Comparative Slow-Blasting in Rice Grown Under Upland and Flooded Blast Nursery Culture

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ABSTRACT

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Evaluation of rice for resistance to rice blast disease caused by *Pyricularia grisea* usually is conducted in upland (nonflooded) disease nurseries, although all commercial U.S. rice is produced under flood irrigation. Upland rice is more susceptible to leaf blast than is flooded rice, and the magnitude of this differential susceptibility can vary among cultivars. This 2-year study was undertaken to determine (i) the relationship between rates of disease development (slow-blasting) in upland and flooded rice and (ii) the value to a rice breeding program of establishing a flooded blast nursery, a facility far more difficult to manage than an upland nursery. Among 200 rice lines compared for leaf blast susceptibility under upland and flooded cultures, only 7 were rated as slightly more resistant under upland culture and 136 rated more resistant under flooded culture. Disease ratings under upland and flooded cultures were highly correlated ($R = 0.819$). Among 14 preselected cultivars over 2 years, disease development curves under upland and flooded cultures were highly correlated ($R = 0.990$). The cultivars with intermediate susceptibility under upland culture appeared to benefit most from flooded culture. Upland culture provided more opportunities to assess slow-blasting than did flooded culture, since many lines that produced susceptible-type lesions in upland culture failed to do so under flooded culture. It was concluded that adequate information on comparative leaf blast resistance among rice lines was attainable from upland blast nurseries and that routine evaluation of rice breeding lines for blast resistance in flooded nurseries was not necessary.

Rice blast caused by *Pyricularia grisea* (Cooke) Sacc. (teleomorph: *Magnaporthe grisea* (Hebert) Yaegashi & Udagawa) is among the most serious constraints to rice grain yields worldwide. The pathogen comprises many races (pathotypes), numbering in the hundreds worldwide (13). Consequently, many national rice varietal improvement programs have emphasized breeding for race-nonspecific resistance, partial resistance, or field resistance (3,9), also called slow-blasting (11). In the southern United States, breeding lines have been evaluated for slow-blasting since 1962 in upland (nonflooded) nurseries, even though all commercial U.S. rice is produced under irrigated (flooded) culture (14). There is much empirical and some experimental evidence that leaf blast develops more slowly in rice grown under flooded culture than in upland rice (5,13). Upland nurseries, how-

ever, provide many advantages over flooded nurseries for blast resistance evaluation, the most important being flexibility in planting over an extended period and ability to extend leaf wetness periods sufficiently for development of leaf blast epidemics. Under Texas conditions, additional leaf wetness is required to ensure sufficient infection for evaluation. Flooded nurseries would require an elaborate system of levees and drainage to allow serial plantings in order to accommodate the large numbers of breeding lines to be evaluated. Flooded nurseries also are subject to weather-related delays in planting, a major concern along the Gulf Coast of Texas. Screening for leaf blast resistance under flooded conditions may justify the resources and effort required to conduct and maintain an irrigated nursery, if the data from flooded nurseries are more indicative of relative rice line susceptibility to leaf blast than data from upland nurseries.

The 2-year study reported here was undertaken to determine the range of effects of flooding on leaf blast symptom development with selected lines and to determine the relationship between disease ratings under upland and flooded cultures.

MATERIALS AND METHODS

Experiments were conducted in an established 16-bed disease nursery at Beau-

mont, TX (8). Rather than attempt to compare blast nursery bed results with flooded field results, we chose to modify several nursery beds for flooding, thereby reducing the probability of confounding the treatment with variables such as soil type, leaf wetness duration, and fertilization. Two pairs of blast nursery beds, each bed 1.1×16.2 m, were surrounded by an 8-cm-high levee of stacked planks that were caulked with silicone and bolted to the concrete walkways between the beds. The two sets of beds prepared for flooding were separated by four upland beds. Each of two sets of experiments was conducted in 1995 and in 1996. The first experiment simulated our usual screening procedure in which test lines are rated once whenever most of the susceptible spreader rows in the beds are killed by leaf blast. The second experiment involved serial estimates of percent diseased leaf area in replicated "miniplots" of selected rice cultivars in order to construct disease progress curves and calculate area under the disease progress curves (AUDPC) (8).

In 1995, the Uniform Rice Regional Nursery (URRN) comprising 200 advanced rice breeding lines and cultivars from the Arkansas, Louisiana, Mississippi, and Texas rice-breeding programs were sown on two dates, 27 June and 24 July, in 60-cm rows across the blast nursery beds, with 10-cm row spacing and 2 g of seed per row. The experiment was designed as a factorial with 200 lines and two cultural regimes, upland and flooded, with seeding dates as replications. Two outside rows and every third row of each bed were planted to 'M-201', a highly blast-susceptible California rice cultivar, which placed each test row adjacent to a "spreader" row (Fig. 1). A single spreader row of 'M-201' was planted the length of each bed on the windward (south) side. Two beds were planted to each treatment, with each bed planted to 100 test lines and 53 rows of 'M-201' spreader. Prior to planting, ammonium sulfate (21% N) was incorporated into the soil at 112 kg of N per ha. The irrigated beds were flooded 2 to 3 cm deep with canal water 8 days after seedling emergence. Leaf wetness periods were extended by sprinklers operating over both the upland and flooded beds for 6 s every 6 min daily from 4:00 to 8:30 p.m. and from 9:00 to 10:00 a.m. Rice

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lines were evaluated for leaf blast severity 33 days after emergence (mid-tillering stage) using a 0 to 9 scale, in which 0 (immune) = symptomless; 1 to 3 (resistant) = pinpoint to 1- to 2-mm oval brown nonsporulating lesions, the larger with small ashen centers; 4 to 8 (susceptible) = typical sporulating spindle-shaped lesions of increasing size; and 9 = dead (4,8).

Immediately following evaluation of the first planting of the URRN, the beds were drained, mowed to the ground, allowed to dry, and then retilled. On 5 September 1995, two upland and two floodable beds

were planted to two replications per bed (four replications per treatment) of 14 rice cultivars in three-row plots separated by two-row buffers of highly blast-resistant 'Jasmine 85' (8,10) (Fig. 1). Row spacing, length, orientation, and seeding rate were as described above. Cultivars were randomized within replications. Lengthwise of each bed, a single row of susceptible 'M-201' was sown on the windward side and resistant 'Jasmine 85' on the leeward side to increase inoculum concentration and reduce wind movement within and among beds, respectively (Fig. 1). Plots were fertilized

and leaf wetness periods were extended as described above. Beginning 25 days after emergence (5 October), percent diseased leaf areas were estimated visually four times at 5-day intervals in order to plot disease progress curves and calculate AUDPC (8).

Both experiments were repeated in 1996. Single sets of the URRN were seeded on 5 June and 17 June. The disease development experiment with 14 rice cultivars was seeded 31 July. Diseased leaf areas were estimated four times at 5-day intervals beginning 25 days after emergence (19 August).

In both years, inocula of four broadly pathogenic pathotypes of *P. grisea* (races IB-1, IB-49, IC-17, and IE-1K) (7) were being generated from seven windward beds in which breeding lines were being evaluated concurrently. In over 20 years of leaf blast evaluation, obtaining the desired broad distribution of airborne inoculum over the 15 × 24-m nursery has posed no problems. All of the test lines selected for the disease development experiment were susceptible to at least one of the four pathotypes known to be present in the nursery. Isolates from representative cultivars were collected at the conclusion of the experiments each year and identified as to pathotype on the international rice blast differential rice varieties (1) to verify their presence.

The data on the URRN entries from upland and flooded beds in the 1995 plantings were compared for overall differences across the 200 lines and subjected to paired *t* tests; regression analysis was used to show the magnitude of the differences, with upland ratings as the independent variable. The two plantings were analyzed separately. The disease development data were analyzed by analysis of variance, and means were compared using protected least significant difference (release 6.12; SAS Institute, Cary, NC).

RESULTS

Disease development in the spreader rows of 'M-201' was uniform across the test beds in all the plantings, indicating adequate distribution of inoculum. Of the four plantings of the 200-line URRN, only the two in 1995 were successful. In 1996, underground seepage from flooded to upland beds could not be controlled adequately, and the flood could not be maintained in the flooded beds. Essentially, both upland and flooded beds were saturated but not submerged. Consequently, disease severities were similar across treatments in 1996. The two replications of the URRN in flooded beds had an average leaf blast rating of 4.37, and in the upland beds, 4.35 (data not shown). The first planting in 1995 produced significantly different ($t = 2.50$, $\alpha = 0.013$) average leaf blast disease ratings of 3.4 in upland and 3.1 in flooded beds. This test was confounded to some degree because of seepage from the flooded to upland beds, causing the upland beds

Fig. 1. Layouts of plantings in the blast nursery showing relative positions of test lines, spreaders, and buffers in single-row plots of the 200 Uniform Rice Regional Nursery entries and in three-row miniplots of 14 lines selected for varying susceptibilities to leaf blast. Beds were 16.2 × 1 m and rows were 60 cm, with 10 cm between rows.

Fig. 2. Relationship between leaf blast severities of 200 Uniform Rice Regional Nursery rice lines grown under upland and flooded culture. The numbers indicate the frequencies of lines with each combination of upland/flooded rating (e.g., 22 lines were rated 4 under upland and 1 under flooded culture). The values in parentheses represent the numbers of lines with differences of one, two, three, or four between ratings in upland and flooded culture, with 57 lines rated the same under both cultures. Beaumont, TX, 1995.

to be saturated, though not submerged. In the second planting, the upland beds were separated from the flooded beds by an unused bed and, therefore, were not affected by seepage. In this test, the average leaf blast rating was 4.1 in upland and 2.9 in flooded beds ($t = 14.75$, $\alpha = 0.0001$).

In the second 1995 planting, only 7 of the 200 entries were rated more susceptible in flooded than in upland culture, all of them occurring in the upland bed in the 1 to 4 scale range (Fig. 2). Under upland conditions, 136 entries were rated more susceptible, 81 of which were two or more ratings higher. The first planting, in which the upland beds were saturated, showed a similar trend, but with 40 entries with lower leaf blast ratings in upland beds, 78 lines with lower ratings in flooded beds, and the remaining 82 lines the same ratings in both beds. The overall severity of leaf blast in the first planting, as measured by disease severity in the upland beds, was less, with no upland ratings above 7; only 18 lines rated above 5 in upland culture, and only 7 lines rated above 5 in flooded culture. Only one entry produced higher blast ratings in flooded beds than in upland beds in both 1995 plantings.

Disease development in the three-row plots of 14 test cultivars was slowed significantly by flooding in both years (Table 1). Though the trends were the same in both years, overall disease severity appeared higher in 1995 than in 1996 according to average AUDPC values across all cultivars. Flooded culture had a positive impact on slow-blasting regardless of inherent susceptibility, but the impact generally was greatest among cultivars with upland AUDPC values between 30 and 60, with exceptions (Table 1, Fig. 3). A comparison of average upland/flood AUDPC ratios between years (Table 1) and the relative slopes of the regression curves (Fig. 3) indicates an inverse relationship between the impact of flooding and disease severity; that is, the more favorable the environment for blast development, the less the impact of flooding on reducing blast development.

The cultivar Dixiebelles is of special interest, not only because of its recent release for commercial production (12), but also because of the extraordinary effects of flooding on blast development. In 1995, the disease progress curve of 'Dixiebelles' under upland culture was similar to that of susceptible 'Maybelle' under flooded culture (Fig. 4). Under flooded culture, however, disease development was similar to that of 'Gulfmont' under flooded culture. 'Gulfmont' has been rated as moderately resistant to resistant to leaf blast under field conditions (2). In 1996, when blast was less severe, the response of 'Dixiebelles' to flooding was similar, though the above relationships to 'Maybelle' and 'Gulfmont' were less notable (Table 1).

Prior knowledge of cultivar race-specific susceptibilities facilitated efficient sampling to recover all of the introduced pathotypes from the test miniplots; e.g., 'Kaybonnet' is susceptible only to race IE-1K, 'Jefferson' only to IB-49, 'Mars' and 'Bengal' are most susceptible to IB-1, and 'Cypress' and 'Gulfmont' are most susceptible to IC-17.

Most of the other cultivars, though susceptible to all four pathotypes, mostly yielded isolates of races IB-49 and IC-17.

DISCUSSION

The favorable effects of flooding on resistance of rice to leaf blast has been common knowledge among rice scientists and

Table 1. Area under disease progress curves (AUDPC)^a of 14 rice cultivars with leaf blast in a blast nursery under upland and flooded culture in 1995 and 1996 at Beaumont, TX

Cultivar	1995 ^b			1996		
	Upland	Flooded ^c	U/F ratio ^d	Upland	Flooded	U/F ratio
Mars	98	84 ns	1.2	96	55*	1.8
Rosemont	77	50**	1.5	29	13**	2.2
Bengal	76	57**	1.3	69	25*	2.8
Maybelle	70	45**	1.6	39	12**	3.3
LaGrue	63	41**	1.5	23	6.6**	3.5
Newbonnet	57	29**	2.0	32	9.6**	3.3
Rico 1	46	25**	1.8	22	10**	2.2
Pecos	43	24**	1.8	4.3	1.3*	3.3
Dixiebelles	39	10**	3.9	22	6.6**	3.3
Jodon	37	18**	2.1	27	8.0**	3.4
Gulfmont	19	8**	2.4	16	5.6**	2.9
Cypress	16	5**	3.2	7.6	2.6**	2.9
Jefferson	1.1	0.2**	...	0.2	0.0*	...
Kaybonnet	0.3	0.2 ns	...	0	0.0 ns	...
Average ^d	53.4	33	2.0	36.4	14.6	2.9

^a Percent of maximal AUDPC (=15) based upon four ratings of percent diseased leaf area at 5-day intervals beginning 25 days after seedling emergence. Means of four replications.

^b Years were significantly different ($F = 51$; $df = 1,27$; $P < 0.0001$).

^c Differences within cultivars between AUDPC under upland and flooded culture were significant at $P < 0.07$ (*), $P > 0.02$ (**), or not significant at $P > 0.07$ (ns) by t test.

^d Upland/flooded AUDPC ratios. 'Jefferson' and 'Kaybonnet' were excluded from the averages since their upland values were minimal.

Fig. 3. Relationship between leaf blast development in rice grown in upland and flooded nursery culture in 2 years with different disease severities. Fourteen rice cultivars were selected to provide a range of susceptibilities from highly susceptible to resistant. Area under disease progress curves (AUDPC) were calculated based on four ratings of percent diseased leaf area at 5-day intervals beginning 25 days after seedling emergence and then converted to a percent of maximal AUDPC (=15). Beaumont, TX, 1995 to 1996.

Fig. 4. Leaf blast disease development curves of 'Maybelle', 'Dixiebelle', and 'Gulfmont' rice grown under upland (Up) and flooded (Fl) culture in a blast nursery. Beaumont, TX, second planting of 1995.

producers and is a moot point in commercial rice production in the United States, since virtually all U.S. rice production is flood-irrigated. Most blast nurseries worldwide, however, are grown under upland conditions, to increase (and in many cases, to allow) disease pressure and to facilitate the screening of large numbers of breeding lines under uniform environmental conditions. Our concern was the degree of failure in detecting potentially effective slow-blasting by not evaluating blast resistance under flooded culture.

Our results indicate that rice lines rated resistant under upland conditions will be at least as resistant and probably more resistant under flooded culture. With only upland evaluation, we would err conservatively in detecting slow-blasting. In 1995 in the URRN, there were 57 lines for which we could have had no measure of slow-blasting because of the absence of susceptible-type lesions under flooded conditions; i.e., those lines rated 3 or less under flooded and 4 or more under upland culture. The performance of those 57 lines under flooded conditions could have been attributed to race-specific major-gene resistance (9).

If we also consider the other evaluations to which breeding lines are subjected, both for other traits (e.g., yield, milling and

cooking quality, growth habit, etc.) and in subsequent seasons for the same traits, many lines will be dropped from further consideration for other undesirable traits or advanced in spite of disease susceptibilities because of other superior qualities. In the former case, disease reactions are of no consequence; in the latter, there will be opportunities for more rigorous evaluation, perhaps including evaluation under flooded culture. We conclude that, in the context of blast resistance breeding, routine evaluation under flooded culture for slow-blasting ability would not be an efficient use of resources and effort.

The data in Table 1 present several interesting between-year inconsistencies, of which 'Rosemont', 'LaGrue', and 'Pecos' are good examples. Although blast appeared less severe overall in 1996, these three cultivars showed disproportionate differences between AUDPC values under upland and flooded culture compared with 1995. One can only speculate as to the mechanisms involved and whether they were weather-sensitive directly or indirectly, as weather or some other environmental factor affected disease pressure. According to experiment station weather records, daily average air temperatures during the rating period and 10 days preceding were 22 and 26°C in 1995 and 1996, respectively. Other

factors such as leaf wetness enhancement and N-fertilizer inputs were consistent between years, but fertilizer efficiency, plant susceptibility, and infection rate can be influenced by temperature (6). The three cultivars mentioned above were among those with no race-specific resistance to any of the four races present in the nurseries.

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