	20 days	30 days	Yield (increase [%])[c]
$HpaG_{Xooc}$							
1	ns	ns	59.9 (11.3)	ns	ns	14.3 (28.6)	16.9 (9.7)
2	29.4 (26.5)	39.1 (23.3)	46.7 (18.5)	0.66 (79.5)	1.2 (77.4)	9.0 (43.8)	16.0 (3.9)
3	33.2 (22.8)	39.9 (22.5)	45.2 (22.9)	0.5 (82.8)	0.9 (80.4)	1.0[Lpb] (78.8)	15.8 (2.6)
4	47.0 (15.8)	54.5 (18.0)	62.0(18.4)	2.6 (83.3)	3.1[Lpb] (85.6)	3.8[Lpb] (86.2)	15.8 (2.6)
$HpaG_{10-42}$							
1	ns (14.5)	ns	67.0	ns (66.7)	ns	9.5 (20.1)	18.5
2	21.0 (62.4)	23.5 (64.7)	26.0 (65.8)	3.8 (75.6)	4.1 (82.2)	5.5 (80.7)	18.4 (19.5)
3	21.5 (61.5)	25.0 (62.4)	28.5 (62.5)	1.2 (92.3)	1.7 (92.6)	1.8[Lpb] (93.7)	18.6 (20.8)
4	26.0 (53.4)	31.5 (52.6)	35.0 (53.9)	1.7 (89.1)	1.6[Lpb] (92.6)	2.5[Lpb] (90.9)	17.5 (13.6)
CK							
1	ns	ns	67.5	ns	ns	20.0	15.4
2	41.0	51.0	57.5	3.2	5.3	16.0	15.4
3	43.0	51.5	58.6	2.9	4.6	5.2[Lpb]	15.4
4	55.8	66.5	76.0	15.6	23.0, 21.5[Lpb]	28.5, 27.5[Lpb]	15.4

[a] Diseases were investigated at 10-day intervals after treatments; ns = not surveyed. Results are given as means evaluated as in Table 4. Lpb denotes mixture of leaf blast and panicle blast scored together; otherwise, only leaf blast was evaluated.

[b] Application stages: 1 = nursery, 10 days before transplant; 2 = late turning-green stage; 3 = late tillering stage; and 4 = early heading stage. Solutions containing $HpaG_{10-42}$ and $HpaG_{Xooc}$ at 6 and 12 µg/ml, respectively, were applied once to plants of the *indica* rice cv. HaiLuHong 2 at the indicated stages of rice growth.

[c] Grain yields (kg/22 m^2) are means from two study locations. The plot size represents one-third of a "Fen," a conventional unit of land area in China equal to 66 m^2.

The effectiveness of HpaG$_{10-42}$ in protecting against diseases and increasing yields of rice was tested on a 30-ha area distributed across the three townships (Fig. 1). Bacterial blight of rice, rice blast, and sheath blight were investigated. The mildness of diseases was visually recognizable in HpaG$_{10-42}$ plots (Figs. 4 and 5). Quantitative assessment showed that sheath blight, leaf blight, and panicle blast each were alleviated markedly ($P < 0.01$) (Fig. 6A). Leaf blight was 86.3% less severe in the *indica* cv. HaiLuHong 2 and 66.4% less severe in the *japonica* cv. ChuJingXiang 1. Sheath blight occurred little in ChuJingXiang 1 and was 55.0% less severe in HaiLuHong 2 at the R2 stage. During seed maturation, panicle blast severity was decreased by 93.2% in HaiLuHong 2 and 79.1% in ChuJingXiang 1 (Fig. 6A). Grain yield was significantly higher in treated plants ($P < 0.01$), presumably as a result of protection form diseases (Fig. 6B). In some locations, ≈80% of panicles and 70% of the grain in CK plots became shrunken, evidently due to blast, but only a small proportion of HpaG$_{10-42}$-treated plants showed such symptoms (Figs. 5 and 6A). Ultimately, HpaG$_{10-42}$-treated plants of the *indica* rice cultivar and the *japonica* rice cultivar had 25.7 and 26.8% greater grain yield, respectively, than CK (Fig. 6B).

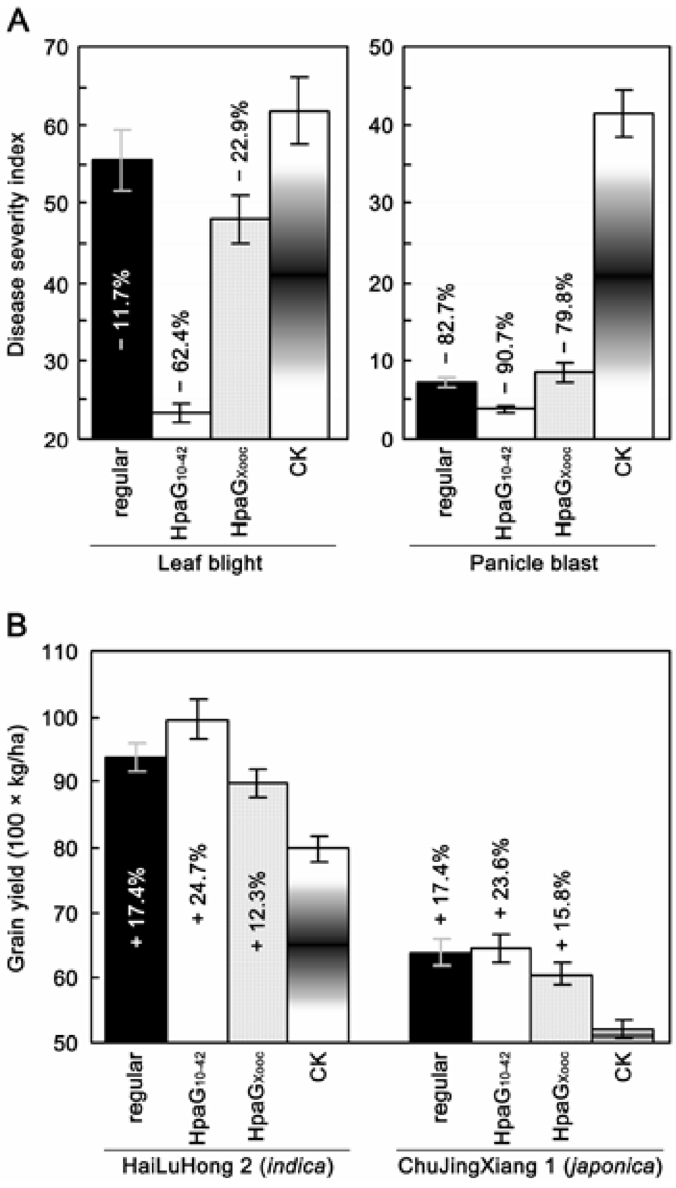

Fig. 2. Effects of HpaG$_{10-42}$ and HpaG$_{Xooc}$ compared with standard local agronomic management practices (Table 2) on diseases and yields of two cultivars of rice (Table 1, test 3). **A,** Disease severities. **B,** Rice grain yields. "Standard" fields (total 135 ha) were managed under local agronomic practices, including the use of chemicals to control diseases. Experimental fields included HpaG$_{10-42}$-treated plots, HpaG$_{Xooc}$-treated plots, and no-spray (CK) plots, each occupying 3.4 ha. Experiments were carried out in three plots with treatments distributed randomly. HpaG$_{10-42}$ was applied four times at 6 µg/ml to nursery seedlings and field transplants at vegetative growth stages V6 and V11 and the reproductive stage R2, respectively. HpaG$_{Xooc}$ was applied at 12, 24, 6, and 12 µg/ml, respectively, to plants at each of these stages. In CK plots, plants were not treated with proteins and disease-controlling chemicals were not applied. Other standard practices, including fertilizer application, were applied to all experimental plots equally. Mean values are shown. Percent increase (+) or decrease (•) is relative to CK.

DISCUSSION

We have conducted field tests at three locations over 3 years with confirmative results. These results demonstrate that HpaG$_{10-42}$ controls diseases and increases grain yields in both *indica* and *japonica* rice cultivars to a greater extent than HpaG$_{Xooc}$ and as effectively as standard management practices at these locations, which include the use of agrichemicals. This conclusion is supported by five tests carried out to assess whether HpaG$_{10-42}$ is a viable option for practical use in rice.

Rate and timing of application were optimized by assessing several combinations (Table 1, test 1) for the ability to enhance growth and impede disease, according to a statistically rigorous design (32). Protein concentrations were chosen based on previous studies. Harpins have been shown to promote plant growth (11) and optimally induce defenses against pathogens (5,31,41), insects (11), and drought stress (12) when applied at ≈0.3 µM in aqueous solution. This molar concentration amounts to HpaG$_{Xooc}$ at 5.2 µg/ml. HpaG$_{10-42}$ is much more active than HpaG$_{Xooc}$ (5). Thus, 12 and 6 µg/ml (0.395 and 1.653 µM) were used as minimal doses of HpaG$_{Xooc}$ and HpaG$_{10-42}$, respectively. Application intervals were chosen based on estimates of initiation and duration of plant responses to harpins determined previously. In these studies, induced resistance and enhanced plant growth became evident 5 days after plant treatment and the effects lasted approximately 20 days (5,11,41). However, levels of induced responses declined with time (34,38,41). In addition, rice leaf diseases, including bacterial blight and leaf blight, typically do not appear until the

TABLE 8. HpaG$_{10-42}$ effects on different rice cultivars[a]

Cultivar	Treatment	Disease severity index (decrease [%]) Leaf blight	Panicle blast	Yield (increase [%])[b]
indica rice				
HaiLuHong 2	HpaG$_{10-42}$	2.0 (20.0)	1.4 (88.1)	20.3 (5.2)
	CK	2.4	11.8	19.3
DaLiXiang	HpaG$_{10-42}$	ns	2.1 (54.4)	26.2 (11.5)
	CK	ns	4.6	23.5
MaZhaGu	HpaG$_{10-42}$	ns	2.1 (79.0)	18.7 (17.6)
	CK	ns	10.0	15.9
XiangYiu 1	HpaG$_{10-42}$	22.0 (14.7)	29.9 (15.5)	16.3 (28.3)
	CK	25.8	35.42	12.7
98Yu10	HpaG$_{10-42}$	7.8 (71.0)	9.5 (71.1)	16.8 (52.7)
	CK	26.9	32.9	11.0
99E3	HpaG$_{10-42}$	6.3 (35.1)	5.9 (63.6)	20.5 (10.8)
	CK	9.7	16.2	18.5
japonica rice				
ChuJingXiang 1	HpaG$_{10-42}$	3.1 (60.8)	1.6 (80.5)	15.5 (14.8)
	CK	7.9	8.2	13.5
HeXi 24	HpaG$_{10-42}$	1.2 (82.6)	0.6 (91.3)	18.0 (11.8)
	CK	6.9	6.9	16.1
YuYiu 1	HpaG$_{10-42}$	0.3 (97.4)	0.2 (97.5)	16.5 (10.0)
	CK	11.2	8.0	15.0

[a] Protein application, experimental design, and plant surveys were similar to those presented in Table 7; CK = untreated control; ns = not surveyed.

[b] Grain yields (kg/22 m^2) are means from two study locations. The plot size represents one-third of a "Fen," a conventional unit of land area in China equal to 66 m^2.

V6 stage, whereas panicle blast, more devastating than leaf blast and stem blast to grain yield formation, typically appears at the middle heading stage of rice (38,57). These factors were figured into the choice of timing of applications, according to plant growth stage. Application of $HpaG_{10-42}$ at 6 μg/ml at each of four stages of rice growth was more effective than $HpaG_{Xooc}$ at 12, 24, 6, and 12 μg/ml applied in turn. Therefore, $HpaG_{10-42}$ is more effective than $HpaG_{Xooc}$ in both beneficial effects.

With optimized concentrations, the proteins have been evaluated for four other aspects important to practical use. A single application of the proteins was tested in its effects on rice (Table 1, test 2) to seek an economical method for agricultural use. However, a single application of the proteins is less effective than multiple applications, and no single-application time point provided optimal effects on yield and each of the three diseases. This might be because a single protein application did not induce full

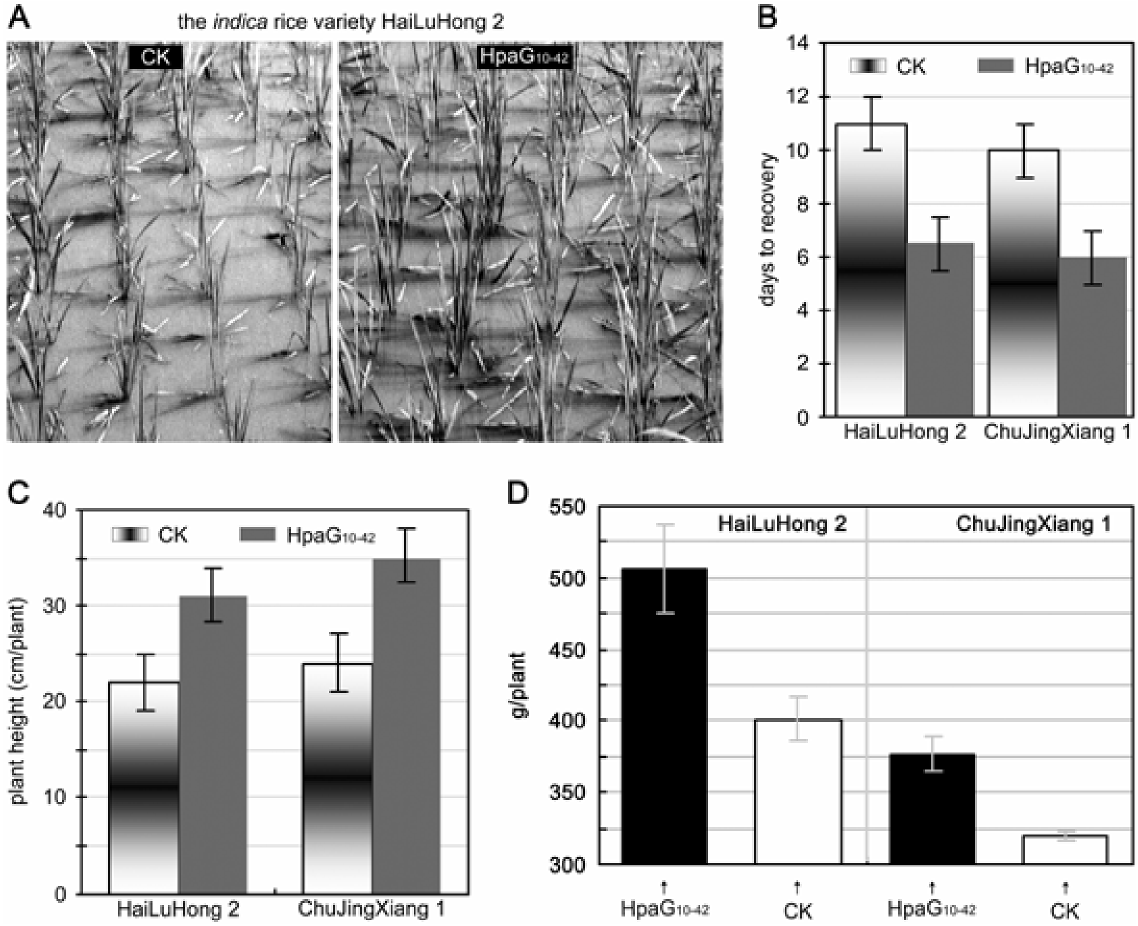

Fig. 3. Effects of $HpaG_{10-42}$ on early growth of rice. Nursery seedlings remained untreated (CK) with proteins or were sprayed with a 6 μg/ml solution of $HpaG_{10-42}$ at 10 days prior to transplant. Plant growth in fields was monitored after transplant. **A,** HaiLuHong 2 plants 7 days after transplant. Photos are representative of three plots for this cultivar. Similar results were observed for ChuJingXiang 1 plants and are not shown. **B,** Days to recovery judged by reinitiation of plant growth in whole plots. **C,** Plant height 20 days after transplant. **D,** Plant fresh weight at the V6 stage. In **C** and **D**, 300 plants in each plot were surveyed. In **B** and **C**, histograms represent means ± standard deviation (n = 3 plots).

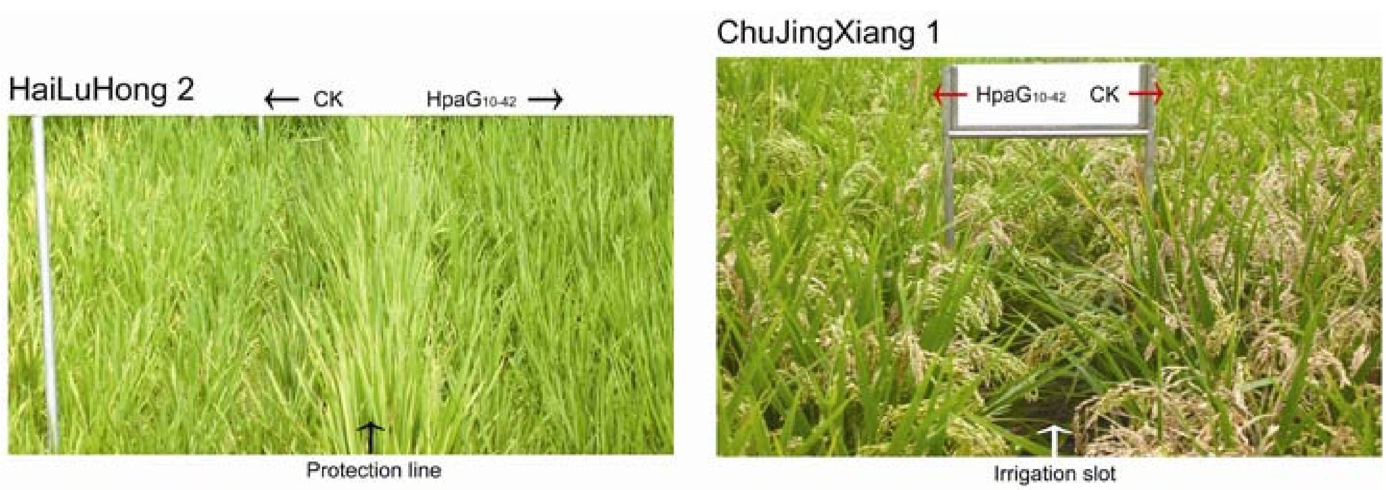

Fig. 4. Field plants with leaf blight (left) and panicle blast (right) during epidemic development. Photos represent populations of the indicated cultivars growing in the plots described for Figure 1 and Table 1. CK = untreated.

Fig. 5. Magnified views of rice blast symptoms on the *indica* rice cv. HaiLuHong 2 growing in the plots described for Figure 1 and Table 1. Top photos show the presence of both leaf blast and panicle blast in plants during seed saturation. Bottom photos show effects of panicle blast on seed maturation. CK = untreated.

effects throughout the rice life cycle. When surveys were extended to the regular fields to which multiple agronomic management measures were applied (Table 2), $HpaG_{10-42}$ was shown to be comparable with local practices (Table 1, test 3) and exceeded $HpaG_{Xooc}$ in protecting nine cultivars of rice from diseases and increase their yields (Table 1, test 4). Moreover, the utility of $HpaG_{10-42}$ was confirmed in large-scale tests (Table 1, test 5). Overall, these results establish the practical potential of $HpaG_{10-42}$ application in rice production.

Bioactive products from plant pathogens are noted resources of secure crop production (9,34,54). Functional fragments have been identified from other harpins (24,27,40,49–52,56) in addition to $HpaG_{Xooc}$. Thus, the efficacy of $HpaG_{10-42}$ in rice provides good prospects for application of functional fragments of harpins to

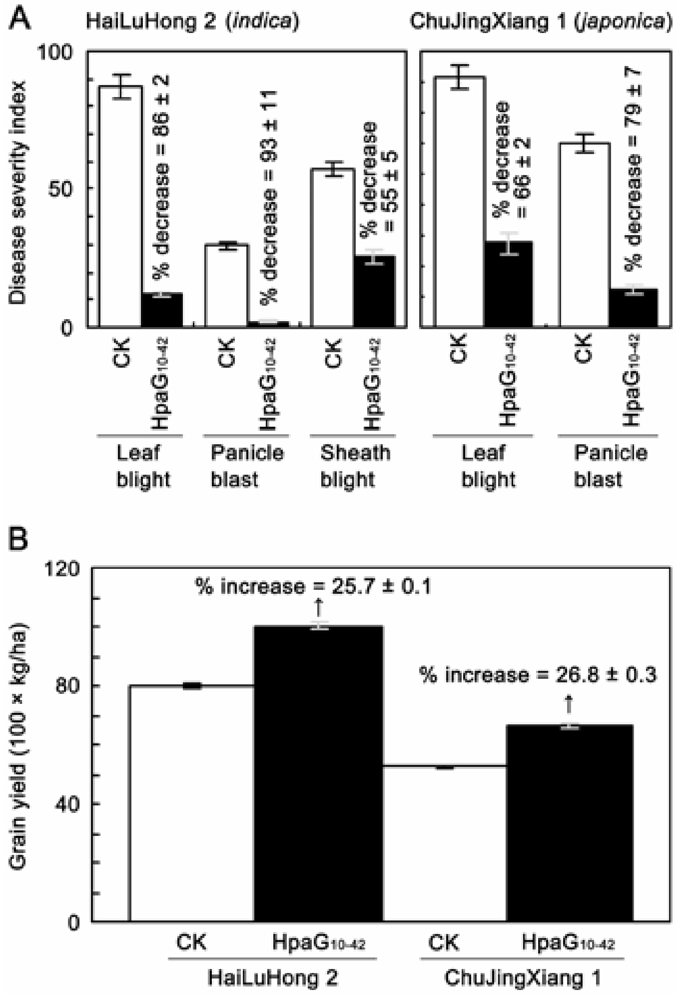

Fig. 6. Disease severities and grain yields in large-scale tests of $\text{HpaG}_{10\text{-}42}$ application to fields of rice cvs. HaiLuHong 2 and ChuJingXiang 1 at locations 2 and 3 (Fig. 1), respectively. CK = untreated. **A,** Disease severities. **B,** Rice grain yields. Experiments were done as in Figure 2. Diseases were scored at the V6 stage. Trends in differences between treatments were similar at the three study sites. Actual grain yields were determined by surveying all plants in three plots and are given as kg/ha after converting plot size into the standard unit. Data represent mean ± standard deviation of results from three plots at each site with 200 plants investigated per plot. Percent increase or decrease is relative to CK.

other crops. In addition to the results presented here, $\text{HpaG}_{\text{Xooc}}$ fragments have been shown to increase yield and improve quality of tea (53). In tea, application of harpin increased content of catechols, compounds with medicinal properties (6). Therefore, use of the proteins could be expected to enhance not only crop protection and yield but also utility.

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