Pathogenic Variation in *Puccinia substriata* var. *indica* in the Southeastern United States and Screening for Resistance in Pearl Millet Germ Plasm

H. Tapsoba, Department of Plant Pathology, University of Georgia, Athens 30602; and **J. P. Wilson**, Research Plant Pathologist, USDA-ARS Forage and Turf Research Unit, University of Georgia Coastal Plain Experimental Station, Tifton 31793-0748

ABSTRACT

Tapsoba, H., and Wilson, J. P. 1996. Pathogenic variation in *Puccinia substriata* var. *indica* in the southeastern United States and screening for resistance in pearl millet germ plasm. Plant Dis. 80:395-397.

Rust caused by *Puccinia substriata* var. *indica* can be very damaging on pearl millet in the southeastern United States. Breeding rust-resistant pearl millet cultivars is complicated by unknown pathogenic variation in the pathogen population. In the present study, 15 single-uredinial isolates of the pathogen were evaluated for pathogenic variation on seedlings of 29 resistant pearl millet germ plasm lines. Eleven races were identified based on seedling reactions. The reaction of the races on the germ plasm lines ranged from virulence to 15 lines to virulence to all 29 lines. Although some lines were susceptible to all races, others were resistant to as many as seven races and constitute new potential sources for genetic resistance. Field studies conducted in 1993 and 1994 indicated that most of the 11 races are probably not yet predominant in the pathogen population at Tifton, Georgia, since some lines susceptible to many races showed low levels of disease in the field. These races will be important as tools for future screening in pearl millet germ plasm for additional sources of resistance to *P. substriata* var. *indica*.

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is increasingly recognized as a high quality annual forage and grain crop for the southeastern United States (1,2,11). In this region, however, rust caused by *Puccinia substriata* Ellis & Barth. var. *indica* Ramachar & Cummins has occurred annually since 1972 (10) and become an important limiting factor for production. Eggplant (*Solanum melongena* L.) and a few other *Solanum* species can serve as alternate hosts of the pathogen (7,12). However, the importance of the sexual stage and the alternate hosts in the epidemiology of the disease in North America remains to be clarified (122). Some resistance sources have already been identified. Two named genes, Rr_1 in Tift 85DB (3) and Rpp_1 in ICML 11 (5) have already been incorporated into released germ plasm. Two additional dominant resistance genes were recently identified in two backcross derivatives of BF 122 and BF 201 from Burkina Faso (9). A recent shift of virulence in the pathogen population is, however, complicating breeding efforts. PS92-1, the first race virulent to Rr_1 in the U.S., was isolated a few years after the release of Tift 85DB (8). The present study was undertaken to investigate pathogenic variation in the pathogen population, evaluate various germ plasm lines of pearl millet for their usefulness as differential hosts, and identify potentially new sources of resistance in pearl millet germ plasm.

Corresponding author: H. Tapsoba

Cooperative investigation of USDA-ARS and the University of Georgia, College of Agriculture and Environmental Sciences, Agricultural Experiment Stations.

Accepted for publication 15 December 1995.

Publication no. D-1996-0202-05R

MATERIALS AND METHODS

Germ plasm lines used. Twenty-nine resistant pearl millet germ plasm lines including 22 backcross derivatives of pearl millet landraces, wild *P. glaucum* (L.) R. Br. subsp. *monodii* (Maire) Brunken and improved inbreds from Burkina Faso, Senegal, and India in a Tift 23DB background, and seven released resistant lines were used in this study. The breeding line Tift 23DB was used as the susceptible check. All backcross derivatives, as well as Tift 23DB, Tift 85DB, Tift $89D_2$, and Tift 8677, were developed at the USDA-ARS Forage and Turf Research Unit at Tifton, GA. ICML 11, ICMP 501, ICMP 83506, J104, and 700481-21-8 were developed in India at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Isolation procedures. Bulk inoculum consisting of urediniospores collected in the field in 1991 at Tifton, Georgia was used to inoculate seedlings of the 22 backcross derivatives in the greenhouse. Seedlings of each backcross derivative at the four- to five-leaf stage were inoculated with the bulk inoculum (approximately 30,000 urediniospores per ml) and placed in a mist chamber for 18 h in the dark at about 25°C. From plants showing a mixture of infection types, single-pustule isolations were made from selected susceptible uredinia and increased on Tift 23DB plants. The inoculum was then stored at −80°C until use.

Greenhouse screening. The 29 resistant pearl millet germ plasm lines were evaluated as differential hosts using the isolates collected during this study and PS92-1 (8). For each isolate, seedlings of each germ plasm line were grown in two 15-cm-diameter pots and inoculated with an aqueous suspension of urediniospores as described in the previous paragraph at the four- to five-leaf stage. Two weeks after inoculation, the plants were evaluated for infection type using the rating system of Stakman et al. (6). Because some lines still segregated for resistance during the first screening, susceptible plants (infection types 3 and 4) were rogued and the remaining plants were inoculated a second time. If at the last evaluation these plants still exhibited a resistant reaction (infection types 0, 1, 2) the germ plasm line was considered resistant to the isolate tested and the rust isolate thus considered avirulent on that line. Otherwise, the line was susceptible and the isolate, therefore, virulent. The entire process was conducted twice for each isolate and, when necessary, a third or fourth screening was performed to clarify ambiguous results.

Field evaluation. Field experiments were conducted to evaluate the resistance of the germ plasm lines to the natural population of the pathogen, and to estimate the relative predominance of the different races in that population. A randomized complete block design with six replications and 30 treatments (germ plasm lines) was used in two trials in 1993 and 1994 at Tifton, GA. In each block, the lines were planted on 22 July 1993 and 28 July 1994 in solid-seeded, 3-m long, single row plots spaced 0.9 m apart. The blocks were separated from each other by two spreader rows of Tift 23DB. In both years, the rust epidemic was initiated approximately a month after planting by inoculating the spreader rows with a bulk rust inoculum collected in the field in 1991 at Tifton. Although the inoculum used to

initiate the rust epidemics in both years was collected in 1991, the inoculation of the spreader rows was done after plants in the plots as well as plants in other pearl millet plots around Tifton were already infected by the naturally occurring inoculum of the pathogen. There was, therefore, no exclusion of the natural population of the pathogen as it existed in the field in 1993 and 1994 at Tifton. An aqueous suspension of the inoculum (approximately 30,000 urediniospores per ml) was applied to the foliage of the plants early in the evening with a hand sprayer. For each plot, the final rust severity was rated using standard diagrams (4) 6 weeks after 50% anthesis in the plot. All effects were considered fixed and analysis of variance was conducted after log(x+1) transformation, with the sums of squares separated into replication and treatment (germ plasm lines) effects. Differences in the means of final rust severities among the lines were determined by Fisher's least significant difference. The ranking of the lines over the two experiments was analyzed using Spearman's rank correlation.

RESULTS

Identified races of the pathogen. Fourteen isolates of the pathogen were collected from seven germ plasm lines. Based on the reactions to the differential set used, 10 new races of *P. substriata* var. *indica* were identified in addition to PS92-1. The new races are as follows: PS93-1 isolated from BF 122; PS93-2 from BF 221; PS93-3 from BF 296; PS93-4 and PS93-5 from BF 34; PS93-6 from BF 49; PS93-7, PS93-8, and PS93-9 from BF 137; and PS93-10 from PS 191. Five isolates from BF 221 all exhibited similar reactions to the 29 lines tested and were therefore considered to be multiple isolations of race PS93-2. Overall, the reactions of the 11 races ranged from virulence to 14 lines (PS93-2) to virulence to all lines (PS93-9) (Table 1). Of the 10 new races collected in 1993, none was identical to PS92-1. All were virulent to Rpp_1 in line ICML 11, and four races (PS93-1, PS93-2, PS93-5, and PS93-9) were virulent to Rr_1 in Tift 85DB. PS92-1 is virulent to Rr_1 and was likewise virulent to 15 germ plasm lines including ICML 11. PS92-1 and PS93-2 exhibited similar reactions on 25 of the 29 lines. PS93-1 was avirulent on BF 122 although it was isolated from that same germ plasm line.

The reactions of seedlings of the 29 lines ranged from susceptibility to all races to resistance to seven races. The lines 700481-21-8, ICML 11, and PS 191 were susceptible to all races tested. The lines BF 17 and BF 178 were each resistant to only one race. Some lines exhibited identical reactions across all races. BF 299 and PS 748 were both resistant to PS93-4, PS93-5, PS93-7, PS93-8, and PS93-10, and susceptible to all the others. Six lines (BF 34, BF 53, BF 137, BF 201, BF 296, PS 727) were resistant to PS92-1 and PS93-2 but susceptible to all other races. BF 156 and BF 334 differed from those six by being resistant to one additional race, PS93-1 and PS93-11, respectively. Three lines, BF 19, BF 337 and ICMP 83506, expressed identical reactions. Many other subsets of lines showed partial similarities in their reactions to one or more races. Of all the lines, BF 122 and PS 756 were susceptible to the fewest number of races.

Field evaluation. The final disease severity ranged from 0% on Tift $89D_2$ and J104 to about 43% on Tift 23DB in 1993, and from 7% on BF 17 and BF 53 to 93% on Tift 23DB in 1994 (Table 1). In both years, the rust severity on Tift 23DB was significantly higher than on any of the 29 lines. In 1993, 22 of the 30 lines had disease severity less than 10%. Due to conducive conditions in 1994, rust severity on all lines was higher than in 1993. How-

Table 1. Reaction of 30 pearl millet germ plasm lines to eleven races of *Puccinia substriata* var. *indica* in the greenhouse and rust severity in the field at Tifton, GA, in 1993 and 1994

		Reaction to race[a] (PS93-)											Final rust severity (%)[b]	
Resistance source		1	2	3	4	5	6	7	8	9	10	PS92-1	1993	1994
Tift 23DB		.	.	.	.	.	.	.	.	.	.	.	42.6	92.5
700481-21-8	Rel[c]	.	.	.	.	.	.	.	.	.	.	.	1.7	11.4
ICML 11	Rel	.	.	.	.	.	.	.	.	.	.	.	7.7	22.5
PS 191	BC_2	.	.	.	.	.	.	.	.	.	.	.	9.5	41.0
BF 17	BC_2	.	R	.	.	.	.	.	.	.	.	.	0.0	7.0
BF 178	BC_4	.	.	.	R	.	.	.	.	.	.	.	5.7	20.0
J104	Rel	.	R	R	.	.	.	.	.	.	.	.	0.0	22.0
BF 49	BC_2	.	.	.	.	.	.	R	.	.	.	R	3.4	13.0
BF 34	BC_2	.	R	.	.	.	.	.	.	.	.	R	0.4	25.0
BF 53	BC_3	.	R	.	.	.	.	.	.	.	.	R	1.4	7.0
BF 137	BC2	.	R	.	.	.	.	.	.	.	.	R	13.1	46.5
BF 201	BC_3	.	R	.	.	.	.	.	.	.	.	R	1.0	8.0
BF 296	BC_3	.	R	.	.	.	.	.	.	.	.	R	1.7	11.0
PS 727	BC_3	.	R	.	.	.	.	.	.	.	.	R	0.6	18.0
BF 19	BC_4	.	R	.	R	.	.	.	.	.	.	R	4.4	23.0
BF 337	BC_3	.	R	.	R	.	.	.	.	.	.	R	3.8	14.0
ICMP 83506	BC_2	.	R	.	R	.	.	.	.	.	.	R	2.3	12.7
BF 156	BC_3	R	R	.	.	.	.	.	.	.	.	R	2.7	28.0
BF 334	BC_2	.	R	.	.	.	.	R	.	.	.	R	2.2	19.0
ICMP 501	Rel	.	.	R	.	.	R	R	.	.	.	.	0.5	17.0
PS 202	BC_3	.	.	R	.	R	R	.	.	.	.	.	0.5	17.0
BF 221	BC_3	.	.	R	.	.	R	R	R	.	.	.	21.2	74.0
BF 41	BC_2	.	R	.	.	R	R	R	R	.	.	.	9.3	27.5
BF 299	BC_2	.	.	.	R	R	.	R	R	.	R	.	23.3	70.0
PS 748	BC_1	.	.	.	R	R	.	R	R	.	R	.	5.2	36.0
Tift 8677	Rel	.	.	.	R	.	.	R	R	.	R	R	21.2	78.0
Tift $89D_2$	Rel	.	R	R	.	R	R	.	.	.	.	R	0.0	24.0
Tift 85DB	Rel	.	.	R	R	.	R	R	R	.	R	.	24.0	75.0
BF 122	BC_2	R	.	R	R	R	R	R	R	.	.	.	25.8	80.5
PS 756	BC_2	.	.	R	R	R	R	R	R	.	R	.	23.9	81.5
LSD ($P = 0.01$)													2.6	12.6

[a] . = susceptible line; R = resistant line.
[b] Rust severity evaluated 6 weeks after 50% anthesis.
[c] Rel = released line; BC_n = n^{th} backcross to Tift 23DB.

ever, the ranking of the lines over the 2 years was not significantly affected (r_s = 0.85, P = 0.0001) by environment or pathogen race. Tift 85DB had about 24 and 75% disease severity in 1993 and 1994, respectively. ICML 11 had less than 8% final rust severity in 1993, whereas in 1994 its disease severity reached more than 20%. BF 17, BF 49, BF 53, BF 178, BF 201, BF 296, PS 727, and 700481-21-8 were all resistant to two or fewer races but disease severity on any of them did not exceed 20% in 1994. In contrast, some lines that were resistant to many races had high final disease severities. BF 122 and PS 756, which were each resistant to seven races, were as susceptible as Tift 23DB in 1994. BF 299, PS 756, and Tift 85DB all showed more than 20 and 70% rust in 1993 and 1994, respectively, despite being resistant to five or more races. BF 299 and PS 748, which exhibited identical reactions to the races, differed significantly for rust severity in both years. All lines resistant to PS92-1 and PS93-2, except for BF 137, showed less than 5 and 30% disease in 1993 and 1994, respectively. On average, lines susceptible to these two races had more than twice as much disease as lines resistant to the two races.

DISCUSSION

These results demonstrate that pathogenic variation in *P. substriata.* var. *indica* exists in the U.S. At least 10 new races were identified in addition to PS92-1. Although the number of isolates tested was relatively small, the identification of these new races confirms a future threat to pearl millet production. Races such as PS93-1 and PS93-9 can be very damaging if no effective resistance is found.

The lack of correlation between the greenhouse reactions and rust severity in the field may be explained by at least two factors. First, most races are not yet common in the natural population of the pathogen at Tifton. In such a situation, lines susceptible to rare races of the pathogen might show in the field lower disease levels than lines susceptible to the most common race(s). The isolation method used was selective and designed to isolate races that occur at very low frequencies. From the reactions of the germ plasm lines in the greenhouse and the field, it can be concluded that PS92-1 and PS93-2 are probably the dominant races in the current pathogen population at Tifton. Indeed, susceptibility to these two races generally resulted in higher disease severity. The second reason is the nature of the resistance exhibited. Greenhouse screening of seedlings may not detect lines with slow-rusting or adult plant types of resistance. Such lines, however, may have low rust severity in the field as was found with 700481-21-8 (10).

The fact that PS93-1 was avirulent on BF 122 from which it was isolated is probably due to segregation occurring in the line while the original isolates were being selected.

Rust resistance in pearl millet is available for use in breeding programs. Many lines evaluated in this study were resistant either in the greenhouse or field and may be useful sources of resistance to the pathogen. The races identified in this study and other races of the pathogen will be useful for resistance screening in pearl millet germ plasm collections. The development of monogenic differential hosts will be valuable in future studies of the variability in the pathogen. Given the pathogenic diversity in the pathogen population, any cultivar with single gene resistance would quickly select for virulence. Development of multilines or lines with two or more resistance genes may possibly create more durable resistance.

ACKNOWLEDGMENTS

We thank Andy Hornbuckle for technical assistance.

LITERATURE CITED

1. Burton, G. W. 1951. Quantitative inheritance in pearl millet (*Pennisetum glaucum*). Agron. J. 43:409-417.
2. Burton, G. W. 1983. Breeding pearl millet. Plant Breed. Rev. 1:162-182.
3. Hanna, W. W., Wells, H. D., and Burton, G. W. 1985. Dominant gene for rust resistance in pearl millet. J. Hered. 76:134.
4. Peterson, R., Campbell, A. B., and Hannah, A. E. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can. J. Res. 26:496-500.
5. Singh, S. D., Andrew, D. J., and Rai, K. N. 1987. Registration of ICML 11 rust-resistant pearl millet germplasm. Crop Sci. 27:367-368.
6. Stakman, W. W., Stewart, D. M., and Loegering, W. Q. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. U.S. Dep. Agric. Bull. Entomol. Plant Quar. Bull. E-617 rev.
7. Wells, H. D. 1978. Eggplant may provide primary inoculum for rust of pearl millet caused by *Puccinia substriata* var. *indica*. Plant Dis. Rep. 62:469-470.
8. Wilson, J. P. 1993. Identification of virulence in *Puccinia substriata* var. *indica* to Rr_1 in pearl millet. Plant Dis. 77:100.
9. Wilson, J. P. 1993. Two new dominant genes for rust resistance in pearl millet. (Abstr.) Phytopathology 83:1395.
10. Wilson, J. P. 1994. Field and greenhouse evaluation of pearl millet for partial resistance to *Puccinia substriata* var. *indica*. Plant Dis. 78:1202-1205.
11. Wilson, J. P., Gates, R. N., and Hanna, W. W. 1991. Effect of rust on yield and digestibility of pearl millet forage. Phytopathology 81: 233-236.
12. Wilson, J. P., and Phatak, S. C. 1995. Alternate host range of *Puccinia substriata* var. *indica*. (Abstr.) Phytopathology 85:1199.