A Screening Technique to Evaluate Resistance of Rice to *Rhynchosporium oryzae*

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ABSTRACT

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A simple and uniform method for testing rice cultivars for resistance to *Rhynchosporium oryzae* in a glasshouse was developed. Leaves of 30-day-old plants grown in plastic trays were inoculated with 6- to 7-day-old mycelial disks and incubated under moist conditions for 96 hr. Lesion extension from the point of inoculation was used as a measure of resistance. Susceptibility of test cultivars relative to a standard check cultivar was calculated. Of 40 local and introduced rice cultivars screened, 21 showed relative resistance to one isolate of *R. oryzae*.

Additional key words: leaf scald, varietal resistance

Leaf scald of rice, caused by *Rhynchosporium oryzae* Hashioka & Yokogi, is an important disease in the humid tropic regions of the world (2,3,6). First reported in Brazil in Braganca, Pará, in 1973 (F. C. Albuquerque, *personal communication*), leaf scald has since been recorded in both irrigated and upland regions of rice cultivation in most Brazilian states (4,5). Although the disease is widespread wherever rice is grown, it constitutes a particular problem in the Brazilian state of Amazonas. In other regions, its occurrence is conditioned by long periods of leaf wetness from heavy dews or continuous rains.

A reliable screening method to evaluate disease resistance to initiate a breeding program is not available. Although screening under field conditions is desirable, incidence and spread of leaf scald are subject to weather conditions, resulting in variable disease levels. We attempted to develop a precise, uniform method for rapid evaluation of germ plasm under controlled conditions.

MATERIALS AND METHODS

Test plants were grown in soil in 42 ×34×8 cm plastic trays. Uniform fertility was maintained with ammonium sulfate (50 kg N/ha), superphosphate (60 kg P_2O_5/ha), and potassium chloride (30 kg K_2O/ha). Two rows of three test cultivars were seeded and later thinned to eight plants per 34-cm row. The fully expanded top leaf and penultimate leaf of 30-day-old plants (32 leaves for each cultivar) were inoculated.

Inoculations were made with agar disks of fungal mycelium. Pure cultures of the fungus were grown in 9-cm petri dishes at 24 C for 6–7 days. Equal quantities (7 ml) of potato-dextrose agar were dispensed in petri dishes to obtain thin agar disks of uniform thickness. Disks of mycelium 4 mm in diameter were removed from margins of growing colonies with a sterilized cork borer and placed approximately in the center of the adaxial portion of each leaf. Disks adhered readily to the leaf surface with gentle pressure from an inoculation needle. The inoculated plants in trays were incubated in a dew deposition chamber at 21–25 C.

Disease reaction was measured by lesion length (cm) from the point of inoculation 96 hr after incubation. Mean lesion size was calculated based on measurement of 32 lesions. Relative growth rate of the lesion was determined on the top two leaves of 30-day-old plants of the cultivar IAC 47.

The minimum sample size required for inoculation was determined from the following formulas (1): $n = n_o/[1 + (n_o/N)]$ and $n_o = (t^2 \times S^2)/d^2$, where N is the number of elements in the presample, t is the table value for the t test at the 0.001 level, S^2 is the sample variance, and d^2 is the percentage of mean admissible error (10% of the mean). Mean differences in lesion extension of test cultivars were compared with the susceptible check IAC 120 by Student's t test at the 0.001 level.

To give a score to the disease reaction based on lesion extension, a post-analysis check cultivar, or "standard check," was used. The cultivar Catalão, which had the

Table 1. Mean lesion extensions of the top two leaves of 30-day-old rice plants inoculated with mycelial disks of *Rhynchosporium oryzae*[a]

Cultivar	Top leaf (cm)	Penultimate leaf (cm)
IR 20	3.6±0.45	3.7±0.32
IR 22	3.9±0.59	3.9±0.57
IR 841-63-5-L-9-33	3.2±0.37	3.2±0.48
EEA 404	4.4±0.70	4.6±0.54

[a] Mean lesion extensions on each leaf are not significantly different for any of the four cultivars at the 0.001 level. Standard deviations for 12 leaves are given after the means.

Table 2. Mean lesion length on leaves and disease severity index of rice cultivars to infection by *Rhynchosporium oryzae*

Cultivar	Mean lesion length[a] (cm)	Disease severity index[b]
IAC 120 (susceptible check)	5.53 ± 0.64	1.16
Parazinho	5.38 ± 0.51	1.13
Dourado Precoce	5.25 ± 0.39	1.10
IAC 5100	5.24 ± 0.55	1.10
IAC 435	5.20 ± 0.65	1.09
Edith longo	5.19 ± 0.67	1.09
IAC 47	5.19 ± 0.39	1.09
Labelle	5.21 ± 0.51	1.09
IAC 25	5.15 ± 0.36	1.08
IAC 416	5.06 ± 0.83	1.06
Iguape redondo 1	4.83 ± 0.58	1.06
IAC 5544	5.06 ± 0.63	1.05
Taichung	5.04 ± 0.63	1.05
Agulha	4.94 ± 0.45	1.03
De Abril	4.93 ± 0.62	1.03
Fernandes	4.91 ± 0.66	1.03
IAC 1246	4.88 ± 0.76	1.02
Iguape redondo 2	4.87 ± 0.58	1.02
IRAT 13	4.77 ± 0.55*	1.00
Pratão precoce	4.67 ± 0.47*	0.98
Amarelão	4.62 ± 0.54*	0.97
Carioca	4.53 ± 0.45*	0.95
EEA 404	4.51 ± 0.62*	0.94
Montanha Liso	4.50 ± 0.63*	0.94
Pérola	4.38 ± 0.61*	0.92
Dawn	4.21 ± 0.73*	0.88
IR 36	4.22 ± 0.72*	0.88
IPEACO 562	4.20 ± 0.77*	0.88
Bico ganga	4.09 ± 0.73*	0.86
TKM-6	4.12 ± 0.39*	0.86
IR 22	4.01 ± 0.52*	0.84
Batatais	3.96 ± 0.44*	0.83
IRAT 9	3.96 ± 0.35*	0.83
IR 20	3.68 ± 0.39*	0.77
EEA 408	3.65 ± 0.42*	0.76
IR 8	3.63 ± 0.46*	0.76
Tainan	3.48 ± 0.30*	0.73
IR 841-63-5-L-9-33	3.28 ± 0.47*	0.69
Ratna	3.24 ± 0.34*	0.68
Catalão (standard check)	4.78 ± 0.58	1.00

[a] Means marked with an asterisk are significantly different from the susceptible check, IAC 120, at the 0.001 level of probability. Means are the average of 32 top and penultimate 30-day-old leaves. Standard deviations are given after the means.

[b] Calculated by dividing the mean lesion length of the test plant by the mean lesion length of the standard check cultivar Catalão. Values less than 1 are relatively resistant; those greater than 1 are relatively susceptible.

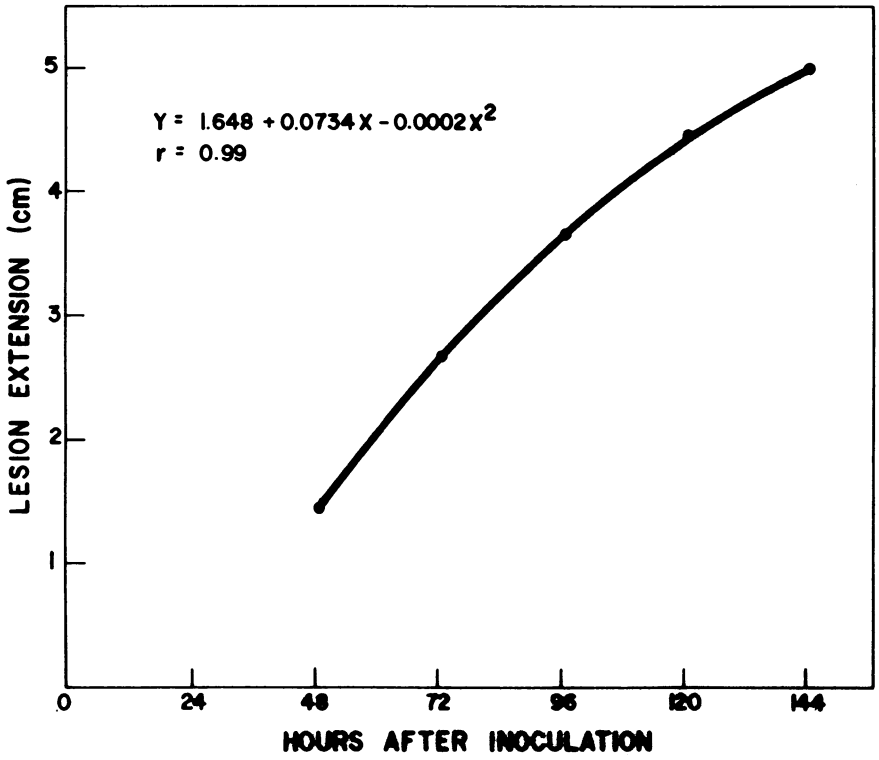

Fig. 1. Increase in lesion size of leaf scald of rice with time. Leaves of rice plants were inoculated with mycelial disks of *Rhynchosporium oryzae*, and lesion extension was measured at intervals during the 96-hr incubation in a moist chamber. Data points represent the average of 32 lesion measurements.

smallest lesions of those cultivars that did not differ significantly from the susceptible check, was chosen for this purpose. The disease severity index of each test plant was calculated as the ratio of the lesion extension of the test plant to the lesion extension of the standard check. Cultivars with disease severity index values less than 1 were regarded as relatively resistant, and those with values greater than 1 were regarded as relatively susceptible.

RESULTS AND DISCUSSION

Mycelial growth around the agar disk of the test fungus was observed 24 hr after inoculation. Lesion extension could be measured from 48 hr until 144 hr after inoculation, when the proximal end of the inoculated leaf dried. Lesion development stopped soon after removal of the plant from the dew chamber, indicating that continuous leaf wetness was required. White fungal mycelium extended in advance of the lesion, followed by a necrotic zone that became clearly demarcated a few hours after the plant's removal from the chamber.

Lesions grew linearly from 24 hr until 120 hr after inoculation at 0.036 cm/hr. The growth rate decreased after 120 hr (Fig. 1). The growth rate of lesions up to 120 hr after inoculation may be used as a measure of partial resistance of cultivars.

Because leaf positions and the susceptibility of individual plant organs such as leaves to fungal pathogens are often variable, the differential susceptibility to lesion development of the top two leaves was studied. Mean lesion extensions of top and penultimate leaves of 30-day-old plants did not differ significantly in any of the four cultivars used for comparison (Table 1). These results indicated that the lesion measurement could be based on the average lesion extension on the top two leaves. The minimum number of plants required for the inoculation test was determined to be six, or 12 leaves per cultivar (Table 1).

Table 2 presents mean lesion size of 40 local and introduced rice cultivars. The lesion lengths of all cultivars were less than that of the susceptible check. Mean lesion length varied from 3.2 ± 0.34 (Ratna) to 5.5 ± 0.64 (IAC 120). Mean lesion length was significantly different from the susceptible check in 21 cultivars.

The selection of a standard check on which the disease severity index is based should depend on the desired level of resistance. However, the susceptible check IAC 120 had to be maintained in all trials to obtain comparable results under different environmental conditions. We tried to obtain uniform levels of infection by spraying plants with a spore suspension, but even with incubation in moist conditions for prolonged periods, the results were not consistent. Inoculations with mycelial disks have shown that growing mycelium is pathogenic, is superior to inoculations of rice with conidia of *R. oryzae*, and can be used to screen rice germ plasm for resistance to *R. oryzae*.

Studies on the correlation between disease reactions in rice inoculated with mycelial disks in the glasshouse and rice grown under field conditions are in progress. Preliminary screening of many entries can be done rapidly with this method of inoculation in a glasshouse, which is more exacting than field screening. Inoculation of young plants with mycelial disks offers the following advantages: 1) quantitative measurements of lesions permit studies on variation in pathogen and host interaction and establishment of epidemiological relationships; 2) large numbers of cultivars may be screened simultaneously, depending on the size of the moist chamber; 3) disease reactions can be compared under similar environmental conditions; 4) the tests can be repeated many times in a year; and 5) the results of different tests are comparable.

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