Determination of Host Responses to *Magnaporthe grisea* on Detached Rice Leaves Using a Spot Inoculation Method

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ABSTRACT

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Through the use of standard assays, where conidia of the pathogen *Magnaporthe grisea* are sprayed onto rice, it is impossible to determine the exact number of conidia in any given area and to predict the locations of disease lesions in the rice blast system. To develop a localized, quantitative inoculation of *M. grisea*, a novel spot method was investigated. Serially diluted Tween 20 was added to *M. grisea* conidial suspensions in 0.25% (wt/vol) gelatin to promote adherence of conidia on detached rice leaves. Standard assays indicated no deleterious effects of Tween 20 to rice blast development and 0.02% (vol/vol) Tween 20 was necessary for promoting adherence of spore suspensions to the detached leaves. The spot method was evaluated using three well-characterized races of *M. grisea* and confirmed with standard assays. Disease reactions of rice to four predominant races of *M. grisea* were tested concurrently using the spot method and standard assays. Successful application of this assay will help identify novel sources of rice blast resistance and evaluate virulence of *M. grisea* to aid in breeding resistance to rice blast.

Additional keywords: *Pyricularia grisea*

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. One of the most devastating diseases of rice worldwide is blast, caused by the fungal pathogen *Magnaporthe grisea* Cav. (anamorph *Pyricularia grisea* (Cooke) Sacc.). The pathogen can infect rice plants at any growth stage from seedling through grain formation, and causes leaf blast, collar rot, nodal blast, neck blast, or panicle blast (25).

The most efficient way to minimize rice blast is to grow resistant cultivars. However, the development of resistant cultivars is slowed by the need for researchers to use F_3 progeny to determine resistance or susceptibility to multiple fungal races (3,28). A method using a single F_2 individual would greatly speed up analysis of disease reactions to a wide range of races of *M. grisea*. Determination of disease reactions to different races of *M. grisea* in

the same plant is also advantageous when the quantity of the seed is limited. Limited seed is commonly encountered in identifying resistance genes from wild relatives of rice (*Oryzae* spp.; 9).

Resistance genes have been identified by performing infection assays on rice plants in the greenhouse using local isolates of the blast pathogen (2,23). Quarantine restrictions frequently prohibit the use of the greenhouse to perform infection assays with exotic isolates because of the possibility that such isolates may escape into the surrounding rice-growing region. Therefore, a pathogenicity assay in a closed-system laboratory environment is needed to evaluate advanced breeding lines for a durable, broad-spectrum blast resistance to a wide range of races.

One of the limiting factors in identifying differentially expressed genes in the rice blast system is the necessity of isolating infected leaf tissue after infection with a given number of conidia; however, standard pathogenicity assays (2,4,5,18,23) often result in infection by an unpredictable number of conidia within any given area. This can result in problems with analysis of mRNA because mRNA may not be isolated from infected areas (8,14,20,21).

The objectives of this study were to develop a method to obtain uniform infection by using three well-characterized races of *M. grisea* on detached rice leaves in a controlled environment, and to validate this method by evaluating reactions of rice to four predominant races of *M. grisea*.

MATERIALS AND METHODS

Plant materials and growth. Seven rice cultivars and one experimental line were used for this study: Cypress, Drew, Katy, Kaybonnet, Koshihikari, LaGrue, M-202, and RU010193 (an experimental line). Seeds were germinated on moist filter papers in petri dishes for 3 days in a 30°C dark incubator. Seedlings were transplanted to 127-cm pots containing a mixture of one part sterilized local soil to one part RediEarth potting mix (Hummert, Earth City, MO). Plants were grown in a greenhouse at 24 to 30°C with a light and dark cycle of 16 and 8 h, respectively, for 2 weeks, until they were at the four-leaf stage.

Fungal isolates and culture. Four predominant races of *M. grisea* in the United States were used for this study: race IB-49 (ZN61), race IC-17 (ZN57), race IG-1 (ZN39) (6), and race IH-1. Dried cellulose filter papers containing fungal mycelia were placed on oatmeal agar plates (22). The inoculated plates were placed under a light and dark cycle of 16 and 8 h, respectively, at 24°C for 7 days. Hyphal tips were transferred to oatmeal agar (22) and cultures were incubated for an additional 12 days for production of conidia.

Conidial suspensions. Conidia were harvested in a sterile 0.25% (wt/vol) gelatin (ICN Biomedical Inc., Costa Mesa, CA) solution by scraping fungal mycelia on the culture plates. The conidial suspension then was filtered through cheesecloth to remove mycelia and pieces of agar (23). The concentration of the initial conidial suspension was determined using the method described by Sambrook and Russell (19). Briefly, a conidial suspension was delivered to both chambers of a hemacytometer by capillary action. The number of conidia in five of the nine large squares in both chambers was summed and

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Fig. 1. Spot inoculation and uniform infection of detached leaves with *Magnaporthe grisea* conidial suspensions. **A**, Uniform inoculation of conidial suspensions on a detached leaf. Seven 5- μ l droplets are shown. **B**, Uniform infection by *M. grisea* and differential host responses. Healthy leaf after 6 days = 0. Resistant: type 1, uniform dark brown pinpoint lesions without visible centers (these lesions typically are barely visible); type 2 (0.1-cm diameter) and type 3 (0.2-cm diameter), small lesions without distinct centers. Susceptible: type 4, large spot lesions with sporulated mycelia and conidia, and no visible resistance. Types 1 to 3 represent Katy inoculated either with IB-49 (1×10^4 conidia/ml), IG-1 (5×10^4 conidia/ml), or IH-1 (5×10^5 conidia/ml). Type 4, M-202 inoculated with conidial suspensions of IB-49 (1×10^4 conidia/ml). **C**, M-202 was inoculated with IB-49 (5×10^5 conidia/ml) containing Tween 20 in 0.25% gelatin using standard pathogenicity assay (23).

Fig. 2. Infection of M-202 rice by *Magnaporthe grisea* with and without Tween 20 in 0.25% gelatin using standard pathogenicity assay. Four-leaf seedlings sprayed with conidia of IB-49 (5×10^5 conidia/ml) with the indicated concentration of Tween 20 in 0.25% gelatin. Blast infection was allowed to develop for 8 days. No visible differences in symptomatology were observed between two treatments.

multiplied by 1,000 to give the number of conidia per milliliter.

Determining Tween 20 concentration.

Tween 20 (polyoxyethylene sorbitan monolaurate; Qbiogene Inc, Carlsbad, CA) was added to 0.25% (wt/vol) gelatin to promote adherence of conidia to rice leaves. Four-leaf staged rice cv. Katy was used to determine the minimum Tween 20 for promoting attachment of conidial suspension to the leaf surfaces. A 5-cm segment of detached leaf was inoculated with appropriate volumes of Tween 20 and sterile 0.25% (wt/vol) gelatin to the conidial suspension to attain conidial concentrations of 1×10^4 , 5×10^4 , 1×10^5 , 5×10^5 , and 1×10^6 conidia/ml, each in Tween 20 concentrations of 1, 0.2, 0.1, 0.02, and 0.01% (vol/vol). To eliminate the possibility that Tween 20 and gelatin would create injury lesions on detached leaves, the same dilution series of Tween 20 in 0.25% gelatin without conidia was spot inoculated on the detached leaves as controls. The adhesion was observed immediately after 5- μ l droplets of each treatment were applied to the detached leaf.

Pathogenicity assay. The standard pathogenicity assay was modified from a procedure described by Valent et al (22). For all experiments, plants were grown in greenhouse at 24 to 30°C with a light and dark cycle of 16 and 8 h, respectively, to four-leaf stage in 127-cm plastic pots, and then each plant was inoculated with 2 ml of conidial suspension using an airbrush (Paasche IVL double action airbrush). Plants were inoculated inside a plastic bag that was then sealed to maintain the high humidity required for fungal penetration of the host plant cuticle (11). Each race was inoculated onto separate plants, and there were four replications of each race. Plants were exposed to 24 h of continuous fluorescent light (10 to $12 \mu\text{Em}^{-2} \text{ s}^{-1}$) at room temperature (21 to 24°C); then, plants were removed from bags and returned to the greenhouse. Disease development was monitored daily and plants were maintained in a greenhouse at 24 to 30°C with a light and dark cycle of 16 and 8 h, respectively, until characteristic disease symptoms were observed (7 to 11 days; 21).

Spot inoculation. Plants were grown in a greenhouse at 24 to 30°C with a light and dark cycle of 16 and 8 h, respectively. At the four-leaf stage (2 weeks after emergence), the youngest leaves were removed and cut into 5-cm segments. Segments of detached leaves (four per plate) were immediately placed into petri dishes lined with moist filter paper. Each leaf segment was inoculated with four to seven 5- μ l droplets of conidial suspension with four replications (four leaf segments) for each conidial suspension (for example, 1×10^4 conidia/ml in 0.02% Tween 20 [vol/vol] and 0.25% [wt/vol] gelatin; Fig. 1A). The petri dishes were placed on a laboratory

bench and maintained at 21 to 24°C under continuous fluorescent light (10 to 12 $\mu\text{Em}^{-2} \text{s}^{-1}$) for 24 h. The light intensity was determined using a CI-310 CO₂ gas analyzer (CID, Inc. Camas, WA). The droplets then were removed by blotting with a laboratory tissue. Double-deionized water was added to filter paper to maintain moisture levels and avoid desiccation of detached leaves during incubation. Detached leaves were monitored each day for development of the necrotic lesion (6 to 10 days). Disease reactions were rated based on incidence of lesion type (at least 70% of spots). Resistant reactions were classified into three types based on lesion size. Type 1 was a uniform dark brown, pinpoint lesion without a distinctive center. These lesions typically were barely visible. Type 2 was approximately 1 mm in length and type 3 was approximately 2 mm in length; both were small lesions without distinct centers. A susceptible reaction was manifested as a type 4 with large spot lesions and sporulated mycelia and conidia. A suspension of 0.02% (vol/vol) Tween 20 and 0.25% (wt/vol) gelatin was used for the control.

Experimental design and statistical evaluation. Statistical analysis of the types of the disease reactions was made assuming a completely randomized design with four replications. One replication for the spot inoculation was one segment of the detached leaf and one replication for the standard method was each plant in each pot. The data were analyzed using SAS (version 8.2; SAS Institute, Cary, NC). Specifically, PROC GENMOD of SAS was used to compare evaluation methods because it was a multinomial response. For development of the inoculation protocol, each pathogen race, each conidia concentration, and each rice cultivar was analyzed separately. For the study of race reactions, each cultivar was compared separately for the four different pathogen races because all the controls for each cultivar was 0. The level of significance was at $P \leq 0.01$.

Inoculation method development and race reactions. Blast-resistant cv. Katy (17) and susceptible cv. M-202 (7) were used to develop the spot inoculation method. Four conidial concentrations of 1×10^4 , 5×10^4 , 1×10^5 , and 5×10^5 for three isolates (IB-49, IG-1, and IH-1) were used for this experiment and the types of disease reactions were determined using the rating system described above. Resulting data were analyzed using statistical procedure described above.

Six rice cultivars Cypress, Drew, Katy, Kaybonnet, Koshihikari, and LaGrue, and one experimental line, RU010193, were used for the study of race reactions. The lowest concentration of the conidia, 1×10^4 conidia/ml, was sufficient for the spot inoculation in the previous experiment; therefore, this conidial concentration was used to evaluate race reactions. To further validate the spot inoculation method, a

comparison of spot versus standard inoculation methods also was performed each time with a control. The experiment was repeated three times and the reaction types were analyzed as described above.

RESULTS

Determination of appropriate Tween 20 concentrations. Regardless of number of conidia, 0.02% (vol/vol) Tween 20 (in 0.25% [wt/vol] gelatin) was sufficient to promote adherence of the conidial suspension to the leaf surface. Distinct localized disease reactions were observed when conidial suspensions were applied in a defined location (Fig. 1B). Visible injury resulting from Tween 20 and gelatin was not observed when the suspensions without conidia were spotted on detached leaves. Tween 20 alone caused no response when sprayed on leaves of Katy and M-202. M-202 exhibited classic blast disease lesions in both cases (Fig. 2), whereas Katy showed little or no infection in both cases, typical of a resistant reaction.

Optimizing inoculation levels for spot inoculations. Distinct localized resistant reactions were obtained using different conidial concentrations. Lower inoculum levels, such as 1×10^4 conidia/ml, resulted in a type 1 reaction. Greater inoculum levels increased the resistant reaction from type 2 to type 3 (Fig. 1B). The susceptible reaction was readily distinguishable as large lesions with sporulated mycelia and conidia. The locations of a susceptible reaction on M-202 were unpredictable using the standard pathogenicity assay (Fig. 1C). The resistant reaction of Katy and susceptible reaction of M-202 to fungal isolates as determined by spot inoculation were consistent with disease reactions obtained from the standard pathogenicity assay (Table 1).

Four different conidial concentrations then were used to compare disease reactions in spot inoculation and standard pathogenicity assays. At all concentrations, disease reactions were similar (Table 1). Fewer than 1×10^4 conidia/ml did not cause apparent disease in either spot or standard pathogenicity assay (*data not shown*). A resistant reaction in Katy could be distinguished quickly from a susceptible reaction for either the spot or standard pathogenicity assay with 1×10^5 conidia/ml. However, sporulated mycelia occasionally could be seen only in the resistant reactions for the spot inoculation technique, and not for the standard pathogenicity assay, with 1×10^6 conidia/ml.

Race reactions. Similar disease reactions to four predominant races of *M. grisea* were observed using the spot inoculation and standard pathogenicity assays (Table 2). Drew, Katy, Kaybonnet, and RU010193 were resistant to all four races of *M. grisea*, whereas Koshihikari and LaGrue were susceptible to all four races of *M. grisea* examined. Cypress was resistant to IG-1 and IH-1 and was susceptible to IB-49 and IC-17 (Table 2).

Statistical evaluations. Disease reactions to the *M. grisea* isolates tested were identical as to being susceptible or resistant reactions using either spot inoculation or standard inoculation, and statistical proof was not possible. The selection of different rating scales for the two methods introduced statistical significant differences, although proper transformation of the rating scales was attempted (*data not shown*).

DISCUSSION

Spot inoculation of *M. grisea* on detached leaves has not been well documented; however, a needle-pricking meth-

Table 1. Disease reaction types for spot inoculation versus a standard assay to different conidial concentrations of *Magnaporthe grisea* on two cultivars of rice

Race (isolate)	Conidia/ml	Katy		M202	
		Spot ^a	Standard ^b	Spot	Standard
IB-49 (ZN61)	1×10^4	1 (R)	1 (R)	4 (S)	5 (S)
IB-49 (ZN61)	5×10^4	2 (R)	0 (R)	4 (S)	5 (S)
IB-49 (ZN61)	1×10^5	2 (R)	1 (R)	4 (S)	5 (S)
IB-49 (ZN61)	5×10^5	3 (R)	1 (R)	4 (S)	5 (S)
IG-1 (ZN39)	1×10^4	2 (R)	0 (R)	4 (S)	4 (S)
IG-1 (ZN39)	5×10^4	2 (R)	0 (R)	4 (S)	4 (S)
IG-1 (ZN39)	1×10^5	3 (R)	1 (R)	4 (S)	4 (S)
IG-1 (ZN39)	5×10^5	3 (R)	1 (R)	4 (S)	5 (S)
IH-1	1×10^4	1 (R)	0 (R)	4 (S)	4 (S)
IH-1	5×10^4	2 (R)	0 (R)	4 (S)	4 (S)
IH-1	1×10^5	3 (R)	0 (R)	4 (S)	4 (S)
IH-1	5×10^5	3 (R)	0 (R)	4 (S)	4 (S)

^a Data represent lesion types observed for spot inoculation. The resistant reactions (R) are classified into three types based on lesion size: type 1, uniform dark brown pinpoint lesions without visible centers, barely visible; type 2 (approximately 1 mm in length) and type 3 (approximately 2 mm in length), small lesions without distinct centers. A susceptible reaction (S) is a type 4, large spot lesions with sporulated mycelia and conidia, and no visible resistance.

^b Ratings for standard pathogenicity assays are based on R = resistant reaction 0 or 1, nonpathogenic reactions because affected tissue does not produce conidia and S = susceptible reaction (2 to 5, pathogenic, but differs in extent of colonization within the tissue) (22).

od was developed previously to inoculate rice with multiple fungal isolates (12). This method required additional wounding and disease reactions were evaluated in the greenhouse, where many variables such as wounding, light intensity, and temperature can affect the outcomes of gene expression and, thus, would not be recommended for critical pathogenicity studies and host defense gene isolation and characterization. In the current study, detached leaves were placed in petri dishes in a homogenous environment; reproducible types of mRNA can be isolated easily from the inoculated areas.

Katy is known to be highly resistant to all isolates tested and all commonly found field isolates, and M-202 is known to be highly susceptible to all isolates tested (7,17). These results were confirmed by spot inoculation. Defined concentrations of fungal conidia were applied onto specific areas of detached leaves in a known volume, making the number of fungal conidia inoculated on an area reproducible and consistently producing localized infections (Fig. 1A and B). However, infection is not uniform and does not occur in a predictable area with the use of the standard pathogenicity assay (Fig. 1C). Our method, however, allows isolation of mRNAs from infected areas (spots) after challenge with known numbers of conidia. Thus, the method of spot inoculation could be a valuable tool for identification of novel resistance genes induced by the rice blast pathogen.

A critical pathogenicity study of four well-characterized fungal races toward seven rice cultivars was performed to validate the spot inoculation method. Similar host responses were observed with both the spot inoculation and the standard pathogenicity assay. The response of Katy to IB-49, IC-17, and IG-1 by spot inoculation was consistent with results obtained from

the standard pathogenicity tests performed by Xia et al. (26). The apparent resistant reactions by Katy, Drew, and Kaybonnet to all four isolates examined were consistent with the fact that Drew and Kaybonnet are different progenies of Newbonnet/Katy (10,16). Katy, however, differed from Drew and Kaybonnet in their hypersensitive response toward IG-1 (Table 2), suggesting two possibilities: (i) the physiological response to the germination of fungal conidia is influenced by the surrounding environment or (ii) Katy, Drew, and Kaybonnet possess additional or modifying genes. If the latter is true, the isolation and characterization of the different resistance genes will provide more insight into the early interaction between the interface of the rice and *M. grisea*.

The spot inoculation method allows determination of pathogenicity of *M. grisea* in a controlled environment. All operations, such as fungal culture, inoculation, and observation of disease development, can be performed in the laboratory. All materials can be destroyed upon completion of studies by steam sterilization or incineration. Because all processes are performed in a controlled laboratory setting, the risk of escape of pathogenic isolates into rice fields is reduced. Successful application of this technique would benefit investigators evaluating the pathogenicities of exotic isolates of *M. grisea*.

Use of the spot inoculation technique would allow simultaneous testing of multiple fungal isolates on the same plant in the laboratory. This could shorten the time for development of broad-spectrum blast resistant cultivars. In addition, gene expression profiling upon infection of *M. grisea* could be repeated easily, and resulting knowledge will lead to a broader understanding of the molecular basis of interactions of rice with *M. grisea* (1,13,15,21,24,27).

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LITERATURE CITED

1. Balhadere, P. V., and Talbot, N. J. 2000. Fungal pathogenicity—establishing infection. Pages 1-19 in: *Molecular Plant Pathology: Annu. Plant Rev.* 4. M. Dickinson and J. Beynon, eds. Sheffield Academic Press, Boca Raton, FL.
2. Bonman, J. M., Vergel de Dios, T. I., and Khin, M. M. 1986. Physiologic specialization of *Pyricularia oryzae* in the Philippines. *Plant Dis.* 70:767-769.
3. Chao, C. T., Moldenhauer, K. A. K., and Ellingboe, A. H. 1999. Genetic analysis of resistance/susceptibility in individual F₃ families of rice against strains of *Magnaporthe grisea* containing different genes for avirulence. *Euphytica* 109:183-190.
4. Correa-Victoria, F. J., and Zeigler, R. S. 1993. Pathogen variability in *Pyricularia grisea* at a rice blast "hot spot" breeding site in eastern Colombia. *Plant Dis.* 77:1029-1035.
5. Correa-Victoria, F. J., Zeigler, R. S., and Levy, M. 1994. Virulence characteristics of genetic families of *Pyricularia grisea* in Colombia. Pages 211-229 in: *The Rice Blast Disease*. R. S. Zeigler, P. S. Teng, and S. A. Leong, eds. Commonwealth Agricultural Bureau International, Wilmington, England.
6. Correll, J. C., Harp, T. L., Guerber, J. C., Zeigler, R. S., Liu, B., Cartwright, R. D., and Lee, F. N. 2000. Characterization of *Pyricularia grisea* in the United States using independent genetic and molecular markers. *Phytopathology* 90:1396-1404.
7. Correll, J. C., Shreve, E. S., Boza, E. J., Cartwright, R. D., and Lee, F. N. 2000. Cultivar reactions to rice blast isolates collected during the 2000 growing season. Pages 115-123 in: *Rice Research Studies*. R. J. Norman and J. F. Mullenet, eds. Univ. Ark. Agric. Exp. Stn. Res. Ser. 485.

Table 2. Disease reaction types to four predominant races of *Magnaporthe grisea* on rice cultivars using spot inoculation and standard method

Cultivar	IB-49 (ZN61)		IC-17 (ZN57)		IG-1 (ZN39)		IH-1	
	Spot ^a	Standard ^b	Spot	Standard	Spot	Standard	Spot	Standard
Cypress	4 (S)	4 (S)	4 (S)	3 (S)	2 (R)	1 (R)	2 (R)	1 (R)
Control ^c	0	0	0	0	0	0	0	0
Drew	1 (R)	0 (R)	1 (R)	0 (R)	1 (R)	0 (R)	1 (R)	0 (R)
Control	0	0	0	0	0	0	0	0
Katy	1 (R)	0 (R)	1 (R)	0 (R)	2 (R)	0 (R)	1 (R)	0 (R)
Control	0	0	0	0	0	0	0	0
Kaybonnet	1 (R)	1 (R)	1 (R)	0 (R)	1 (R)	0 (R)	1 (R)	1 (R)
Control	0	0	0	0	0	0	0	0
Koshihikari	4 (S)	4 (S)	4 (S)	4 (S)	4 (S)	5 (S)	4 (S)	4 (S)
Control	0	0	0	0	0	0	0	0
LaGrue	4 (S)	5 (S)	4 (S)	4 (S)	4 (S)	5 (S)	4 (S)	4 (S)
Control	0	0	0	0	0	0	0	0
RU010193	1 (R)	0 (R)	0 (R)	1 (R)	1 (R)	1 (R)	1 (R)	0 (R)
Control	0	0	0	0	0	0	0	0

^a Data represent lesion types observed for spot inoculations. The resistant reaction (R) is classified into three types based on lesion size: type 1, uniform dark brown pinpoint lesions without visible centers, barely visible; type 2, approximately 1 mm in length and type 3, approximately 2 mm in length, small lesions without distinct centers. A susceptible reaction (S) is a type 4, large spot lesions with sporulated mycelia and conidia, and no visible resistance.

^b Ratings for standard pathogenicity assays are based on R = resistant reaction (0 to 1, nonpathogenic reactions because affected tissue does not produce conidia) and S = susceptible reaction (2 to 5, pathogenic, but differ in extent of colonization with the tissue) (21).

^c A suspension of 0.02% (vol/vol) Tween 20 and 0.25% (wt/vol) gelatin without conidia was used as mock inoculation.

8. Derisi, J. L., Iyer, V. L., and Brown, P. O. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278:680-686.
9. Eizenga, G. C., Tai, T. H., Lee, F. N., and Jia, Y. 2002. Progress in identification of disease resistance in the *Oryza* species accessions. Proc. 29th Rice Technical Working Group. Little Rock, AR.
10. Gravois, K. A., Moldenhauer, K. A. K., Lee, F. N., Norman, R. J., Helms, R. S., Bernhardt, J. L., Wells, B. R., Dilday, R. H., Rohman, P. C., and Blocker, M. M. 1995. Registration of 'Kaybonnet' rice. *Crop Sci.* 35:586-587.
11. Howard, R. J., and Ferrari, M. A. 1989. The role of melanin in appressorium function. *Exp. Mycol.* 13:403-418.
12. Inukai, T., Nelson, R. J., Zeigler, R. S., Sarkarung, S., Mackill, D. J., Bonman, J. M., Takamura, T., and Kinoshita, T. 1994. Allelism of blast resistance genes in near-isogenic lines of rice. *Phytopathology* 84:1278-1283.
13. Kim, Y. K., Lee, S.-H., Park, H. C., Bae, C. G., Cheong, Y. H., Choi, Y. J., Han, C., Lee, S. Y., Lim, S. Y., Lim, C. O., and Cho, M. J. 2000. Identification of rice blast fungal elicitor-responsive genes by differential display analysis. *Mol. Plant-Microbe Interact.* 13:470-474.
14. Liang, P., and Pardee, A. B. 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* 257:967-71.
15. MacKill, D. J., and Bonman, J. M. 1992. Inheritance of blast resistance in near isogenic lines of rice. *Phytopathology* 82:746-749.
16. Moldenhauer, K. A. K., Gravois, K. A., Lee, F. N., Norman, R. J., Bernhardt, J. L., Well, B. R., Dilday, R. H., Blocker, M. M., Rohman, P. C., and McMinn, T. A. 1998. Registration of 'Drew' rice. *Crop Sci.* 38:896-897.
17. Moldenhauer, K. A. K., Lee, F. N., Norman, R. J., Helms, R. S., Well, R. H., Dilday, R. H., Rohman, P. C., and Marchetti, M. A. 1990. Registration of 'Katy' rice. *Crop Sci.* 30:747-748.
18. Roumen, E. C., Bonman, J. M., and Parleviet, J. E. 1992. Leaf age related partial resistance to *Pyricularia oryzae* in tropical lowland rice varieties as measured by the number of sporulating lesions. *Phytopathology* 82:1414-1417.
19. Sambrook, J., and Russell, D. W., eds. 2001. Estimation of cell number. A8.6 in: *Molecular Cloning: A Laboratory Manual*, Vol. 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
20. Schena, M. D., Shalon, D., Davis, R. W., and Brown, P. O. 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270:467-470.
21. Valent, B. 1997. The Rice Blast Fungus, *Magnaporthe grisea*. *Plant Relationships*. Pages 37-54 in: *The Mycota V Part B*. G. C. Carroll and P. Tudzynski, eds. Springer-Verlag, Berlin, Heidelberg, Germany.
22. Valent, B., Crawford, M. S., Weaver, C. G., and Chumley, F. G. 1986. Genetic studies of pathogenicity and fertility of *Magnaporthe grisea*. *Iowa State J. Res.* 60:569-594.
23. Valent, B., Farrall, L., and Chumley, F. G. 1991. *Magnaporthe grisea* genes for pathogenicity and virulence identified through a series of backcrosses. *Genetics* 127:87-101.
24. Wang, Z., Taramino, G., Yang, D., Liu, G., Tingey, S. V., Miao, S. V., and Wang, G.-L. 2001. Rice ESTs with disease resistance gene or defense-response gene-like sequences mapped to regions containing major resistance genes or QTLs. *Mol. Genet. Genomics* 265:302-310.
25. Webster, R. K., and Gunnell, P. S. 1992. Rice blast. Pages 14-17 in: *Compendium of Rice Diseases*. R. K. Webster and P. S. Gunnell, eds. American Phytopathological Society, St. Paul, MN.
26. Xia, J. Q., Correll, J. C., Lee, F. N., Ross, W. J., and Rhoads, D. D. 2000. Regional population diversity of *Pyricularia grisea* in Arkansas and the influence of host selection. *Plant Dis.* 84:877-884.
27. Xiong, L., Lee, M.-W., Qi, M., and Yang, Y. 2001. Identification of defense-related rice genes by suppression subtractive hybridization and differential screening. *Mol. Plant-Microbe Interact.* 14:685-692.
28. Yu, Z. H., Mackill, D. J., Bonman, J. M., McCouch, S. R., Guiderdoni, E., Nottenhem, J. L., and Tanksley, S. D. 1996. Molecular mapping of genes for resistance to rice blast (*Pyricularia grisea* Sacc.). *Theor. Appl. Genet.* 93:859-863.