Euphytica **69**: 185–190, 1993.

Latent period to leaf blast in rice and its importance as a component of partial resistance

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Key words: leaf blast, *Magnaporthe grisea, Oryza sativa,* partial resistance, *Pyricularia oryzae,* rice

Summary

In many pathosystems, a prolonged latent period is an important component of partial resistance. Latent period in rice to leaf blast was assessed in cultivars representing a fairly wide range of partial resistance under various conditions that are known to influence the expression of partial resistance considerably. The latent period was slightly more than four days and varied only little between treatments, with a maximum difference of only eight hours between cultivars. The very small differences in latent period were not associated with differences in partial resistance due to cultivar, nitrogen, or leaf age effects. It was concluded that the latent period is of no importance as a component of partial resistance to leaf blast.

Introduction

Selection for higher levels of partial resistance (PR) to *Magnaporthe grisea* (anamorph *Pyricularia oryzae*) in tropical rice cultivars (*Oryza sativa*) is complicated. Many of these cultivars carry one or more effective major genes (Kiyosawa et al., 1986). In tropical fields, a mixture of races is usually present and efficient field evaluation of PR is hindered due to epistatic effects of the major genes (Ezuka, 1979; Notteghem, 1989; Parlevliet, 1983). In addition to the problem of epistatic effects of major genes, PR is very sensitive to changes in environmental conditions (Ou, 1985). Evaluation of components of PR and selection for one or more of the components might serve as an alternative, more efficient approach to improve PR. Among the components often associated with higher levels of PR are a reduced infection efficiency, a longer latent period, and a reduced sporulation capacity (Parlevliet, 1979).

Studies on latent period (LP) to leaf blast are relatively few, and the importance of LP as a component of PR to leaf blast is not clear. Some studies indicated substantial variation for LP among rice cultivars (Rodríguez & Gálvez, 1975; Brodni et al., 1988; Castaño et al., 1989), but other studies indicated little or no variation (Yeh & Bonman, 1986; Silue et al., 1992; Roumen, 1992). Under tropical conditions, the LP of leaf blast is relatively short, about 4 to 5 days (Ou, 1985). Leaf blast thus behaves as a compound interest disease, and even relatively small differences in LP might result in clear cultivar differences for PR (Zadoks, 1971; Zadoks & Schein, 1979). In the present paper, the importance of the LP as a component of PR in rice to leaf blast is investigated.

Material and methods

The isolate Po6-6 was used for inoculations. This isolate induces a susceptible infection type (large elliptical lesions with a grey centre) on the cultivars used in the experiments.

For assessing LP accurately, the beginning of sporulation of lesions has to be measured. For blast lesions, this cannot be done visually. However, sporulation is usually only observed in lesions with a grey centre (Jeanguyot, 1983). To investigate the relation between the start of sporulation and the development of grey centres, a new technique to detect the presence of spores on lesions was used (Experiment I). Three cultivars, IR50, IR64 and IR66, representing a fairly wide range of PR under field conditions (Bonman et al., 1989; Sah & Bonman, 1992) were assessed at several categories of leaf age, since leaf age markedly influences other components of PR to leaf blast in these cultivars (Roumen, 1992).

Experiment I was repeated three times (series) with intervals of four weeks. Per series and per cultivar, 20 pots were planted with seven germinated seeds each. The 60 pots were completely randomized and placed in a 1.5 m^2 block in a greenhouse. Plant cultivation, inoculation and incubation were as described previously (Roumen, 1992). Inoculation was done when most of the plants developed the seventh leaf on the main culm. The days of emergence of leaves five, six and seven on the main culm were recorded for each plant. On the day of inoculation, the point of emergence of the youngest leaf was marked with a felt-tip water proof marker. In the first series, all plants were marked regardless of differences in stage of development between plants, but in the second and third series, subsets of at least 20 plants per genotype with the same day of emergence of leaf seven were selected. Up to nine well separated emerging lesions (visible as small white flecks) were monitored per plant. Per leaf, three emerging lesions were selected near the leaf tip, three in the middle section, and three near the leaf base. The time of first appearance of the lesions was recorded and the growth of grey centres was assessed using a key described previously (Roumen, 1992). The age of the leaf area for each lesion was estimated by interpolation using the time of leaf emergence.

To induce sporulation, a fine mist of water was sprayed over the plants shortly before sunset on day three after inoculation and onwards, until the leaves were covered with a barely visible layer of droplets. The plants were then covered with a plastic cage. Sporulation was measured each morning. Batches of two pots per cultivar were transferred from the cage to a wind-free room. There, the presence of spores was assessed using small agar disks that were stamped from a 2% water-agar layer of circa 1 mm thickness with a small plastic cylinder (3 mm in diameter). The disks were gently pressed on the lesions with a small spatula and were then transferred onto an object-glass. In a small preliminary test, the agar disks were found to be very efficient in removing spores from lesions without damaging these lesions.

The object glasses with the agar disks were stored in Petri-dishes to prevent desiccation and contamination of the samples. The presence of spores on the disks was assessed using a microscope (magnification: 100 ×). The time between inoculation and the time that 50% of the lesions became visible as small white flecks (IC_{50}), and the time between inoculation and the time that 50% of the lesions started sporulating (LP_{50}) was calculated for each cultivar by linear interpolation of the daily measurements.

In experiment II, also carried out three times (series), cultivar differences for LP were assessed. The cultivar IR66 was replaced by the highly susceptible cultivar CO39. The three series were again planted with an interval of four weeks. Per series and per cultivar, 16 pots were planted with seven germinated seeds per pot. Two blocks were formed per series, each with eight pots per cultivar. The pots were completely randomized within blocks. Nitrogen fertilizer (ammoniumsulfate) was applied in three splits. Depending on the block, either 5-5-5, or 5-10-10 g/m^2 N was added to each pot at leaf stage two, at leaf stage four, and on the day before inoculation. The two nitrogen levels were included because increased supply of nitrogen markedly enhances the susceptibility of rice to blast (Kwon et al., 1974; Matsuyama, 1975; Tokunaga et al., 1966). Changes

Table 1. Incubation and latent periods[1] (hours) in leaves of three rice cultivars after artificial inoculation to a virulent isolate of the blast pathogen in three series of experiment I

Cultivar	Incubation period				Latent period			
	Series			Mean[2]	Series			Mean[2]
	1	2	3		1	2	3	
IR50	83	87	82	84	100	91	98	96
IR66	94	82	81	86	103	90	106	100
IR64	88	87	85	87	99	99	105	101
Mean[2]	88	85	83		101	93	103	

[1] Period from inoculation until 50% of the selected lesions became visible and started sporulating, respectively, estimated by interpolation of data.

[2] Means were not significantly different ($\alpha = 0.05$) according to the F-test.

in the nitrogen supply thus are also likely to affect any important component of resistance.

From the fifth leaf onwards, the point of emergence of the topmost leaf was marked every other day, enabling the calculation of the age of each leaf part by interpolation. Inoculation and actions to induce sporulation were as in experiment I. Assessments were made on a sample of four (series 1 and 2) or five pots (series 3) per cultivar for each nitrogen application. The number of sporulating lesions that became visible as small white flecks was counted once daily in series 1 and twice daily in series 2 and 3 for each marked leaf segment until the number stopped increasing. The IC_{50} was calculated for each pot by linear interpolation, as the time (hours) from inoculation until 50% of the finally observed lesions in the leaves on the main culm of the seven plants were visible. In addition, the IC_{50} was calculated per cultivar and per nitrogen level for each of the marked leaf segments differing in age. In each series, at least 20 random lesions per cultivar were sampled with agar disks to estimate the LP_{50}.

Results

Experiment I. Non-sporulating lesions, which appeared mostly as minute dark spots, were observed

Table 2. LP_{50} in hours, lesion size (LS) in mm^2 at LP_{50}, and sample size (n) for three rice cultivars (Cult.) and four categories of leaf age (days) after inoculation with a virulent isolate of the blast pathogen in three series of experiment I

Cult.	Age	Series 1			Series 2			Series 3		
		LP50	LS	n	LP50	LS	n	LP50	LS	n
IR50	0–1	98	0.9	49	89	0.5	59	99	0.6	22
	2–3	98	0.9	56	88	0.3	56	92	0.4	38
	4–5	100	0.7	51	99	0.3	22	97	0.6	28
	6–7	104	0.7	34	111	0.4	12	113	0.7	16
IR66	0–1	100	0.9	33	89	0.3	59	102	0.3	27
	2–3	107	0.7	36	87	0.1	40	107	0.4	41
	4–5	104	0.8	50	92	0.2	35	–	–	0
	6–7	103	0.8	21	98	0.3	38	–	–	0
IR64	0–1	99	0.9	50	99	0.5	39	101	0.5	33
	2–3	99	0.7	47	97	0.4	31	109	0.5	43
	4–5	100	0.7	34	–	–	0	–	–	0
	6–7	–	–	0	–	–	0	–	–	0

Table 3. Incubation period[1] (hours) in leaves of three rice cultivars after inoculation with a virulent isolate of the blast pathogen in three series of experiment II

Cultivar	Series			Mean
	1	2	3	
IR50	88 a[2]	103 a	102 a	98 a
CO39	89 a	104 a	108 ab	100 a
IR64	98 b	107 a	112 b	106 b
Mean	92	105	107	101

[1] The incubation period was calculated as the time between inoculation and the time that 50% of the sporulating type lesions became visible. The latent period was nearly the same as the incubation period.

[2] Within columns, values followed by different letters indicate significant differences between cultivars at $\alpha = 0.05$ according to Bonferroni's test for inequalities.

to develop ahead of the sporulating lesions. Sporulating lesions emerged as minute white or grey flecks. At 71 hours after inoculation, no sporulating lesions were observed at all, but at 87 hours 60% (averaged across cultivars and series), and at 111 hours nearly all of the sporulating lesions were visible. The data confirmed earlier observations on these cultivars that most sporulating type lesions appear within a short time (Roumen, 1992).

The estimated period between inoculation and the time that 50% of the lesions became visible (IC_{50}) or started sporulating (LP_{50}) differed little, if at all, between the cultivars (Table 1). The LP_{50} was about 4 days, ranging from 91 to 106 hours depending on series and cultivar. The average difference between the IC_{50} and LP_{50} was 13 hours. Breakdown of the LP_{50} by leaf age showed a slight tendency for the start of sporulation of lesions to be delayed in older leaves (Table 2). The average size of the grey centre in the visible lesions at the LP_{50} was very small, with a range of 0.1–0.9 mm^2. The data indicate that lesions were capable of sporulation shortly after appearance regardless of the age of the leaf area where it develops.

Experiment II. The agar disk samples showed that, in each series of the second experiment, lesions started sporulating very shortly (less than 10 hrs) after the time that the lesions became just barely visible as small white flecks. The LP_{50} was thus almost identical to the IC_{50} that was actually assessed (Tables 3 and 4), and treatment differences for IC were similar to those for LP. Visually, no differences between cultivars were observed in time of appearance of lesions.

Adding extra nitrogen did not influence the LP. Adding more nitrogen caused an increase of the number of sporulating lesions of 55% in CO39, 66% in IR50 and 118% in IR64, averaged over the three series of the experiment, whereas the LP_{50} was decreased with less than 0.2%. Similar as in experiment 1, the IC_{50} (LP_{50}) was about 4 days (Table 3). In each of the series, the incubation and latent periods were shortest in IR50 and longest in IR64,

Table 4. Incubation period[1] (hours) in leaves of three rice cultivars for five categories of leaf age (days) after inoculation with a virulent isolate of the blast pathogen in three series of experiment II

Age	Cultivar									Mean
	CO39 Series			IR50 Series			IR64 Series			
	1	2	3	1	2	3	1	2	3	
0–1	89	105	107	88	105	103	103	106	112	102
2–3	88	105	107	86	102	102	85	105	113	99
4–5	86	102	108	86	92	101	87	112	114	99
6–7	88	108	109	86	94	105	–	–	–	
≥ 8	90	–	108	–	–	–	–	–	–	

[1] The incubation period was calculated as the time between inoculation and the time that 50% of the sporulating type lesions became visible. The latent period was nearly the same as the incubation period.

the average difference between these culivars across series being about 8 hours (significant at $\alpha = 0.05$). No effect of leaf age on the IC_{50} (LP_{50}) was detected in any of the cultivars (Table 4).

Discussion

Since spores of the blast pathogen are small (19–23 × 7–9 μm) and lack a contrasting colour (Ou, 1985), their presence on blast lesions can only be detected using a microscope, making assessment of the LP a tedious operation. Direct observation of the leaves and lesions under the microscope was done by Rodríguez & Gálvez (1975), Brodni et al. (1988), and Castaño et al. (1989), strongly limiting the number of lesions per cultivar that can be measured. Besides, this method has the disadvantage that leaves are easily damaged, which is likely to influence the measurements. Excised lesions were observed by Yorinori & Thurston (1975), but such observations may not be representative since the authors mentioned that lesion development in detached leaves differed from that in intact leaves. In comparison, the sampling technique using agar disks presented here is relatively fast. Some 200 lesions can be sampled per hour. Moreover, this method causes no or hardly any leaf damage and the flexibility of the experimental setup is improved since the plants don't have to be transferred to a microscope or vice versa.

The measurements of both experiments showed that lesions that developed grey centres began sporulating very shortly after these lesions became visible. The LP of a cultivar may therefore be estimated by measuring the far easier measurable incubation period, as was done by Yeh & Bonman (1986).

In contrast with the results of the present study, considerable cultivar differences for LP have been reported by others. Rodríguez & Gálvez (1975), reported that LP ranged from 5 to 9 days depending on the cultivar. However, examination of their results revealed that the isolates used by these authors appeared to be avirulent to most of the test cultivars. The LP increased when cultivars developed a more resistant infection type, but cultivar differences were not very clear when the isolate was virulent. Comparison of the present results with those of Brodni et al. (1988) and Castaño et al. (1989) is more difficult. Brodni et al. (1988) found a five day difference for LP among seven rice cultivars, but no information on the infection type was supplied. Castaño et al. (1989), studying a group of 69 cultivars, found a difference of about five days between the cultivars with the shortest and the longest LP, but also in this study the infection type of the cultivars was not clear. The cultivars were classified as medium resistant, medium susceptible, or susceptible, using a scale that mixes qualitative with quantitative criteria. Pooled across cultivars, the LP significantly increased with a more resistant classification, but whether significant differences between cultivars within each class were also present was not determined.

Generalizing the finding that sporulating type lesions are able to start sporulating soon after appearance, studies that were restricted to cultivars with a susceptible infection type indicated little or no cultivar differences for LP (Yeh & Bonman, 1986; Roumen, 1992). Silue et al. (1992) did not notice clear genetic variation for incubation period among *Oryza sativa* while finding a six hours shorter period in *Oryza glaberrima.*

The results of the present study indicate that small cultivar differences for LP exist when a susceptible infection type is induced. These differences (up to 8%) are too small to be of use in breeding programs and the differences for LP were not clearly related to cultivar differences of PR measured under field conditions. Among the cultivars used in experiment II, CO39 is by far the most susceptible cultivar in the field and IR64 the most resistant. CO39 may be completely destroyed where IR64 suffers relatively little damage. However, in two out of the three series the LP between these cultivars was not significantly different (Table 3). Moreover, the LP was not affected by increased nitrogen supply although this is well known to increase blast susceptibility (Sakurai & Toriyama, 1967; Yunoki et al., 1970 cited in: Toriyama, 1975). Also leaf age, known to affect the susceptibility in terms of lesion number (Kahn & Libby, 1958; Roumen, 1992), did not affect LP. It is concluded that the LP period is not important as a component of PR to leaf blast.

Acknowledgements

This paper is part of the results of a collaborative project with inputs of the International Rice Research Institute, Los Baños, Philippines, the Dept. of Plant Breeding, Wageningen Agricultural University, The Netherlands, and The Netherlands' Ministry of Development Cooperation, The Hague, The Netherlands. Publication does not constitute and endorsement by the Netherlands Minister for Development Cooperation. The authors thank Ellen Silab, Leonido Angeles and Mang Temyong for providing assistance during the experiments.

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