Genetics of Resistance to *Bipolaris setariae* in Pearl Millet

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ABSTRACT

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In the summer of 1983 *Bipolaris setariae* was identified as the organism causing a previously unobserved severe leaf spot on pearl millet on all the plants in a single plot and a few plants in a second plot in a breeding nursery at Tifton, GA. These plants originated from the fourth selfed generation of the fourth backcross to Tift 23DB pearl millet of an original Tift 23DB × *Pennisetum glaucum* subspecies *monodii* cross. Genetic studies on F_1, F_2, F_3, and testcross progenies showed the data best fit a 195:61 resistant to susceptible ratio and that resistance was controlled by dominant gene action. The 195:61 inheritance ratio is characteristic of a four-independent-gene system with duplicate dominant resistance genes, one inhibitory gene, and one anti-inhibitory gene. The gene symbols Bp_1 Bp_1 Bp_2 Bp_2 (duplicate genes), bp_3 bp_3 (inhibitory gene), and bp_4 bp_4 (anti-inhibitory gene) were assigned.

Additional keywords: disease resistance, Helminthosporium, inheritance, leaf spot.

In the summer of 1983 a severe leaf spot associated with *Bipolaris setariae* (Saw.) Shoemaker was observed on all the plants in one plot and on a number of plants in a second plot of pearl millet, *Pennisetum glaucum* (R.) Br. in a breeding nursery at the University of Georgia Coastal Plain Experiment Station, Tifton. The severely affected plants had large elliptical to oblong lesions with considerable necrosis, whereas the remaining pearl millet plants in the nursery and nearby fields either had no leaf spot damage or no more than the usual amount of mild to moderate leaf flecking commonly associated with *B. setariae* in the Tifton area. Affected plants were from advanced breeding lines that carried immunity to rust, *Puccinia substriata* Ell. & Barth. var. *indica* Ramachar & Cumm. and Pyricularia leaf spot caused by *Pyricularia grisea* (Cke.) Sacc. (6). *Bipolaris setariae* causes a common leaf spot of minor importance on pearl millet, but it is sometimes more serious as a seedborne pathogen and seedling blight on this host (1,9–14). It also has been reported as a pathogen of minor importance on a number of other Gramineae (8,11). Studies reported in this paper were undertaken to determine if the indigenous *B. setariae* could cause this severe leaf spotting and to determine the genetics of inheritance of the increased susceptibility to the pathogen.

MATERIALS AND METHODS

The susceptible plants that were originally observed to have the severe Bipolaris leaf spot in the field were of the fourth selfed generation (S_4) of the fourth backcross (BC_4) to Tift 23DB (2,3) of an original Tift 23DB pearl millet × *P. glaucum* (L.) R. Br. subspecies *monodii* (Maire) Brunken cross. We selfed susceptible plants and crossed them both as male and female with Tift 23B. Pollination was controlled by bagging inflorescences before stigmas were exerted. Because of disease severity in the field, selfed and hybrid seed on the susceptible plant in the field did not mature and germinate. The seeds produced from the crosses on 23B females were viable and were used to produce the F_2, F_3, and testcross generations reported in this study.

The F_1 and F_2 generations were grown in the field. Selfed seeds from each plant in each generation were grown in the greenhouse to determine reaction to *B. setariae*. Testcrosses were made in the greenhouse using remnant seed to establish susceptible plants. Numbers of progeny observed in each F_3 segregating line (Table 1) ranged from 30 to 50 plants.

Plants in the greenhouse were established (20–40 seedlings/pot) and maintained in 16-cm plastic pots containing equal parts, v/v/v, coarse building sand, peat, and vermiculite amended with agricultural gypsum 8.8 g/L and Osmocote (3–4 mo release formulation) 14.0-6.1-11.6% (N-P-K) fertilizer (Sierra Chemical Company, Milpitas, CA) at 5.2 g/L. Moisture was maintained with an automatic, timed drip irrigation system. Ten isolates of *B. setariae* derived from single conidia isolated from pearl millet seed grown approximately 6 km from the area showing the severe leaf spot symptoms were used as inoculum. Each of the 10 isolates of *B. setariae* were grown on 20% V-8 juice agar in 9-cm-diameter petri dishes for 7 days under continuous cool-white fluorescent light (approximately 60 μEs^{-1} m^{-2} constant daylight fluorescent light), wounded by scraping off the aerial mycelia and then returned under light for 3 days. Lids were removed from dishes and cultures were allowed to dry until agar was brittle. Conidia and mycelial fragments were scraped from the surface. Conidia and mycelial fragments of the 10 isolates were combined by lightly stirring with a rubber policeman, sealed in 25- × 25-mm plastic bags, and placed in an ultra low freezer at −73 C. More than adequate inoculum was produced and stored before initiation of the tests to perform all of

TABLE 1. Expected (4) and observed genetic ratios for F_3 segregating lines for reaction to *Bipolaris setariae* in pearl millet

F_3 Ratio[w]	Frequency/666 F_3 Lines			
	Expected from			
	3:1 F_2	193:63 F_2	195:61 F_2	Observed
1:0	167	174	127	78
15:1	0	0	73	35
3:1[x]	332	328	244	368
9:7[y]	0	0	62	80
1:3[z]	0	15	78	37
1:15	0	31	0	0
1:63	0	21	0	0
0:1	167	97	82	68
Total Lines	666	666	666	666

[w] Resistant:susceptible theoretical ratios.
[x] Includes 45:19, 193:63, 195:61, 49:15, and 13.3 ratios.
[y] Includes 9:7 and 39:25 ratios.
[z] Includes 1:3, 15:49, and 3:13 ratios.

TABLE 2. F_2 inheritance data from a Tift 23B × susceptible cross for resistance to *Bipolaris setariae* in pearl millet

F_2[z] Family	Total plants observed		Goodness to fit to theoretical ratios[y]			
			3:1		195:61	
	Resistant	Susceptible	X^2	P value	X^2	P value
13347	330	119	0.54	0.30–0.50	1.77	0.10–0.20
13348	282	102	0.51	0.30–0.50	1.61	0.20–0.30
13339	408	146	0.54	0.30–0.50	1.95	0.10–0.20
13350	341	94	2.67	0.10–0.20	1.18	0.20–0.30
13351	319	81	4.81	0.02–0.05	2.78	0.05–0.10
13352	305	104	0.04	0.80–0.90	0.58	0.30–0.50
Composite	1,985	646	0.28	0.50–0.70	0.83	0.30–0.50

[y] Resistant:susceptible. Each X^2 value has 1 df.
[z] Each number represents a separate resistant × susceptible cross.

TABLE 3. Testcross data for resistance to *Bipolaris setariae* in pearl millet from susceptible × F_1 of Tift 23B × susceptible cross

1986 Plot	Total plants observed		X^2[z] 9:7	P value
	Resistant	Susceptible		
556	39	34	0.24	0.50–0.70
558	41	43	1.89	0.10–0.20
562	32	16	2.12	0.10–0.20
563	50	46	0.68	0.30–0.50
565	8	9	0.58	0.30–0.50
566	10	16	3.34	0.05–0.10
575	18	6	3.43	0.05–0.10
581	9	10	0.61	0.30–0.50
Composite	207	180	1.19	0.20–0.30
Heterogeneity X^2 (7 df) = 11.70, P = 0.10–0.20.				

[z] Resistant:susceptible ratio.

the required inoculations conducted during the period of several years. At time of use, the inoculum packets were retrieved and placed directly into a hot water bath at 40 C for 4 min, comminuted with deionized water in a blender, and atomized to runoff onto foliage of test plants. Inoculum concentrations were approximately 400 conidia/ml. Plants were exposed to intermittent fog in a humidity chamber for approximately 20 hr (1700 hours to 1300 hours the following day) and returned to the greenhouse bench. Plants in the greenhouse were 4 wk old at time of inoculations. Isolations also were made from inoculated leaves with small flecks only and from inoculated leaves with symptoms typical of the severe symptoms noted in the field. Spores from single spore isolates reisolated from both reaction types were used separately to inoculate Tift 23B.

Disease ratings on a score of 0–5 were made 7 days after inoculation. Ratings were 0 = no symptoms; 1 = small dark flecks; 2 = moderate size dark flecks; 3 = small elliptical spots; 4 = numerous large elliptical spots; and 5 = large elliptical spots with most leaves dead. Plants receiving a rating of 0–2 were considered resistant and plants receiving ratings of 3–5 were considered susceptible. F_2 and testcross data were analyzed by chi-square tests to determine goodness-of-fit to various theoretical ratios (5). Heterogeneity chi-square tests (7) on F_2 progeny within F_2 families in Table 2 showed good agreement among progeny so data shown are total of five to eight progeny within each family. All data were analyzed using a specially developed CHISQA program (5).

RESULTS AND DISCUSSION

Pathogenicity. Field symptoms of the severe leaf spot first observed in 1983 consisted of numerous elliptical to oblong leaf spots that were brown or had a gray center with brown margins. The gray centers occurred primarily in the older leaf spots. Leaf spots varied in width from 3 to 7 mm with most of the spots being from 5 to 7 mm wide. They varied in length from slightly more than width up to three times their width. On older leaves, leaf spots were numerous and coalesced, resulting in tip-burn and dying. Affected plants died before seed matured. In greenhouse inoculations Tift 23B plants showed only slight to moderate flecking with no elliptical spots. No tip-burn or dying of leaves was observed on inoculated Tift 23B. Segregating populations had two distinct types of reactions: resistant plants that had no more than moderate fleckings and were rated a 2 or less and susceptible plants with large elliptical leaf spots that rated a 4 or 5 seven days after inoculation. The intermediate-type reaction that would have been rated a 3 was not observed. The *B. setariae* reisolated from both the fleck and elliptical leaf spots caused only dark flecking when used to inoculate additional Tift 23B plants. The normally occurring endemic biotype of *B. setariae* isolated from seed of pearl millet is capable of causing both the severe leaf spot and mild dark fleck symptoms, thus the severe leaf spot is apparently a result of a change in the host.

Inheritance. F_1 plants from resistant × susceptible crosses were resistant to *Bipolaris* leaf spot in the field and when inoculated in the greenhouse.

Chi-square tests on F_2 data for goodness-of-fit to various theoretical ratios showed a good fit to a 3:1, 195:61 (Table 2), 49:15, and 193:63 ratios for resistant to susceptible genotypes. Data and goodness-of-fit for chi-square are reported in Table 2 only for 3:1 and 195:61 theoretical ratios. The reaction of the F_1 hybrids to the disease indicates dominant gene action for resistance. The good fit of the data to the various theoretical ratios was expected, since the 49:15, 195:61, and 193:63 ratios all approximate a 3:1 ratio. Testcross segregation ratios can be used to distinguish between the 3:1 and 49:15 F_2 ratios because they should give 1:1 and 5:3 testcross ratios, respectively (4). Six of the eight testcross progenies in Table 3 showed a good fit ($P \geqslant 0.05$) to a 1:1 ratio and none showed a good fit ($P \leqslant 0.05$) to the 5:3 ratio (chi-square data not reported). On the basis of the testcross data we eliminated the 49:15 ratio as a basis for explaining the inheritance to *B. setariae*. All eight testcross progenies showed a good fit ($P \geqslant 0.05$) to a 9:7 theoretical ratio (Table 3). A 9:7 testcross ratio would be expected from 195:61 and 193:63 theoretical F_2 ratios (4). Because the testcross data showed a good fit to a 9:7 ratio and a relatively good fit to a 1:1 ratio it was necessary to study the segregation behavior of F_3 segregating lines to establish whether resistance to *B. setariae* was being controlled by a single gene or by a four-gene system. Data in Table 1 indicated that resistance was not controlled by a single gene. In a single gene system selfed susceptible F_2 plants should breed true, but 35% of the susceptible F_2 plants segregated. Consequently, the observed frequencies of truebreeding classes were also quite different than what would be expected from a 3:1 ratio. Although it was not possible to obtain an exact frequency of each ratio observed because of the 30–50 plant progenies studied, it was possible to determine frequencies of various groups of ratios. As an example, 37 of the 666 plants studied segregated in a frequency that showed a good fit to the 1:3, 3:13, and 15:49 group. Larger plant progenies or a progeny test of each plant would be needed to distinguish among each of the three ratios which approximated 1 resistant: 3 susceptible plants. The resistant: susceptible segregation in this latter group of plants is characteristic of 195:61 and 193:63 ratios with the 3:13 and 15:49 F_3 ratios (observed for those progeny) only characteristic of a 195:61 ratio. No 1:15 and 1:63 ratios were observed for those progeny,

which is characteristic only of a 193:63 F_2 ratio. Eighty (12%) of the 666 total plants showed a good fit to the 9:7 and 39:25 F_3 ratio group which are characteristic only of a 195:61 ratio. Theoretically, 9.4% (4) of the segregating genotypes should have been of these two classes. It was not possible to distinguish between these two ratios because of the similarity of the ratios and the population size we used. The 9:7 and 39:25 F_3 ratios are strong supporting evidence that resistance to *B. setariae* is controlled by a 195:61 ratio four-gene system. The 9:7 and 39:25 F_3 ratios do not occur in a 193:63 ratio system.

A 195:61 ratio is indicative of a four-gene system with duplicate dominant resistance genes, one inhibitory gene, and one anti-inhibitory gene. The duplicate loci are Bp_1 and Bp_2, while Bp_3 is the inhibitory locus and Bp_4 is the anti-inhibitory locus. Genotypes with Bp_1 ___ and/or Bp_2 ___ plus Bp_3 ___ ___ ___ or bp_3 bp_3 bp_4 bp_4 are resistant to *B. setariae*. Genotypes bp_1 bp_1 bp_2 bp_2 ___ ___ ___ ___ and Bp_1 ___ and/or Bp_2 ___ bp_3 bp_3 Bp_4 ___ are disease susceptible. Homozygous bp_3 (inhibitory gene) cancels the dominant effects of Bp_1 ___ and/or Bp_2 ___ while homozygous bp_4 (anti-inhibitory gene) cancels the inhibitory effects of bp_3 bp_3. Susceptible phenotypes with Bp_1 ___ and/or Bp_2 ___ bp_3 bp_3 Bp_4 bp_4 would segregate for resistance.

It is likely that genes for increased susceptibility were donated by each parent, since neither parent had shown symptoms of severe leaf spot. Tift 23B probably contributed one of the duplicate loci and was homozygous for either Bp_1 or Bp_2, Bp_3, and bp_4 since this inbred has been selfed for a number of generations and never produced any susceptible plants. The open-pollinated wild *monodii* subspecies probably had the genotype Bp_1 ___ or Bp_2 ___ plus Bp_3 bp_3 Bp_4 ___. The complex four-gene inheritance system controlling resistance to *B. setariae* in a cross-pollinated species such as pearl millet is probably the reason this leaf spot is of minor significance on this crop plant.

LITERATURE CITED

1. Bhowmik, T. P. 1972. *Bipolaris setariae* on two new hosts in India. Indian J. Phytopathol. 25:590-591.
2. Burton, G. W. 1969. Registration of pearl millet inbreds Tift 23B, Tift 23A, Tift 23DB, and Tift 23 DA1. Crop Sci. 9:397-398.
3. Burton, G. W. 1981. Registration of pearl millet inbreds Tift 23 DBE, Tift 23 DAE, and Tift 756. Crop Sci. 21:804.
4. Deokar, A. B., and D'Cruz, R. 1962. Interactions involving three or more pairs of factors. Poona Agric. Coll. Mag. (India) 52:29-31.
5. Hanna, W., Mullinix, B., and Grimes, L. 1978. Computer programs for analysis of inheritance and linkage data. Crop Sci. 17:517.
6. Hanna, W. W., Wells, H. D., and Burton, G. W. 1985. Dominant gene for rust resistance in pearl millet. J. Hered. 76:134.
7. LeClerg, E. L., Leonard, W. H., and A. G. Clark, A. G. 1966. Field Plot Technique. Burgess Publishing Company, Minneapolis, MN. 373 pp.
8. Luttrell, E. S., Harris, H. B. and Wells, H. D. 1974. Bipolaris leaf blight of *Panicum fasciculatum*: Effects of host age and photoperiod on susceptibility. Phytopathology 64:476-480.
9. Misra, A. D., Prakash, O., and Mishra, B. 1974. An eye spot disease of bajra caused by *Helminthosporium sacchari* from India. Indian J. Phytopathol. 27:101-102.
10. Shetty, H. S., Mathur, S. B., Neegaard, P., and Safeeulla, K. M. 1982. *Drechslera setariae* in Indian pearl millet seeds, its seed-borne nature, transmission, and significance. Trans. Br. Mycol. Soc. 78:170-173.
11. Sprague, R. 1950. Diseases of Cereals and Grasses in North America. The Ronald Press Company, New York. 538 pp.
12. Wells, H. D. 1967. Effects of temperature on pathogenicity of *Helinthosporium setariae* in seedlings of pearl millet, *Pennisetum typhoides*. Phytopathology 57:1002.
13. Wells, H. D., and Burton, G. W. 1967. *Helminthosporium setariae* on pearl millet, *Pennisetum typhoides*, as affected by age of host and host differences. Crop Sci. 7:621-622.
14. Wells, H. D., and Winstead, E. E. 1965. Seed-borne fungi in Georgia-grown and Western-grown pearl millet seed on sale in Georgia during 1960. Plant Dis. Rep. 49:487-489.