Resistance to *Gerlachia oryzae* in Rice

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ABSTRACT

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Two hundred and eighty-eight rice germ plasm accessions from six subspecific cultivar groups of *Oryza sativa* were tested for resistance to the leaf scald pathogen, *Gerlachia oryzae*. Included were indica cultivars (group I); japonica, tropical upland, and *bulu* cultivars (group VI); two smaller groups (groups II and V); and two satellite groups of deepwater cultivars (groups III and IV). A detached leaf method used to measure lesion growth gave results comparable to those of tests using intact plants ($r^2 = 0.81$, $P = 0.01$). Rice cultivars in groups III, IV, and V and the temperate accessions of group VI had the shortest lesions and the highest proportions of resistant entries. Over all test cultivars and within groups II and VI, lesion length was positively correlated with leaf width. Cultivars Labelle and Dourado Precose inoculated with two isolates of *G. oryzae* showed evidence of pathogenic specialization. Such specialization may be responsible for disparities in the results of resistance tests reported from different countries.

Leaf scald is an important disease of rice (*Oryza sativa* L.) in certain high-rainfall environments (4,10,12,16,17). Breeding for resistance is one option for managing the disease, but few sources of scald resistance in rice are known.

Some efforts have been made to identify resistance in rice (2,3,5) and other species of *Oryza* (15). Faria and Prabhu (5) described a method to assess resistance using mycelial disks from cultures of the pathogen, *Gerlachia oryzae* (Hashioka & Yokogi) W. Gams (= *Rhynchosporium oryzae*). They screened 40 rice cultivars, all of which were infected but showed different lesion lengths.

Field observations in Sierra Leone indicated that cultivars with broad leaves tended to have more severe leaf scald than those with narrow leaves (16). Leaf blade width is one of the main characters used to differentiate morphological types in rice germ plasm (11). Variation in blade width is associated with subspecific indica-japonica discrimination (13), with differences in culture type (9), and with geographic origin (7).

Recent isozyme analysis of rice germ plasm by Glaszmann (6) identified six groups: two main subspecific groups, comprising indica cultivars (group I) and japonica, tropical upland, and *bulu* cultivars (group VI); two smaller groups, one mainly from Bangladesh and northeast India and comprising Aus rices (group II) and the other found in a geographic belt from Iran to Burma and including Basmati rices (group V); and two satellite groups comprising deepwater rices from Bangladesh and northeast India (groups III and IV).

Whether resistance to leaf scald can be related to any subspecific classification scheme is of primary importance to breeding strategies. Our purpose was to develop a detached leaf method for assessing the growth of leaf scald lesions and to use the method to measure resistance in rice germ plasm from the subspecific groups. We also investigated pathogenic specialization in the leaf scald fungus.

MATERIALS AND METHODS

Screening methods. In the detached leaf method of assessing resistance to leaf scald, 7-cm pieces were cut 5–10 cm from the tip of the first fully expanded leaves of 30-day-old plants. Before being placed in petri dishes containing 2% water agar, excised leaves were dipped in distilled water with 0.02% Tween 20 to help them adhere to the agar surface. Inoculum consisted of 4-mm disks transferred from the margins of *G. oryzae* colonies growing on potato-dextrose agar and placed at the center of each excised leaf. The petri dishes were sealed with Parafilm to maintain high moisture and incubated at 25 C in darkness. Lesion length was measured 72 hr after inoculation.

Lesion growth on detached and attached leaves was compared using 20 rice

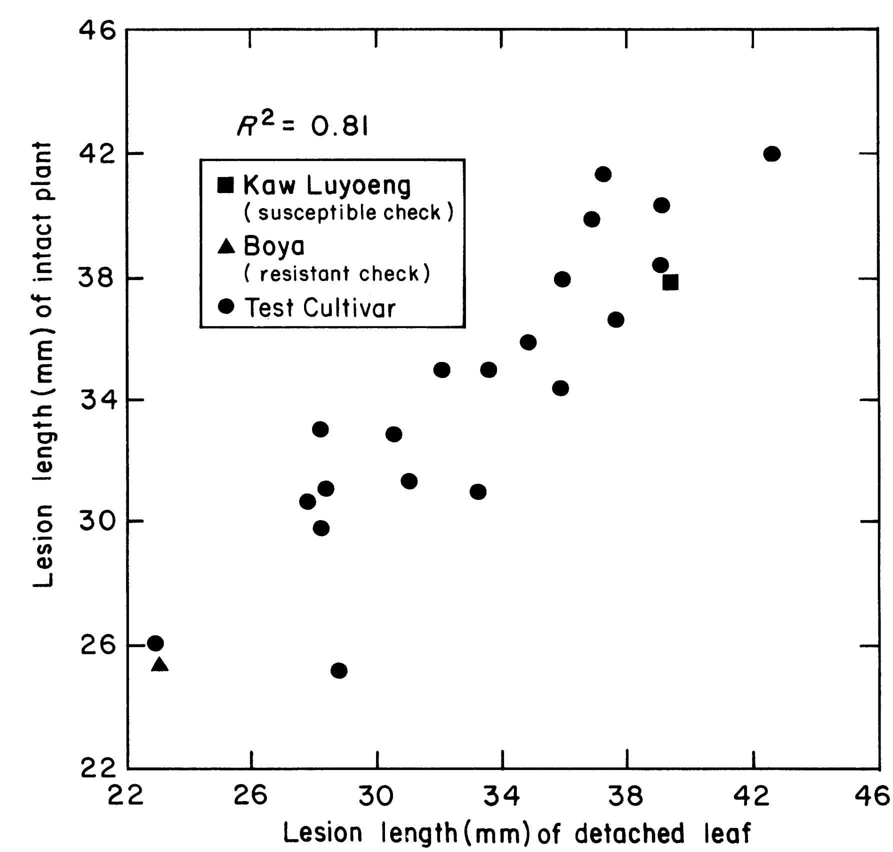

Fig. 1. Correlation between detached leaf and intact plant methods of assessing leaf scald resistance in rice.

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cultivars known to have different levels of resistance. Seedlings were grown in the greenhouse in 9-cm-diameter pots, 12–14 pots per cultivar. Each pot was planted with eight seeds and thinned to three plants 10 days later. At 10 and 23 days after seeding, 0.5 g of ammonium sulfate was applied to each pot. Eight pots with uniform plants were selected for the comparison.

Resistance of intact plants was assessed using the method of Faria and Prabhu (5). Inoculum disks were placed 5–10 cm from the tips of the first fully expanded leaf on each plant. Inoculated plants were incubated in a dew chamber for 72 hr at 25 C. Because lesion width was limited by the edges of the leaf, lesion size was recorded as the length on the longitudinal axis of the leaf blade.

Two sets of plants were inoculated 30 days after seeding. Each set consisted of four pots per replication. Petri dishes containing excised leaves and pots containing intact plants were placed in the same dew chamber to ensure uniform temperature. The experiment had four replications, and each was placed in a separate chamber. The experiment was repeated three times with different isolates to ensure that results were reproducible.

Screening germ plasm accessions. Rice germ plasm accessions from the International Rice Germplasm Center (IRGC) of the International Rice Research Institute (IRRI) were selected to represent each of the six subspecific cultivar groups of rice identified in previous isozyme analysis (6). IRGC data on country of origin were used to classify accessions within group VI as either temperate or tropical. The few high-elevation tropical accessions of group VI were present in the sample and were classified as temperate.

The detached leaf method was used to test 288 accessions: 118 from group I, 95 from group VI, 34 from group V, 29 from group II, 7 from group IV, and 5 from group III. We tested 15 leaves per accession. The experiment was done twice, each time using a different freshly isolated hyphal tip culture of *G. oryzae* taken from infected plants at the IRRI experimental farm.

Data on leaf length and width were taken from IRGC records. Because the number of accessions in each leaf size category differed, we used a weighted regression to compare leaf length and width with lesion length (1). Leaf width data were not available for 15 accessions, so data for only 273 accessions were used for the regression analysis.

Pathogenic specialization. Both detached leaf and intact plant methods were used to test for pathogenic specialization in *G. oryzae*. On the basis of preliminary tests, four cultivars and two isolates were selected. Test cultivars were Labelle (IRRI accession 24274), Dourado Precose (IRRI accession 26011), resistant check Boya (IRRI accession 58920), and susceptible check Kaw Luyoeng (IRRI accession 27716). The test isolates were NV88-1 (from a lowland rice field in Nueva Viscaya Province, Philippines) and L88-1 (from an upland rice field at the IRRI research site in Cavinti, Laguna Province). The cultivars were tested against the isolates three times by the detached leaf method and twice by the intact plant method, five replications per trial and five leaves per replicate.

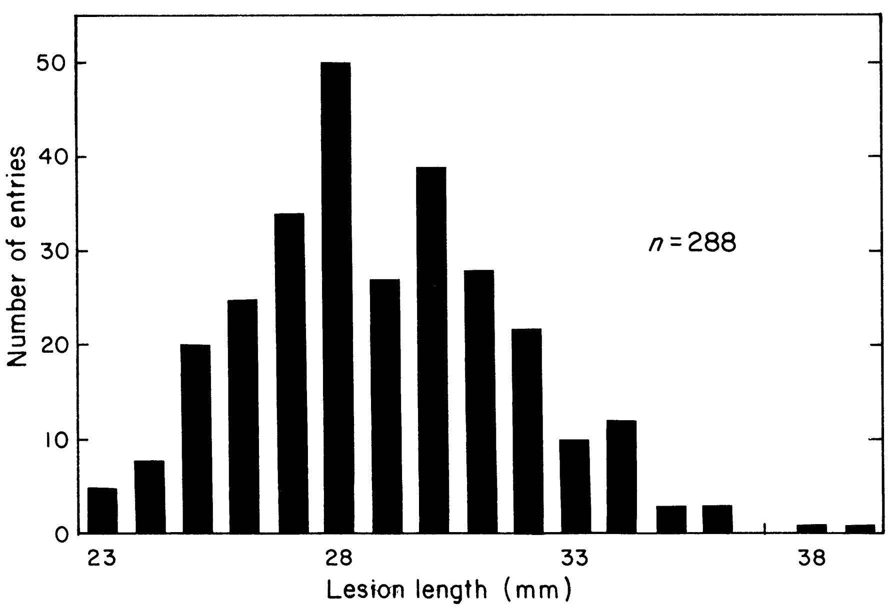

Fig. 2. Frequency distribution of leaf scald lesion length for 288 rice accessions.

Table 1. Length of leaf scald lesions, mean width of leaves, and number of resistant accessions in six rice cultivar groups

Cultivar group[v]	Number of accessions	Resistant accessions[w] No.	%	Lesion length (mm)[x]	Leaf width (cm)[y]
I	118	34	29	29.8 b	1.33
II	29	3	10	30.4 a	1.28
III	5	3	60	27.7 d	1.28
IV	7	7	100	25.8 e	1.44
V	34	23	68	27.5 d	1.23
VI Temperate[z]	37	30	81	26.7 de	1.14
Tropical	58	25	43	28.8 c	1.64
Total	288	125			

[v] After Glaszmann (6).
[w] Not significantly different from check by LSD ($P = 0.01$).
[x] Mean of two trials. Means followed by the same letter or letters are not significantly different by Duncan's multiple range test ($P = 0.05$).
[y] Mean values from International Rice Germplasm Center data.
[z] Based on data for accession origin from the International Rice Germplasm Center and includes high-elevation tropical accessions.

RESULTS AND DISCUSSION

Results of the detached leaf method were similar to those of the intact plant method. Lesion lengths as measured by the two methods were positively correlated in each trial ($r^2 = 0.74$, $r^2 = 0.52$, $r^2 = 0.73$, $P = 0.01$), as were the

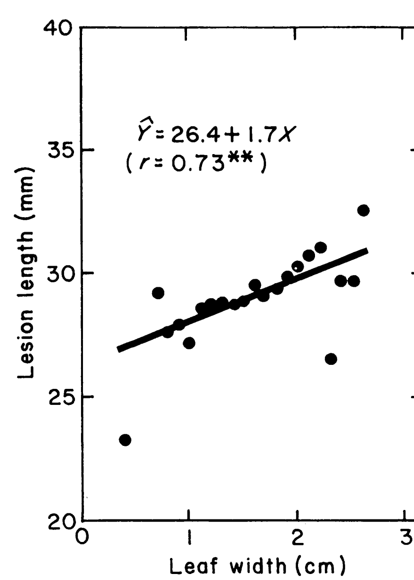

Fig. 3. Relationship between rice leaf width and mean leaf scald lesion length for 273 accessions. Each point represents the mean value for a leaf-width category. ** = Significant at $P = 0.01$.

means of the three trials (r^2 = 0.81, P = 0.01) (Fig. 1). The detached leaf method is more convenient to use when screening a large number of accessions because intact plants require incubation in a dew chamber.

A range of lesion sizes was observed among the 288 accessions screened (Fig. 2). Some accessions had longer lesions than those on the susceptible cultivar Kaw Luyoeng (mean = 34.7 mm), but none had lesions significantly shorter than those on the resistant cultivar Boya (mean = 23.2 mm). As in previous studies, pathogen growth was not completely inhibited in any rice cultivar.

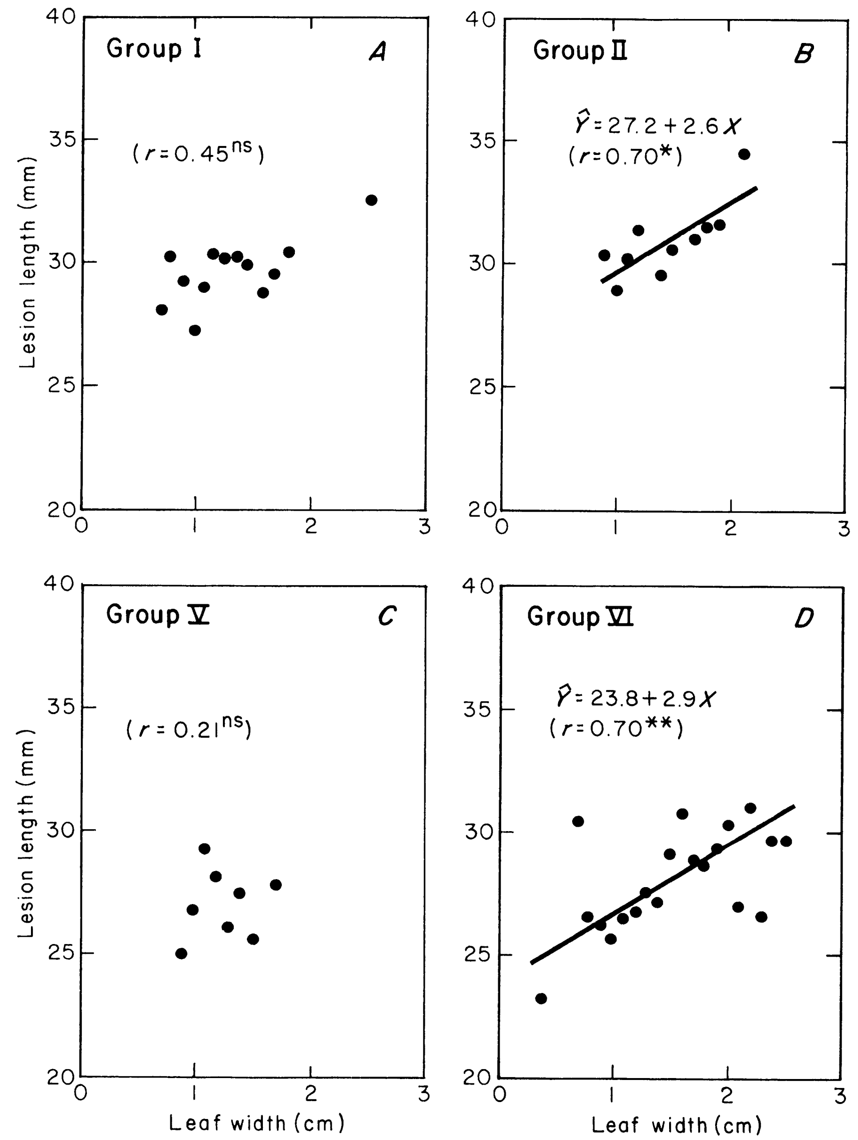

Fig. 4. Relationship between rice leaf width and mean leaf scald lesion length for **(A)** 105 accessions from group I, **(B)** 29 accessions from group II, **(C)** 34 accessions from group V, and **(D)** 94 accessions from group VI. Each point represents the mean value for a leaf-width category. ns = Not significant, * = significant at P = 0.05, ** = significant at P = 0.01.

Table 2. Lesion length (mm) produced by two isolates (L88-1 and NV88-1) of *Gerlachia oryzae* on four rice cultivars using two inoculation methods[y]

	Detached leaf method			Intact plant method		
Cultivar	**L88-1**	**NV88-1**	**Diff.**	**L88-1**	**NV88-1**	**Diff.**
Labelle	24.7 c	29.5 c	4.8**[z]	25.1 c	31.2 c	6.1**
Dourado Precose	31.0 b	32.2 b	1.2 ns	33.2 b	34.2 b	1.0 ns
Kaw Luyoeng (susceptible check)	35.7 a	36.3 a	0.6 ns	37.7 a	38.8 a	1.1 ns
Boya (resistant check)	24.5 c	23.7 d	0.8 ns	25.1 c	25.5 d	0.4 ns

[y] Means of three trials with detached leaf method and two trials with intact plant method. Within a column, means followed by the same letter are not significantly different by LSD (P = 0.05).

[z] ** = Significant by LSD (P = 0.01), ns = not significant.

Accessions in cultivar groups III, IV, and V and accessions of temperate origin within group VI had the shortest mean lesion length and the highest proportion of resistant entries (Table 1).

Results from the weighted regression analysis of all accessions indicate that lesion length increased with increasing width of leaf blades; about one-half of the variation in lesion length could be explained by this relationship (Fig. 3). When cultivar groups I, II, V, and VI were considered separately, only groups II and VI showed a significant relationship (Fig. 4). When temperate and tropical accessions of group VI were considered separately, no significant relationship between lesion length and leaf width was found. Thus, the trend within group VI as a whole (Fig. 4) reflected the wider leaves and the higher susceptibility of the tropical accessions compared with the temperate accessions (Table 1). These results support the common field observation that lines with wide leaves are often more susceptible to leaf scald (16). Satellite groups III and IV were exceptions—they have relatively wide leaves yet were relatively resistant (Table 1).

No significant relationship between leaf length and lesion length was found over all accessions. Thus, lesion length should be a reasonable measure of resistance, because longer lesions would denote higher diseased leaf area. When group VI was considered separately, accessions with longer leaves tended to have longer lesions, but only about 17% of the variation in lesion length could be explained by this relationship (r = 0.41, P = 0.01).

Group IV consists of Rayada floating rices from Bangladesh. Our sample of these rices was small because only a few Rayada rices exist. Combined groups III and IV rices represent only about 1% of all traditional rice cultivars (8). Group IV is genetically the most narrow of the two satellite groups; these cultivars are currently being grown on only about 5,000 ha in Bangladesh (14). These areas have 4–6 m of water during the wet season, and the crop is harvested 12 mo after sowing (14). Because of their unusually long life cycle, group IV cultivars have probably undergone strong selection pressure for disease resistance.

The resistance shown in some cultivar groups may be related to pathogenic specialization. Results from inoculation of cultivars Labelle and Dourado Precose support the hypothesis that some degree of specialization occurs within *G. oryzae*. With both inoculation methods, Labelle was consistently more resistant to isolate L88-1 than to isolate NV88-1, whereas Dourado Precose and the two check cultivars gave similar reactions to both isolates (Table 2).

The inoculum used in this study was from the Philippines. The cultivar groups of primarily tropical origin, such as groups I and II and the tropical accessions of group VI, tended to have a higher proportion of susceptible accessions. Perhaps the accessions in these groups were relatively more susceptible because they were inoculated with tropical isolates.

Pathogenic specialization within *G. oryzae* also might explain why our results differed from those of Faria and Prabhu (5). We tested several of the same cultivars, and some that were relatively resistant in their tests were relatively susceptible in our tests, and vice versa. For example, they found De Abril to be susceptible and IR8 to be resistant, whereas the opposite was true in our experiments, in which mean lesion lengths were 24.5 mm for De Abril and 32.8 mm for IR8. Field data have shown similar anomalies; multilocation leaf scald resistance tests conducted through the International Rice Testing Program in the early 1980s showed evidence of location × cultivar interactions (D. V. Seshu, *personal communication*). Normally, reversals in the rank order of resistance of cultivars are needed to establish the presence of physiologic races, and currently only circumstantial evidence indicates that races of *G. oryzae* might exist. Therefore, future work should investigate the extent and importance of pathogenic specialization in *G. oryzae*.

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