Leaf Age Related Partial Resistance to *Pyricularia oryzae* in Tropical Lowland Rice Cultivars as Measured by the Number of Sporulating Lesions

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ABSTRACT

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To study the effects of leaf age on resistance, plants of four rice cultivars were inoculated with a virulent isolate of *Pyricularia oryzae*. Susceptibility of leaves declined rapidly with increasing leaf age. In older leaves, fewer sporulating lesions developed per unit of leaf area, and eventually leaves became completely resistant. The period during which newly formed leaves were susceptible, as well as the initial level of susceptibility of new leaves, differed greatly between cultivars. Cultivars with high levels of partial resistance to leaf blast showed typical susceptible lesions, but the resistance in leaves rapidly increased with age, and the initial level of susceptibility of new leaves was low.

Additional keywords: *Magnaporthe grisea*, rice blast disease.

The number of sporulating lesions that develop after inoculation is an important parameter of partial resistance to blast disease on rice (*Oryza sativa* L.), caused by *Pyricularia oryzae* Cavara, the imperfect stage of *Magnaporthe grisea* (Hebert) M. E. Barr (13,15,16). Resistance to blast is influenced by plant and leaf age. The infection frequency is less in older than in younger plants (1,6). In plants of the same age, it is less in older leaves, and after a certain time the resistance of leaves becomes virtually complete. This resistance appears to be common if not ubiquitous in rice cultivars and is expressed in various environments (4,6, 8,15).

Although resistance to blast increases with leaf age, there is little information available about possible cultivar differences for the increase of this age-related resistance or for the period after which newly formed leaves become highly resistant. Cultivars that rapidly build up high resistance levels might be expected to develop fewer lesions after inoculation, because the susceptible leaf area is reduced. The experiments reported in this paper help determine the extent to which age-related resistance differs between rice cultivars.

MATERIALS AND METHODS

The research was done in a greenhouse at the International Rice Research Institute (IRRI) at Los Baños, the Philippines. The tropical lowland rice cultivars CO39, IR50, IR66, and IR36 were used. CO39 lacks effective major gene resistance against many tropical blast isolates (3,7) and served as the susceptible check. The cultivars IR36 and IR50 were included for comparing the results with those obtained from earlier work (15). IR66 was included as representative of a recently released Philippine cultivar.

Isolate P06-6 of *P. oryzae* was used for each inoculation. In preliminary tests, after plants were inoculated with this isolate at the six- or seven-leaf stage, the four cultivars developed a susceptible infection type, according to the classification of Yamasaki and Kiyosawa (14).

Three series of plants were grown with an interval of 1 mo between the series from October to December 1987. To ensure the presence of all stages of leaf expansion at the time of inoculation, stagger planting was done on four consecutive days per series for IR36, IR50, and IR66 and on six consecutive days for CO39. Seeds were germinated in petri dishes, and the seedlings were planted to 10-cm-diameter plastic pots, with four plants per pot. Nine pots per day were planted for each cultivar, and these were equally divided over three 1.5-m^2 blocks. On completion of the planting of a series, each block contained 12 pots of IR36, IR50, and IR66 and 18 pots of CO39. Within the blocks, pots were completely randomized. A few days after planting, nitrogen fertilizer was added by the application of an ammonium sulfate solution to each pot at 0.157 g N per pot (equivalent to 20 g/m^2). The soil moisture in the pots was monitored twice daily, and watering was done as necessary to avoid moisture stress. Care was taken to keep pots well drained, and plants were grown under nonflooded soil conditions.

We inoculated the three blocks of each planting series on different, usually consecutive days to spread the work load of the data collection. Expression of partial resistance to blast is known to be very sensitive to changes in environmental conditions (9). Thus, a different predisposing effect on the plants, because of variations in weather conditions, may have occurred between the different days of inoculation. Furthermore, fresh inoculum was prepared for each block; each block (=inoculum day) was regarded as a separate test for a total of nine inoculation series.

On the day of inoculation, we made a small mark, with a felt-tip pen, at the point of emergence of the youngest leaf to determine the leaf stage of each plant. After the leaves were marked, the pots were again completely randomized within the block. Inoculum was produced as described by Bonman et al (3). The plants were inoculated just before sunset with 400 ml of a conidial suspension containing 3×10^4 conidia per milliliter. Inoculum was sprayed as a fine turbulent mist; a nozzle attached to a portable air compressor was used. To ensure uniform distribution of the inoculum, we directed the turbulent mist beam at all plants in the same manner in slow systematic movements, covering the plants from different directions. After inoculation, the plants were covered with a plastic cage and placed in a humid glasshouse room that was kept at 25 C. The cage was removed the following morning, and the plants were kept in the same room until evaluation 6 or 7 days later.

The number of sporulating lesions on each leaf of the main culm was counted, and the leaf length was measured. Only lesions with a grayish center were considered sporulating (5). Long narrow sporulating lesions growing along the leaf edge were excluded from the count because of their atypical shape. In each plant, the number of leaves on the main culm with at least one sporulating lesion and the total number of sporulating lesions on leaves of the main culm were counted. Leaf width was measured on a sample of 15 plants per cultivar in series 4, because it was observed that leaf width in CO39 was larger than in the other cultivars. Variation in leaf width between individual leaves within a genotype was small, and the coefficient of variation for the samples varied between 4.2 and 5.8%. Leaf area (cm^2) was calculated as 0.7 $\times$ leaf width $\times$ leaf length. Lesion density was calculated by dividing the number of lesions per leaf by the leaf area. Adjustment for leaf width had little influence on relative differences between cultivars and did not affect cultivar ranking.

In series 6, only the presence or absence of sporulating lesions in leaves was scored. In series 8 and 9, lesion density on CO39 was too high to distinguish individual lesions, and assessment was hindered on CO39. Seed of IR66 used in the first planting appeared to be impure because two phenotypes could be distinguished, and one of these was completely resistant to isolate P06-6. Because of the possibility that there were other genotypes present in IR66 that could not be separated visually, the results of IR66 in series 1–3 were excluded from the analysis. The seed of IR66 used in the remaining series was increased from a single panicle of the IR66 source that was used in the first planting.

Analysis of variance showed that residuals of the measurements were not normally distributed, and this could not be solved by transformation. Therefore, appropiate nonparametric tests were used (12). Analysis of the data for comparisons across series was done by using the Wilcoxon ranked sign test with the treatment means per series as the experimental unit, whereas comparisons within series were done by using the Kruskall-Wallis test with the plant values as the experimental unit.

For the analysis of the number of sporulating lesions per square centimeter of leaf area, plants were classified under three age categories on the basis of leaf stage and leaf expansion. The first category had plants of the most common leaf stage with young expanding leaves of less than 15 cm in length; the second included leaves between 15 and 30 cm in length; and the third included expanded or nearly expanded leaves exceeding 30 cm. The third group included leaves of plants on which the next leaf was already emerging, as long as this newly expanding leaf was shorter than 15 cm.

TABLE 1. Average number of leaves with sporulating lesions on the main culm of four rice cultivars inoculated with *Pyricularia oryzae* in nine series

	Cultivar			
Series	CO39	IR50	IR66	IR36
1	1.90	0.97	...	0.66
2	1.83	0.75	...	0.65
3	2.47	1.44	...	1.15
4	2.46	1.56	1.63	1.05
5	2.45	1.48	1.39	1.15
6	2.50	1.41	1.47	1.11
7	3.12	1.34	1.86	1.13
8	...	1.90	2.46	1.28
9	...	1.72	1.93	1.21
Weighed mean[z]	2.60 a	1.40 b	1.59 b	1.04 c
Relative to CO39	100%	54%	61%	40%

[z] Different letters indicate significantly different means (Wilcoxon signed rank test; pairwise comparison between cultivars at $\alpha = 0.05$).

RESULTS

At the time of scoring, sporulating lesions on leaves of IR36, IR50, and IR66 showed dark margins, but dark margins were not always observed around sporulating lesions on CO39. Lesions on older leaves developed a dark margin faster than lesions on younger leaves. The dark margins surrounding sporulating lesions on leaves of IR66 were well developed and were darker than those on the other cultivars. Each of the cultivars also developed nonsporulating lesions, ranging from barely visible to larger (to 2–3 mm in diameter) reddish-brown flecks. These lesions were observed mostly on older leaves.

The gray center of lesions appeared to be smaller on older leaf parts regardless of cultivar. However, in series 7–9 the lesions of CO39 with the largest gray centers were seen on slightly older leaves. In these series, the density of sporulating lesions on young leaves of CO39 was high, and lesions began coalescing soon after appearance. In all cultivars, larger lesions developed on leaves of tillers than on leaves of the main culm.

In all series, the average number of leaves with sporulating lesions on the main culm was consistently highest for CO39 and lowest for IR36, whereas IR50 and IR66 had intermediate scores. The average number of leaves with lesions was generally lower in IR50 than in IR66, but the difference between these cultivars was not significant (Table 1). The number of leaves that developed lesions varied between series and increased in all cultivars when conditions favored infection, as expressed by the total number of lesions per plant (Table 2). Relative differences between cultivars did not depend on the general infection level. The average Pearson correlation coefficient for the number of leaves with lesions beween the cultivars over the series was 0.82.

The number of sporulating lesions that developed differed greatly among series, indicating a strong influence of environment on infection (Table 2). Between the series 2 and 7, the number of lesions on CO39 varied more than sevenfold, and the number of lesions on IR36 and IR50 varied more than 10-fold. Despite the large environmental effect, the average number of sporulating lesions per plant was consistently highest in CO39 and lowest in IR36. Averaged over all series, the number of sporulating lesions in CO39 was about eightfold of that in IR36. The number of sporulating lesions on IR50 and IR66 differed little, except in series 9, in which the number of lesions per plant in IR66 was about half that of IR50. Both cultivars developed about one-fifth the number of lesions in CO39.

In all cultivars, most lesions were found on the youngest leaf closest to the top. At the same time, the proportional distribution of the lesions over the leaves varied between genotypes and series (Table 3). In IR36, nearly all lesions were located on the top leaf in all series (range 93–98%). Only a few lesions developed

TABLE 2. Average number of sporulating lesions per plant on leaves of the main culm of four rice cultivars inoculated with *Pyricularia oryzae* in nine series

		Cultivar			
Series	Predominant leaf stage	CO39	IR50	IR66	IR36
1	6	17.9 a[y]	4.2 b	...	1.3 c
2	7	14.9 a	1.6 b	...	1.0 b
3	7	32.2 a	6.3 b	...	5.0 b
4	7	22.0 a	8.0 b	6.7 bc	3.8 c
5	7	46.7 a	10.3 b	10.6 b	6.8 b
7	7	113.2 a	19.5 b	20.5 b	13.0 b
8	8	...	20.4 b	17.6 b	9.1 c
9	8	...	15.8 b	8.4 bc	6.1 c
Weighed means[z]		43.2 k	9.6 l	8.5 l	5.1 m
Relative to CO39		100%	22%	20%	12%

[y] Different letters indicate significantly different cultivar means within series according to the Kruskall-Wallis rank test; multiple comparison at $\alpha = 0.05$.

[z] Different letters indicate significantly different cultivar means over all series according to the Wilcoxon signed rank test; pairwise comparison of cultivars at $\alpha = 0.05$.

on the second youngest leaf, and the third leaf from the top developed no lesions. The third leaf from the top was also completely resistant in IR50, but in this cultivar relatively more lesions developed on the second youngest leaf (mean of 12% over series). The proportion of lesions on the second youngest leaf was considerably higher in IR66 and CO39 (means of 39 and 33%, respectively). In these cultivars, a small proportion of the lesions was found on the third youngest leaf. In CO39, in series 7, a few sporulating lesions were observed on the fourth leaf from the top.

The number of sporulating lesions per unit of leaf area of expanding seventh leaves varied greatly between cultivars, between age categories, and between series (Table 4). CO39 was the most susceptible cultivar, consistently showing the highest lesion number per unit of leaf area despite large differences in lesion number between series. IR36 had the fewest lesions per unit of leaf area.

Except in CO39, the number of lesions per unit of leaf area decreased considerably with increasing leaf age at inoculation. For CO39, in some series, more lesions per unit of leaf area occurred on leaves that were in the middle of leaf expansion than on leaves of the youngest age group.

The relative difference between cultivars increased when leaves matured. For example, the resistance of IR36, in terms of lesions per unit of leaf area, was about twice that of CO39 in young, newly expanding leaves. But on fully expanded leaves, IR36 was 16 times more resistant (Table 4).

DISCUSSION

Resistance to infection of rice leaves by *P. oryzae* was strongly dependent on age and stage of leaf expansion. Resistance rapidly increased in more expanded (older) leaves, resulting in a reduced number of sporulating lesions per leaf area.

Similar results were obtained in the United States, Japan, and the Ivory Coast (4,6,8) for other cultivars and isolates. The rapid increase of resistance with increased leaf age appears to be a general phenomenon in rice that can be observed in diverse environments. More importantly, the present study shows that the increase in resistance with aging of leaves differs among cultivars.

Because leaves become increasingly resistant to infection with time, the total number of successful infections resulting in sporulating lesions for a certain amount of inoculum will depend on the initial level of susceptibility of newly emerging leaves and the rate of increase in resistance of aging leaves. Successive leaves from the top are increasingly older and, thus, more leaves from the top will be infected in cultivars with leaves that remain susceptible during a longer time after appearance. New leaves of IR36 became completely resistant faster than new leaves of IR50 or IR66, which in turn became resistant faster than leaves of CO39. The rapid buildup of age-related resistance in IR36 compared to the other cultivars was demonstrated by a consistently low number of infected leaves, by a high proportion of the total number of lesions located on the top, youngest leaf, and by a relatively large increase in resistance of the top leaf during expansion. Cultivar differences also were found for the initial level of susceptibility of the newly emerging leaves.

Partial resistance has been defined as a reduced epidemic in the field despite a susceptible infection type (11). Components associated with higher levels of partial resistance are a reduced infection frequency, longer latent period, and reduced sporulation capacity (10). The infection frequency, or related parameters such as the infection efficiency, has been reported as an important component of partial resistance to *P. oryzae* in rice (13,15,16). Because a more rapid buildup of resistance in young leaves over time causes a reduction in the total number of successful infections resulting in sporulating lesions, this characteristic should be associated with the partial resistance level of rice cultivars. A low initial level of susceptibility in young leaves would further add to the resistance. On the basis of the initial susceptibility of young leaves and subsequent rate of increase of resistance with aging among the tested cultivars, CO39 should have the lowest partial resistance level, followed by IR50 and IR66, whereas partial resistance is expected to be highest in IR36. These findings agree with data available from field studies (2,15).

The time that newly emerged leaves remain susceptible and

TABLE 3. Percentage of sporulating lesions on the youngest (N), second youngest (N−1), and third youngest (N−2) leaves of the main culm of four rice cultivars inoculated with *Pyricularia oryzae*

	Cultivar											
	CO39			IR50			IR66			IR36		
Series	N	N−1	N−2	N	N−1	N−2	N	N−1	N−2	N	N−1	N−2
1	73	26	1	98	2	0	...	...	...	96	4	0
2	80	19	1	93	7	0	...	...	...	97	3	0
3	53	45	2	87	13	0	...	...	...	97	3	0
4	67	30	3	84	16	0	78	21	1	97	3	0
5	64	34	2	91	9	0	61	39	0	98	2	0
7	51	43	6	92	8	0	81	19	0	98	2	0
8	...	...	...	74	26	0	29	63	8	94	6	0
9	...	...	...	85	15	0	42	53	5	93	7	0
Mean	65	33	2	88	12	0	58	39	3	96	4	0

TABLE 4. Number of sporulating lesions on the seventh leaf per 100 cm^2 of leaf for three categories of leaf expansion of four rice cultivars inoculated with *Pyricularia oryzae* expressed relative to mean values in young leaves of CO39

		Cultivar[x]											
		CO39			IR50			IR66			IR36		
Series	Lesions[y]	1[z]	2	3	1	2	3	1	2	3	1	2	3
2	88.6	100 a	125 a	94 a	44 a	16 ab	8 b	...	...	...	24 a	9 ab	1 b
3	148.9	100 b	161 a	84 b	85 a	43 b	...	...	...	...	78 a	38 a	9 b
4	229.2	100 a	61 b	44 b	66 a	33 b	...	50 a	31 a	10 b	38 a	17 b	10 c
5	293.9	100 a	95 a	97 a	66 a	40 a	...	40 a	36 a	...	58 a	23 b	4 b
7	529.9	100 a	102 a	80 a	62 a	32 a	16 b	59 a	29 ab	21 b	49 a	32 a	3 b
Mean		100	109	80	65	33	12	50	32	16	49	24	5

[x] Different letters indicate significant difference between leaf age categories within cultivars (Kruskall-Wallis; multiple comparison at $\alpha = 0.05$).
[y] Mean number of lesions on young leaves of CO39 (equal to 100%).
[z] Leaf expansion categories are 1, leaves shorter than 15 cm; 2, leaves between 15 and 30 cm; and 3, leaves longer than 30 cm.

the initial level of susceptibility may be partly independent. For example, the initial level of susceptibility of new leaves of IR66 was similar to that of IR36, but additional resistance increased more slowly in IR66. In addition, the number of infected leaves per plant and the proportion of sporulating lesions on older leaves were not only higher on IR66 compared to IR36, but also compared to IR50.

The leaf age related resistance studied in this paper was partial in its effect because each of the rice genotypes showed a susceptible infection type after inoculation. Considering the inability of *P. oryzae* to infect old leaves, even of highly susceptible cultivars, it is postulated that cultivar resistance characterized by a susceptible infection type and by a rapid increase of age-related resistance is less likely to be quickly overcome by new strains of the fungus than cultivar resistance based on a resistant infection type.

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