Field and Greenhouse Evaluations of Pearl Millet for Partial Resistance to *Puccinia substriata* var. *indica*

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ABSTRACT

Wilson, J. P. 1994. Field and greenhouse evaluations of pearl millet for partial resistance to *Puccinia substriata* var. *indica*. Plant Dis. 78:1202-1205.

Pearl millet (*Pennisetum glaucum*) inbreds Tift 383, 700481-21-8, and ICMP 501 were evaluated for partial rust resistance in comparison to the susceptible inbred Tift 23DB. In field trials, area under the disease progress curves of the three inbreds were less than that of Tift 23DB when disease pressure was severe. Uredinium dimensions on seedlings 10 days after inoculation were smaller for the three inbreds than for Tift 23DB. Seedlings exhibited susceptible infection types when inoculated with four single-uredinium isolates except for a moderately resistant infection type 2 when ICMP 501 was inoculated with isolate PS89-775. Whole-plant latent periods of the three inbreds were longer than that of Tift 23DB when inoculated with a bulk rust collection, but longer only for ICMP 501 when inoculated with isolate PS92-1. No inbred was consistently more resistant than Tift 23DB across all evaluations. Tift 383, 700481-21-8, and ICMP 501 express low to moderate levels of partial resistance, which may be more consistent in expression and useful if they were combined.

Rust is an important late-season disease of pearl millet (*Pennisetum glaucum* (L.) R. Br.) in the southeastern United States. Eggplant (*Solanum melongena* L.), the alternate host of *Puccinia substriata* Ellis & Barth. var. *indica* Ramachar & Cummins (18), is grown in this region, but its importance in the epidemiology of the disease is not clear. At Tifton, Georgia, onset of disease appears to depend on the introduction of aerial-borne inoculum, and infection foci are usually first observed between late July and late August. Dry environmental conditions can limit epidemic development, but rainfall and humidity are often adequate during the fall for consistent infection by the pathogen. When adequate moisture exists, disease levels can continue to increase until plants are killed by frost. Digestible dry matter yield of forage (7,21) and grain yield (13) can be reduced by infection, limiting late-season productivity of forage hybrids and severely affecting grain yield of late-planted grain hybrids.

Accepted for publication 21 September 1994.

Tifleaf 2 was the first rust-resistant forage hybrid released in the United States (4). In 1991, inbreds used to produce the grain hybrid HGM 100 were released for commercial production (2). Both Tifleaf 2 and HGM 100 have the Rr_1 gene for resistance (3). Rr_1 was extremely effective in controlling the disease. However, a virulent biotype of *P. s. indica* was recently identified (20). By the 1993 season, a shift in virulence frequency occurred in the pathogen population, rendering both Tifleaf 2 and HGM 100 susceptible to rust (*unpublished*).

New sources of resistance are needed to reduce rust infection of commercial hybrids to acceptable levels. Heterogeneity for resistance in the host population appears to have potential for reducing the effects of rust in forages (22). A more comprehensive approach to managing the disease would include incorporating slow-rusting or partial resistance into improved inbreds to supplement the few known dominant genes for resistance. This gene-stacking approach should provide greater resistance than that conferred by dominant genes for qualitative resistance (23).

Classic slow-rusting resistance is often expressed as a susceptible infection type of reaction, with reduced infection frequency and uredinium size, longer latent period of infection, and reduced sporulation (23). Several putative slow-rusting pearl millets were evaluated by Sokhi and Singh (14). Slow-rusting selections had lower rust severities, fewer uredinia per unit of leaf area, smaller uredinia, and reduced urediniospore production in the field. Latent period did not differ among the slow- and fast-rusting pearl millets.

Problems exist with evaluating pearl millet for slow-rusting resistance. Although slow-rusting resistance is usually race nonspecific (1,6), little is known about the population structure of *P. s. indica* in the United States. Delayed increase of rust on putative slow-rusting lines in the field may be the result of race-specific resistance that is effective against only a portion of the pathogen population. This requires that greenhouse screening accompany field evaluations. In greenhouse evaluations, latent period differences are usually more pronounced on young flag leaves (8). Taylor and Mims (17) have determined that upper leaves of mature, susceptible pearl millet grown in the greenhouse express a partially resistant reaction, which complicates effective comparisons of latent period of infection and uredinium sizes on flag leaves. Although differences in resistance are often more pronounced on mature plants of other cereals, differences in resistance can also be detected to a lesser extent in immature plants (5,10). It may be possible to evaluate seedlings and modify the evaluation of latent period to identify slow-rusting resistance in pearl millet.

The following studies were conducted to determine if field and greenhouse evaluations of pearl millets selected as potential sources of partial resistance to *P. s. indica* were consistent and could be reliably used to screen additional germ plasm for resistance.

MATERIALS AND METHODS

Pearl millet inbreds Tift 383, 700481-21-8, and ICMP 501 were evaluated for partial rust resistance in comparison with

the susceptible inbred Tift 23DB. Tift 383 and Tift 23DB were developed in the United States at the USDA-ARS Forage and Turf Research Unit, and 700481-21-8 and ICMP 501 were developed in India at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Field evaluations. The inbreds were evaluated in the field at Tifton, Georgia, in 1992 and 1993. In 1992, six replications were planted in a randomized complete block on 16 July. Single-row plots were 3 m long, and the entire test area was surrounded by a rust-susceptible cultivar. In 1993, the four inbreds were planted on three dates (DOP): 13 and 29 July and 12 August, designated DOP-1, DOP-2, and DOP-3, respectively. Five replications of two-row plots 5 m long were planted in a randomized complete block design within each DOP. Tests were surrounded by a border of rust-susceptible Tifleaf 1 in 1992 and Tift 23DB in 1993.

Natural rust severities (percent foliage infected or killed by rust infection) were visually estimated at 7- to 10-day intervals beginning 28 September and ending 29 October in the 1992 evaluation. In 1993, rust severities were evaluated at weekly intervals beginning 15 September and continued until plots were killed by disease or by frost on 2 November. Date when approximately 50% of the panicles reached anthesis was recorded for each plot in both years.

Severity data for each plot were transformed to logits and regressed against days from anthesis by quadratic regression. Severities at 5-day intervals from anthesis to 30 days after anthesis were estimated from regression equations for the 1992 evaluation. In 1993, plants in each DOP were at different stages of growth when ratings began and ended. Rust severities were estimated from regression equations at 5-day intervals from anthesis to 30 days after anthesis for DOP-1, from −10 to to 20 days from anthesis for DOP-2, and from −20 to 10 days from anthesis for DOP-3.

Area under the disease progress curve (AUDPC) for the 30-day interval for each plot was calculated as AUDPC = $\Sigma[(Y_{i+5} + Y_i)/2] \times [X_{i+5} - X_i]$, where Y_i = estimated disease severity at day X_i. AUDPCs were analyzed by analysis of variance. In 1993, sums of squares were partitioned into DOP, replication within DOP, inbred, and DOP × inbred interaction. Means were differentiated by Fisher's LSD.

Greenhouse evaluations. Greenhouse temperatures during evaluations ranged from 35 C during the day to 24 C during the night. Plants were grown under natural light, averaging 7.4×10^4 lm/m^2 in midafternoon on cloudless days.

Uredinium dimensions of seedlings were evaluated twice, with six and 10 replications (pots with three to five seedlings) of each inbred for the two respective tests. Seedlings at the five-leaf stage were placed in an incubation chamber and misted to dripping with inoculum (2.5×10^4 urediniospores per milliliter) consisting of a bulk uredinio-spore collection taken from susceptible cultivars in the field in 1991. Plants were automatically misted with deionized water at 30-min intervals. Inoculation chamber temperatures averaged 16 C during the incubation intervals. After 18 hr of incubation in the dark, plants were allowed to dry and were returned to the greenhouse bench.

The third leaf of one seedling from each pot was removed 10 days after inoculation and preserved in lacto-phenol. Lengths and widths of 10 arbitrarily selected, isolated uredinia from each leaf were measured by a microscope with a calibrated eyepiece. Areas were calculated as (length × width × π)/4. Data were analyzed by analysis of variance with sums of squares partitioned into experiment, replication (leaf) within experiment, and inbred effects. Means were differentiated by Fisher's LSD.

Race specificity of inbred resistance was evaluated by inoculating approximately 25 seedlings at the four-leaf stage as previously described with either the bulk rust collection or single-uredinium isolates PS88-199, PS89-775, PS92-1, and PS92-2. These isolates have been determined to be pathogenically different (19,22). Infection types (16) were evaluated 10–13 days after inoculation. The test was repeated twice, with identical results.

Latent periods of adult plants were evaluated in two tests. Inbreds were grown in 12-cm-diameter pots. Plants with fully emerged flag leaves were inoculated as previously described with the bulk rust collection in the first evaluation and with PS92-1 in the second evaluation. An average of 36 and 25 plants of each inbred were examined in the first and second tests, respectively.

In both evaluations, the percentage of infection sites erupted into uredinia was estimated for the whole plant (generally six to seven leaves), rather than just the flag leaf. Beginning at 7 days after inoculation, estimates were made at 1- to 3-day intervals until all uredinia were determined to have erupted. If the maximum uredinium eruption was less than 100%, the data were corrected as a percentage of the maximum value determined.

Proportions of erupted uredinia were transformed to probits and regressed against days from inoculation. Whole-plant latent periods (T_{50}) were calculated by the method for calculating the T_{50} of flag leaves, as described by Shaner (12). T_{50} values within each experiment were analyzed by analysis of variance and means were differentiated by Fisher's LSD.

RESULTS

Field evaluations. Rust did not develop until late in the season in 1992. Final

Table 1. Area under the disease progress curve (AUDPC) of pearl millet inbreds evaluated for resistance to *Puccinia substriata* var. *indica* in the field at Tifton, Georgia

	AUDPC[y]			
		1993		
Inbred	1992	DOP-1	DOP-2	DOP-3
Tift 23DB	493 a[z]	766 a	814 a	255 b
Tift 383	551 a	239 b	348 b	607 a
700481-21-8	435 a	11 c	29 c	44 c
ICMP 501	162 b	225 b	271 b	167 b
LSD ($P = 0.05$)	153	165	84	96

[y] From anthesis to 30 days postanthesis for 1992 and 1993 DOP-1, from −10 to 20 days from anthesis for 1993 DOP-2, and from −20 to 10 days from anthesis for 1993 DOP-3. DOP = day of planting.
[z] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

Table 2. Uredinium dimensions on the third leaf of pearl millet seedlings 10 days after inoculation with a bulk collection of *Puccinia substriata* var. *indica* in the greenhouse

	Uredinium dimensions		
Inbred	Length (mm)	Width (mm)	Area (mm^2)
Tift 23DB	0.41 ± 0.21 a[z]	0.23 ± 0.10 a	0.09 ± 0.08 a
Tift 383	0.30 ± 0.13 b	0.16 ± 0.06 b	0.04 ± 0.03 b
700481-21-8	0.29 ± 0.13 b	0.18 ± 0.07 b	0.05 ± 0.04 b
ICMP 501	0.18 ± 0.10 c	0.11 ± 0.06 c	0.02 ± 0.02 c
LSD ($P = 0.05$)	0.03	0.02	0.01

[z] Means ± standard deviation within a column followed by the same letter do not differ significantly ($P = 0.05$).

disease severity as estimated from regression equations averaged 47% on Tift 23DB. ICMP 501 had a significantly lower AUDPC value than the other inbreds (Table 1). There were no differences among AUDPCs of Tift 383, 700481-21-8, and Tift 23DB.

Disease pressure was greater in the first two DOPs in 1993. Final severities on Tift 23DB estimated from the regression equations for DOP-1, DOP-2, and DOP-3 were 70, 95, and 40%, respectively. The low disease levels for DOP-3 reflect the stage of epidemic development when an early killing frost occurred on 2 November 93. The DOP × inbred mean square was significant ($P < 0.05$) in the analysis of variance, so AUDPCs were analyzed within each DOP. AUDPCs of Tift 383, ICMP 501, and 700481-21-8 were all less than that of Tift 23DB in the first two DOPs (Table 1). Only 700481-21-8 had an AUDPC less than that of Tift 23DB in DOP-3. Differences in resistance among the inbreds were most apparent in the 1993 DOP-1 and DOP-2 trials, when rust pressure was most severe.

Greenhouse evaluations. Uredinium dimensions 10 days after inoculation with a bulk rust collection were greater on Tift 23DB than the other inbreds (Table 2). Dimensions on Tift 383 and 700481-21-8 did not differ but were larger than those on ICMP 501.

In seedlings inoculated with different isolates of *P. s. indica*, a moderately resistant infection type 2 was observed when ICMP 501 was inoculated with isolate PS89-775 (Table 3). All other inbred × isolate interactions were susceptible, although ICMP 501 and 700481-21-8 tended to express a moderately susceptible infection type 3. Differences in seedling reactions of inbreds were less apparent when evaluated with isolate PS92-1. All inbreds were highly susceptible in the seedling stage to this isolate. Mixtures of infection types were not observed when seedlings were inoculated with the bulk rust collection.

Regressions of probit uredinium eruption against days from inoculation resulted in a minimum r^2 value of 0.66 ($P < 0.01$), with an average r^2 value of 0.88. These values indicated the validity of using a probit transformation for calculating whole-plant T_{50}. Although data were not recorded, uredinium eruption usually began and ended 1–3 days earlier on the lower leaves than on the flag and flag-1 leaves. When a bulk rust collection was used for inoculation, uredinium eruption was delayed on inbreds Tift 383, 700481-21-8, and ICMP 501 compared with Tift 23DB (Table 4). When inoculated with PS92-1, only ICMP 501 had a T_{50} longer than Tift 23DB. Although no statistical comparisons can be made because the two tests were not conducted simultaneously, T_{50} values of Tift 23DB and Tift 383 did not differ greatly between evaluations. T_{50} values of 700481-21-8 and ICMP 501 were considerably shorter with inoculation with PS92-1.

DISCUSSION

Pearl millets Tift 383, ICMP 501, and 700481-21-8 expressed moderate levels of rust resistance compared with Tift 23DB. In general, the inbreds differed for disease development in the field, for uredinium areas on seedlings, and for whole-plant T_{50} values. Race-specific resistance in seedlings of ICMP 501 was detected, but this inbred more frequently expressed a susceptible reaction in field and greenhouse evaluations. It is possible that biotypes avirulent to ICMP 501 do not constitute a high proportion of the pathogen population. Uredinia are smaller with infection type 2 reactions than with the susceptible infection types 3 and 4. When ICMP 501 seedlings were inoculated with a bulk rust collection, an infection type 3 was expressed even though standard deviations of uredinium dimensions were generally smaller on this inbred than on the others (Table 2). If virulent and avirulent biotypes were prevalent in the bulk rust collection, standard deviations of uredinium dimensions for ICMP 501 should have been greater than for the other inbreds.

A race-specific effect on whole-plant T_{50} appears to exist for inbred 700481-21-8. When inoculated with isolate PS92-1, this inbred had the shortest latent periods, which is illuminating, considering the resistance of this inbred in the field. A significant change in virulence frequency in the pathogen population occurred between 1992 and 1993. In 1992, virulence to Rr_1 was very low, and no infection was observed on pearl millet with this resistance gene. In 1993, high levels of infection occurred on pearl millet with Rr_1 (*unpublished*). If PS92-1 became predominant in the pathogen population in 1993, it might be expected that 700481-21-8 would be highly susceptible. The reverse was true, however; 700481-21-8 was consistently the most resistant inbred in 1993 and was more resistant in 1993 than in 1992. These observations suggest that a race(s) virulent to Rr_1 and different from PS92-1 has become widespread.

The inbreds express several components of slow-rusting resistance. However, some variation in inoculum-specific resistance in seedlings and maturing plants exists. Until the effects of an undefined race structure of the pathogen population on evaluating resistance of these inbreds in the field are determined, it may be preferable to state that these inbreds express partial resistance to *P. s. indica*.

Although the selected inbreds expressed a moderate resistance to *P. s. indica*, the inbreds were not consistently more resistant than Tift 23DB in most evaluations. Variations in assessment of cultivar resistance in the field as a result of planting date (15) and disease pressure (11) and in latent periods of plants as a result of isolate × cultivar interactions (9) have been reported. Variations such as these are less frequently reported than a stable expression of resistance across environments or with various pathogen isolates. A great deal is yet unknown concerning minor environmental differences, growth stage of plants, and variability for virulence in the pathogen population in the United States as they affect evaluating resistance in pearl millet. In this study, uredinium dimensions of seedlings and whole-plant T_{50} values from inoculation with a bulk rust collection in the greenhouse gave results more consistent with the ranking of inbred resistance in the field when disease pressure was most severe. Effective screening for partial resistance in pearl millet will require both field and greenhouse trials.

Sokhi and Singh (14) reported that several pearl millets identified with a 700481 prefix expressed slow-rusting

Table 3. Infection types of pearl millet seedlings inoculated with *Puccinia substriata* var. *indica* in the greenhouse

Inbred	Infection types[z]				
	Bulk rust collection	Single-uredinium isolate			
		PS88-199	PS89-775	PS92-1	PS92-2
Tift 23DB	4	4	4	4	4
Tift 383	4	4	4	4	4
700481-21-8	4	3	3	4	4
ICMP 501	3	3	2	4	3

[z] Infection types were rated 10–13 days after inoculation. Types 2, 3, and 4 indicate moderately resistant, moderately susceptible, and susceptible reactions, respectively (Stakman et al [16]).

Table 4. Whole-plant latent periods of infection for pearl millet inbreds inoculated with *Puccinia substriata* var. *indica* in the greenhouse

Inbred	Latent period (days)	
	Bulk rust collection	PS92-1
Tift 23DB	8.4 a[z]	8.5 a
Tift 383	9.2 b	9.6 ab
700481-21-8	14.6 c	8.4 a
ICMP 501	14.3 c	10.9 b
LSD ($P = 0.05$)	0.5	1.4

[z] Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

resistance. In the present evaluation, 700481-21-8, which is probably a related inbred, also expressed a level of partial resistance. Although 700481-21-8 and ICMP 501 were both developed in India, the genes conferring their resistances may differ. Seedlings of the inbreds differed in reaction to two of the four isolates, inoculum-specific differences in latent period appeared to exist between the inbreds, and the inbreds differed for AUDPC in each field trial. Both inbreds may be useful as sources of partial resistance.

The level of resistance expressed by all three inbreds and the agronomic characteristics of 700481-21-8 and ICMP 501 are not suitable for use in hybrid production for the United States. High-quality pearl millet forage requires extremely effective rust resistance, since low levels of rust infection can reduce digestible dry matter yield (21). The resistances from these pearl millets would probably be more useful after they have been combined in an improved agronomic type.

ACKNOWLEDGMENTS

Cooperative investigation of the USDA-ARS and the University of Georgia, College of Agricultural and Environmental Sciences, Agricultural Experiment Stations. I thank S. B. King, ICRISAT, for providing seed of ICMP 501 and 700481-21-8, and A. M. Hornbuckle, for technical assistance.

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