Resistance in Sorghum to Seedling Disease Caused by *Pythium arrhenomanes*

G. A. FORBES, Graduate Research Assistant, Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station; O. ZIV, Professor, The Volcani Research Center, Bet Dagan 50200, Israel; and R. A. FREDERIKSEN, Professor, Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station 77843

ABSTRACT

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Resistance to infection by *Pythium arrhenomanes* was identified in the grain sorghum cultivar QL3(India). Both field and laboratory tests were used to establish QL3(India) and SC748-5 as resistant and susceptible candidates, respectively. The relative resistance in QL3(India) was tested under controlled conditions. Leaf length, leaf dry weight, and root dry weight were reduced 53, 43, and 42%, respectively, in SC748-5 as a result of infection. Reductions in the same variables for QL3(India) were 10, 6, and 15%, respectively. Resistance was associated with differences in secondary root production and lesion size.

Seedling disease is periodically a serious problem of grain sorghum (*Sorghum bicolor* (L.) Moench). The disease is severe when cool, wet soil conditions prevail after planting. *Pythium* spp. have been associated with sorghum seedling disease in the field and were pathogenic on sorghum seedlings in greenhouse tests (3,5,7,11,12,15). Several species have been implicated, including *P. graminicola* (15), *P. arrhenomanes* Drechs. (5), *P. aphanidermatum* (7), and spheral-sporangium isolates (11).

Seedling disease of sorghum caused by *Pythium* spp. has received less attention than other sorghum diseases. We are aware of no report where sorghum germplasm was evaluated in the seedling stage for resistance to *Pythium* spp. Resistance to seedling disease caused by *Pythium* spp. has been reported for beans (2), peas (13), maize (8), and cotton (9).

Resistance to *Pythium* spp. is generally quantitative, and it may represent relatively small differences in disease severity or symptoms (9). In preliminary assessments of sorghum seedlings for reactions to *Pythium* spp., we found that unexplained variability within and among experiments often confounded evaluation. Cultivars that appeared resistant under one set of conditions reacted differently in subsequent tests. Johnson and Palmer (9) found that lesion size and symptom severity of seedling disease of cotton caused by *P. ultimum* were extremely variable within cultivars. This variability was not reduced with three generations of inbreeding. Inconsistent cultivar responses could result from many unidentified sources associated with host (seedling vigor), pathogen (different isolates), or environmental conditions.

Unidentified sources of variability may mask relatively small differences in quantitative resistance to *Pythium* spp., requiring highly controlled and replicated tests. Testing numerous cultivars with many replications can be costly and laborious. Furthermore, results generally represent only one set of environmental conditions. An alternative approach could be a system of experiments similar to those described by Freeman (6). Initially, cursory experiments are used to identify potential sources of resistance (i.e., resistant candidates). These simple experiments need little statistical analysis but can give important information for the design of further studies. Once potential sources of resistance are found, a formal hypothesis can be developed and tested. An advantage to this approach is that initial experiments have few replications and many treatments. In contrast, experiments testing formal hypotheses have few treatments and a greater number of replications.

In 1983, severe seedling disease caused by *P. arrhenomanes* permitted the evaluation of many sorghum cultivars for resistance in the field. The purpose of this paper is to report the reactions of several sorghum cultivars to *P. arrhenomanes* under controlled environmental conditions and to compare that reaction with the 1983 field evaluation. A formal hypothesis about a potential source of resistance was then tested statistically in a controlled experiment.

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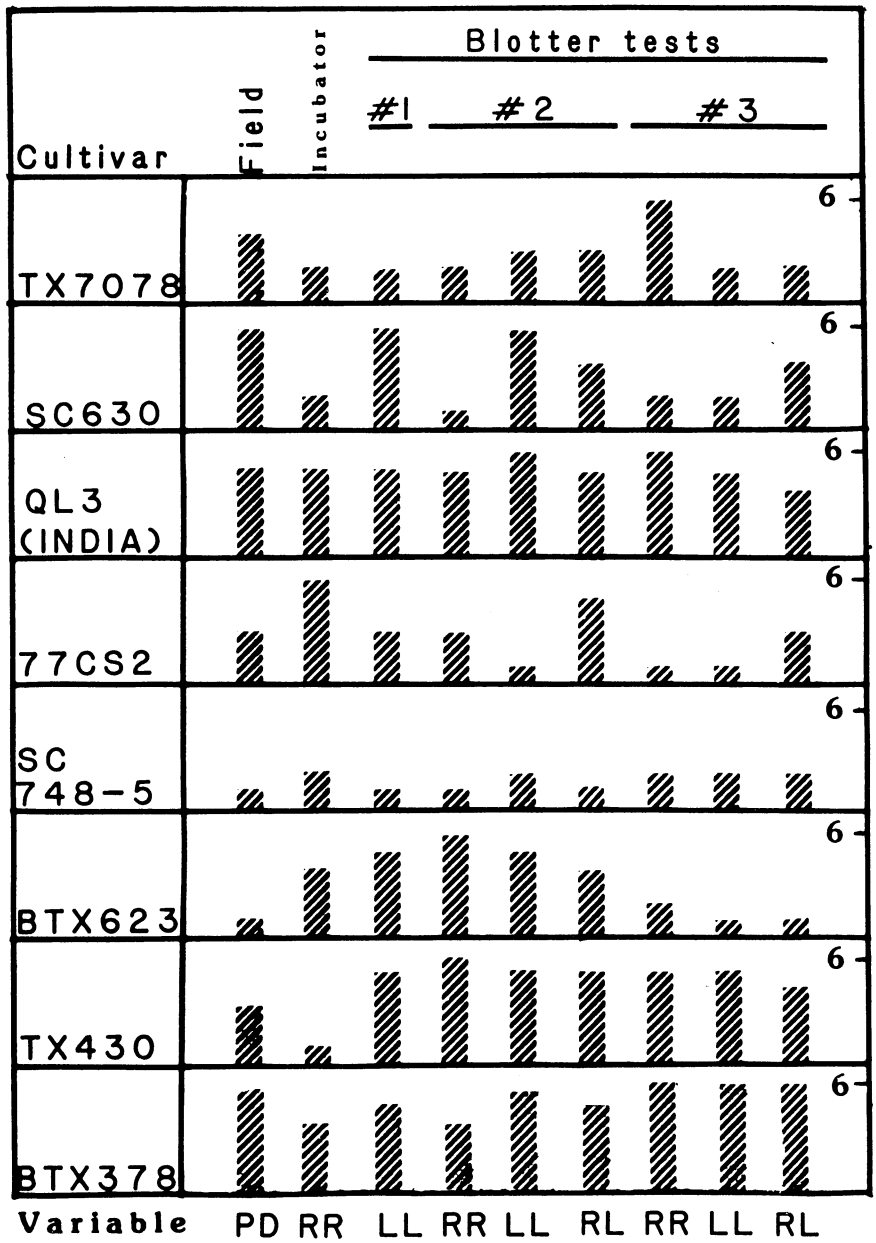

Fig. 1. Rankings of sorghum cultivars for seedling disease resistance. Ranks (1–6) are based on differences between inoculated seedlings and controls, except for the field assessment. PD = postemergence damping-off, RR = root rot, LL = leaf length, and RL = root length; blotter test 1 = isolate A (late infection), 2 = isolate B (early infection), and 3 = isolate B (late infection).

MATERIALS AND METHODS

Cursory tests. Eight sorghum cultivars (inbred lines) were evaluated for resistance to *P. arrhenomanes* in a sorghum disease nursery near La Ward, TX, by assessing percentage of post-emergence damping-off. Plot size was 6 m with 1-m row centers, and each cultivar was replicated two or four times. On the basis of symptoms, random isolations, and pathogenicity tests, we determined that *P. arrhenomanes* was the primary causal agent (5).

In one laboratory evaluation, growing seedlings were placed between two paper blotters (16) and inoculated with 0.5-cm plugs of 6- to 8-day cultures of *P. arrhenomanes*. The test was repeated three times under the following conditions: 1) isolate A of *P. arrhenomanes*, inoculated 3 days after seed germination; 2) isolate B of *P. arrhenomanes*, inoculated 3 days after seed germination; and 3) isolate B of *P. arrhenomanes*, inoculated at the time of seed germination. Isolates A and B originated from field-infected sorghum seedlings. Evaluations were made 10–14 days after seed germination and included one or more of the following measurements: leaf length, leaf dry weight, root length, and root rot based on a scale of 1–5. Cultivars were replicated two or three times. Each replicate consisted of one blotter containing six to 10 seedlings. Cultivars were also assessed in field soil within an incubator. Ten pregerminated seeds of each cultivar were sown 4.5 cm deep in 190-ml Styrofoam cups in either pasteurized or unpasteurized Houston Black Clay soil. Containers were paired (i.e., one pasteurized and one unpasteurized) in the incubator, which was adjusted to a day/night cycle of 20/10 C. The plants were watered daily for 7 days, then removed from the incubator to a greenhouse bench (20–30 C), where they were watered only when soil became dry and before drought symptoms appeared. Thirteen days after plants were removed from the incubator, leaf length, leaf dry weight, and percent emergence were measured.

Table 1. Results of *t* test comparisons between sorghum seedlings inoculated with *Pythium arrhenomanes* and uninoculated controls

	Blotter test 2[a]				Blotter test 3[b]			
	Root length		Leaf length		Root length		Leaf length	
Cultivar	Control	Infected	Control	Infected	Control	Infected	Control	Infected
Tx7078	9.51	4.73***[c]	7.02	5.93*	12.68	9.09***	8.47	7.04***
SC630-11E	8.88	4.51***	5.57	5.45^{ns}	11.91	8.57***	6.66	4.96***
QL3(India)	7.92	5.01***	5.35	5.42^{ns}	8.05	4.94***	5.68	4.70^{ns}
77CS2	7.35	4.99***	7.74	6.55*	8.91	5.60***	7.37	5.80**
SC748-5	11.87	4.04***	5.97	4.44**	12.17	8.72***	6.06	4.65***
BTx623	10.85	6.60***	6.64	6.72^{ns}	10.28	6.54***	6.76	5.25**
Tx430	9.27	6.94**	4.83	4.61^{ns}	10.84	7.77***	5.68	4.77^{ns}
BTx378	8.45	5.84***	6.17	6.32^{ns}	9.73	7.91**	7.60	7.13^{ns}

[a] Test 2 = inoculation 3 days after seed germination (late infection).
[b] Test 3 = inoculation at the time of seed germination (early infection).
[c] Significant at * = $P = 0.05$, ** = $P = 0.01$, and *** = $P = 0.001$; ns = not significant.

The objective of these cursory assessments was to identify potential sources of resistance for further evaluation. Emphasis was placed on the overall reaction of cultivars in field, blotter, and incubator experiments rather than on the results of any one experiment. To identify overall resistance, cultivars were assigned ranks between 1 and 6. Ranks were based on percentage of damping-off in the field and one or more of the variables mentioned before for the blotter and incubator tests. Each rank represented 1 standard deviation from the mean value of the respective variable. For the blotter and incubator tests, ranks represent differences between inoculated seedlings and uninoculated controls. For blotter tests 2 and 3 (those involving *P. arrhenomanes* isolate A), *t* tests were performed. Inoculated seedlings were compared with uninoculated seedlings for significant differences in leaf length and root length. Cultivars were compared separately.

Testing the hypothesis of resistance. The hypothesis of resistance in QL3(India) relative to SC748-5 was tested by the blotter technique (16). After pregermination for 48 hr at 25 C to facilitate the elimination of nonviable or moldy seed, three clean seedlings of each cultivar were placed in a blotter and maintained at 23 C with 12 hr of light per day. Numerous blotters were prepared to allow for further selection. After 24 hr, 12 blotters were chosen for each cultivar on the basis of lack of fungal contamination and uniformity of seedling radicle and plumule length. Six blotters were used as controls, and seedlings in the other six were inoculated with *P. arrhenomanes* isolate A. Inoculum, consisting of a 0.5-cm plug from a 5-day culture on cornmeal agar, was placed at the radicle tip, about 3 cm below the seed. The six replicates of inoculated seedlings and controls were randomly arranged in plastic trays 30 × 16 cm. Ten days after inoculation, resistance was assessed by measuring leaf length, leaf dry weight, and root dry weight. Replicate means (i.e., mean of three seedlings in a blotter) were analyzed by analysis of variance.

RESULTS

The results of the field, incubator, and blotter tests have been summarized in a comparison of resistance ranks (Fig. 1). Several cultivars reacted inconsistently across all tests, appearing resistant in some and susceptible in others. QL3(India) and BTx378 were resistant in all tests, whereas SC748-5 was consistently susceptible. On the basis of *t* tests, differences in root length between uninoculated and inoculated seedlings were significant for all cultivars in both blotter tests (Table 1). QL3(India), Tx430, and BTx378 were the only cultivars for which differences in leaf length were not significant in both tests. Differences in leaf length were not significant for SC630-11E and BTx623 in test 2 but were significant in test 3, which was more severe because of early inoculation. On the basis of these results, QL3(India) and SC748-5 were chosen as resistant and susceptible candidates, respectively.

In the final blotter test, infection by *P. arrhenomanes* caused visible reductions in leaf length of cultivar SC748-5 but not of QL3(India). Lesions on roots of SC748-5 extended acropetally up from the point of inoculation (3 cm below the seed), advancing almost to the seed. Lesions were dark brown and water-soaked. On QL3(India), lesions were much smaller and often delineated by a bright red ring around the root. Lateral root growth was profuse above the lesions on QL3(India) but not on SC748-5.

The effect of greatest importance in the blotter test was the cultivar × treatment interaction (Table 2). The significance of this interaction indicates that cultivars did not react equally to inoculation (i.e., one was more susceptible). This interaction was significant for both leaf length ($P = 0.01$) and leaf dry weight ($P = 0.04$) but not for root dry weight ($P = 0.18$). These interactions are clearly represented in the graphic display of the means (Fig. 2). A slight interaction is apparent for root dry weight, although its level of statistical significance is greater than $P = 0.05$. The important feature of all three variables measured was that infection by *P. arrhenomanes* caused greater reductions with SC748-5 than with QL3(India). For SC748-5, reductions in leaf length, leaf dry weight, and root dry weight were 51, 43, and 42%, respectively. Reductions for same variables for QL3(India) were 10, 6, and 15%, respectively.

DISCUSSION

Resistance to *P. arrhenomanes* was identified in sorghum cultivar QL3(India) relative to susceptible cultivar SC748-5. Although popularized by India, QL3 (India) is actually an Australian genotype that has high levels of resistance to sorghum downy mildew (*Peronosclerospora sorghi* (Weston & Uppal) Shaw) and maize dwarf mosaic virus (MDMV). Recently, Pawar et al (14) showed that four other cultivars of similar geographic origin to QL3(India) also have high levels of resistance to *P. sorghi*. Future attempts to locate sources of resistance to *Pythium* spp. should involve these Australian cultivars. Tx430 and BTx378 were resistant in all or most of the preliminary tests and may serve as other sources of resistance genes.

We are aware of no other reports of resistance in grain sorghum seedlings to *Pythium* spp. Nonetheless, cultivars have been selected for rapid germination and emergence at low temperatures (17). These traits are generally assessed in the field or in pathogen-free greenhouse tests. Field testing does not provide consistent or uniform exposure of seedlings to pathogens. Furthermore,

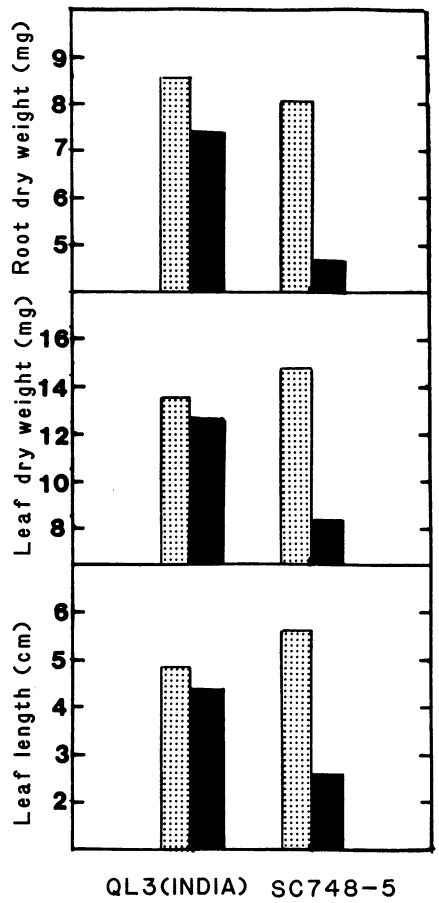

Fig. 2. Comparisons of inoculated and uninoculated (control) seedlings of sorghum cultivars QL3(India) and SC748-5. Solid = inoculated and dotted = control.

Table 2. Analysis of variance for a blotter test comparing levels of resistance in sorghum cultivars QL3(India) and SC748-5 with *Pythium arrhenomanes*

		Mean squares		
Source	df	Leaf length	Leaf dry weight	Root dry weight
Model	3	8.944**[a]	0.045*	0.019**
Cultivar (C)	1	1.042	0.013	0.017*
Treatment (T)	1	17.002***	0.076*	0.033**
C × T	1	8.640**	0.047*	0.007
Error	20	1.098	0.010	0.003

[a] Significant at * = $P = 0.05$, ** = $P = 0.01$, and *** = $P = 0.001$.

rapid germination or emergence at low temperature is not always related to seedling disease resistance. Bird (1) found that resistance in cotton seedlings to *Pythium* spp. and *Rhizoctonia solani* Kühn was related to slow germination and emergence.

Our experiences with sorghum seedling disease indicate that leaf length is a better measurement variable for resistance screening than leaf dry weight, root dry weight, or root length. Any measurements on the roots are hampered by the necessity of removing them from soil, potting medium, or blotters. Leaf dry weight does not seem to afford any advantages that compensate for the labor involved. Furthermore, the use of uninfected controls for each cultivar corrects for intercultivar variation in the leaf weight/length ratio. On the basis of the significance of models (Table 2), leaf length was the best variable for distinguishing between a susceptible and resistant cultivar.

Initial experiments were useful indicators of resistance, when considered collectively, although statistical analyses of leaf length, leaf dry weight, and root dry weight data from individual cursory experiments were generally not significant in analysis of variance models. This was probably due to the low number of replications and/or the high degree of intracultivar variability. The latter was especially high in the incubator study, which relied on field soil as a source of inoculum. The blotter test gave more consistent results within experiments, although intracultivar variability was high enough to negate efforts to find statistically significant differences among cultivars with two or three replications. We do not believe that this variability reflects genetic heterogeneity within cultivars but rather that it arises from sources extraneous to the seed, such as the high level of seed contamination often associated with grain sorghum. This problem was circumvented in the final comparison of QL3(India) and SC748-5 by pregermination followed by two selections of moldfree seedlings. An alternative approach might involve seed produced in a greenhouse or in very dry areas, where molding is minimal. Regardless of the technique used, variability in seed quality must be controlled to effectively assess resistance to soilborne seedling pathogens.

We found QL3(India) resistant to two isolates of *P. arrhenomanes* under different test conditions. This resistance may not be expressed against other isolates of *P. arrhenomanes* or against other species of *Pythium*. Kilpatrick (10) found an interaction between cultivars of wheat, barley, and oats and isolates of *Pythium*. Hooker (8), in contrast, concluded that one pathogenic isolate of *Pythium* sp. was sufficient to identify general resistance in maize seedlings based on the reactions of 40 maize cultivars.

Two characteristics of QL3(India) were observed that may be associated with mechanisms of resistance: 1) smaller lesions (relative to SC748-5) delineated by pigmented bands and 2) production of secondary lateral roots above the point of inoculation. On SC748-5, lesions often progressed upward from the point of infection (3 cm below the seed), almost reaching the seed. Infected SC748-5 seedlings produced fewer lateral roots than infected QL3(India) seedlings, although no difference could be detected between cultivar controls. Smaller lesions and continued lateral root production in QL3(India) may be related to a containment of fungal colonization of the root tissue. Impedance of internal spread of *Pythium* spp. has been proposed as a component of cultivar resistance (4).

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