Leaf Spot, Rust, and Smut Resistance in Pearl Millet Landraces from Central Burkina Faso

JEFFREY P. WILSON, Department of Plant Pathology, GLENN W. BURTON, Department of Agronomy, and HOMER D. WELLS, Department of Plant Pathology, USDA-ARS, University of Georgia Coastal Plain Experiment Station, Tifton 31793; and JEAN-DIDIER ZONGO and IDRISSA OUSMANE DICKO, Ouagadougou University, Ouagadougou, Burkina Faso, West Africa

ABSTRACT

S1. bulks of 111 pearl millet landraces collected in central Burkina Faso were evaluated for resistance to Pyricularia grisea, Puccinia striariata var. indica, Bipolaris setariae, and Moesziomyces pennisicililae. The isolates of the pathogens were collected in the United States. All inoculations were performed in the greenhouse at Tifton, Georgia. Seedlings were inoculated with P. grisea and P. s. var. indica. Maturing plants were inoculated with B. setariae and emerging inflorescences inoculated with M. pennisicililae. Within the landraces, the frequency of plants resistant to P. grisea ranged from 39.2 to 98.2% (x = 80.7%). Frequency of plants resistant to P. s. var. indica ranged from 0 to 47.5% (x = 5.1%). Mesothetic reactions (both resistant and susceptible reactions on the same plant) were common. All plants of all landraces were resistant to B. setariae. Differences in resistance to M. pennisicililae existed among the landraces. Percent seed set ranged from 12.2 to 68.0% (x = 34.3%) and was not correlated (r = 0.03, P = 0.78) with percent florets with sori, which ranged from 1.2 to 18.1% (x = 6.4%). These landrace accessions will provide useful genes for pearl millet breeding as well as genes for characterizing the pathogen populations that affect the crop.

Pearl millet (Pennisetum glaucum (L.) R. Br.) is an annual bunchgrass grown as a grain crop in areas of India, Pakistan, and Africa and for forage in areas of South America, Australia, and the United States (5). In the United States, 0.5 million ha are sown to millet for forage annually. Full realization of its potential use will require further improvement in yield and quality of its forage and grain and expansion of its region of adaptation. An extensive germ plasm base will prove useful to future improvement and cultivation of the crop.

The gene pool of pearl millet is tremendously diverse yet greatly under-}

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problems in breeding nurseries where seed production is necessary and may become a more significant problem when grain-producing cultivars of pearl millet are developed for the United States. In Burkina Faso, the leaf spots are common throughout the country. Rust is also common but occurs late in the season. Little is known about the effects of the leaf spots and rust on grain yield. Smut incidence is sporadic but occasionally becomes severe and can reduce yield.

In this paper we present some of the progress in evaluating the landraces from central Burkina Faso for resistance to several fungal diseases of pearl millet prevalent in the southeastern United States.

MATERIALS AND METHODS

Soil, climate, and vegetation maps were used to select ecologically diverse collection sites in the northeast, central, and southwest regions of Burkina Faso. After harvest in 1983, extension personnel collected 500 g of seed (about 5,000 seeds) from the growers of the best landraces in the northeast region. In 1984 and 1985, similar collections were made in the central and southwest regions of Burkina Faso. Seeds (40 g) of each landrace were sent to ICRAST in India; to Roland Loiselle, Ottawa Research Station, Agriculture Canada, Central Experimental Farm; and to the USDA Forage and Turf Research Unit, Tifton, Georgia.

To satisfy USDA quarantine, landrace seeds were planted only in the greenhouse during the winter when no pearl millet was grown in the field. Some seed from the original landraces (numbers 110–225) were planted in methyl bromide-fumigated soil beds in the greenhouse. Ten hills of each landrace were planted, and the plants were thinned to one per hill. The panicles of all plants were bagged to allow self-pollination. Seed from all the plants within a landrace were bulked, and the bulked S1 seed was used for the evaluations.

S1 seed was planted into 16-cm plastic pots containing equal volumes of coarse building sand, peat moss, and perlite, amended with 8.8 g/L of agricultural gypsum and 5.2 g/L of Osmocote fertilizer (3- to 4-month release, N-P-K 14-6.1-11.6, Sierra Chemical Co., Milpitas, CA). One replicate (one pot with an average stand of 43.5 plants) of each landrace was randomized within each of three blocks on the greenhouse bench. These blocks were grown and inoculated at different times. Greenhouse temperatures were maintained at approximately 30°C, and the plants were grown under natural lighting.

Five days after emergence, albino seedlings were removed, resulting in an average of 40.8 plants per pot. Eight days after emergence, plants at the two- to three-leaf stage were inoculated with a suspension of P. grisea conidia bulked from a number of isolates collected in Georgia. Conidia were scraped off dried colonies on V-8 agar and stored in plastic envelopes at −72°C. Before being used, packets of conidia were submerged in a 40°C water bath for 5 min, then comminuted in deionized water with one drop of Triton B 1956 per 100 ml. The final inoculum concentration was 1 × 10⁴ conidia per milliliter. Plants in an inoculation chamber were misted to dripping with the inoculum. The plants remained overnight (18 hr) in the chamber, where they were automatically misted with deionized water for 1 min every 30 min. After 18 hr, the plants were returned to the greenhouse bench.

Six to seven days after inoculation, the plants were evaluated for reaction to P. grisea on a 0–4 infection-type scale, where 0 = no symptoms, 1 = dark brown flecks, 2 = small flecks or scattered large brown necrotic lesions generally 0.5–3 mm long, 3 = moderately large to large water-soaked lesions (longer than 2–3 mm) generally spiral or elliptical shaped with necrotic gray centers and often associated with chlorosis, and 4 = lesions coalesced, often killing one or more leaves. Types 0, 1, and 2 were considered resistant and types 3 and 4, susceptible. The number of susceptible plants in each pot and the predominant reactions of the resistant plants were recorded. Some of the highly susceptible or small, uncompetitive plants were thinned during the evaluation, resulting in an average of 27.2 plants per replicate for the rust evaluation.

Plants at the four- to five-leaf stage were inoculated with P. s. var. indica. Urediniospores had been collected in Georgia from a field planting of a rust-resistant cultivar in 1985 and were stored in plastic packets at −72°C. Procedures for inoculum preparation and inoculations were identical to those used with P. grisea. The inoculum concentration was 2.5 × 10⁴ urediniospores per milliliter. Nine to eleven days after inoculation, the infection sites on leaves not previously infected by P. grisea were evaluated on a 0–4 infection-type scale (13) in which types 0, 1, 2, and X (mesothetic, both resistant and susceptible reactions on the same plant) were considered resistant and types 3 and 4, susceptible. The numbers of plants per pot with resistant, mesothetic, or susceptible reactions were recorded.

![Fig. 1. Resistance to Pyricularia grisea in pearl millet landrace S1 bulks. Seedlings were inoculated and examined for percentage of resistant plants and the predominant resistant reaction (infection type 0, 1, or 2). Values are the means of three replicates.](image-url)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>P. grisea*</th>
<th>P. s. var. indica*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landrace</td>
<td>110</td>
<td>0.128**</td>
<td>0.620**</td>
</tr>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.749**</td>
<td>0.407*</td>
</tr>
<tr>
<td>Error</td>
<td>218</td>
<td>0.030</td>
<td>0.122</td>
</tr>
</tbody>
</table>

*Data transformed to arcsin (proportion resistant) before analysis.

**Data transformed to log (percent resistant + 1) before analysis.

* and ** = Significant effects at P = 0.05 and 0.01, respectively.
One to three days after the plants were evaluated for rust reactions, the clumps of plants of two replicates were divided and transplanted into each of two 16-cm pots, resulting in four replicates (pots), each pot containing an average of 11.6 plants. At approximately 6 wk of age, plants of the landraces and of controls previously selected for susceptibility (17) were inoculated with an isolate of _B. setarueae_ collected in Georgia. Procedures for inoculum production, preparation, and inoculation were identical to those described for _P. grisea_. The inoculum concentration was 1.5 X 10^6 conidia per milliliter. Seven days after inoculation, the leaves not previously infected by _P. grisea_ or _P. s. var. indica_ were rated on a 0-4 infection-type scale, where 0 = no infection, 1 = small dark brown flecks less than 2 mm long and not associated with necrosis, 2 = mild leaf spot reactions with 2- to 4-mm brown necrotic fleck lesions, 3 = general infection with brown water-soaked early lesions sometimes coalesced, and 4 = abundant brown water-soaked lesions coalesced, killing one or more leaves. Types 0, 1, and 2 were considered resistant and types 3 and 4, susceptible.

When about half emerged from the boot, individual panicles were inoculated with _M. penicillariae_. Sporidial cultures of 14 isolates collected in Georgia were increased on V-8 agar and transferred as necessary. Sporidia of 3- to 10-day-old cultures were comminuted in deionized water with one drop of Triton B 1956 per 100 ml. The final inoculum concentration was 4 X 10^6 sporidia per milliliter. Late in the afternoon, flag leaf sheaths of plants were peeled off to expose the entire inflorescence, and the panicles were misted until dripping with the inoculum. The inflorescence was covered with a prewetted plastic bag to retain a high humidity. After approximately 17 hr, the plastic bag was removed and replaced by a glassine bag. About 1 mo after inoculation, the percentages of the surface area of the panicle occupied by seed and by sori were visually estimated for each plant. The mean percent seed set and mean percent florets with sori of the plants in a pot (average of 5.8 plants per pot) were taken as the value of a replicate.

Because at least one landrace was not represented in one block in all of the inoculations, the percentage of plants resistant to _P. grisea_, the percentage resistant to _P. s. var. indica_, and the percent seed set and percent florets with sori were each analyzed as randomized complete blocks with the general linear model procedure of SAS (12). The data were transformed to arcsin (proportion resistant to _P. grisea_) and to log (percent rust resistant + 1) before analysis to reduce the relationship between these treatment means and variances. The correlation between percent seed set and percent florets with sori was calculated, as were correlations between the percentages of plants resistant to _P. grisea_ and to _P. s. var. indica_ and the percentage of florets infected with _M. penicillariae_.

**RESULTS**

A variety of reactions to the four pathogens was observed among and within the landraces. Resistance to _P. grisea_ was common. All landraces had resistant plants. The frequency of resistant plants ranged from 39.2 to 98.2% (x̄ = 80.7%) (Fig. 1). Highly resistant infection type 0 predominated in five landraces; the others expressed resistant or moderately resistant reactions 1 or 2. In the analysis of variance, effects due both to landraces and to replication were highly significant (Table 1).

In contrast to the resistance to _P. grisea_, susceptibility predominated in the rust evaluations. The frequency of resistant plants ranged from 0 to 47.5% (x̄ = 51.1%) (Fig. 2). Mesothetic reactions, in which a plant has both resistant and susceptible infection sites, were common. The effects due both to landraces and to replication were significant in the analysis of variance (Table 1).

All plants were resistant to _B. setarueae_. the most common reactions were infection type 1. Inoculation of the susceptible control resulted in type 4 infections, indicating that the inoculum was virulent on a susceptible host.

In the _M. penicillariae_ evaluation, differences among the landraces existed for percent seed set, which ranged from 12.2 to 68.0% (x̄ = 34.3%), and percent florets with sori, which ranged from 1.2 to 18.1% (x̄ = 6.4%) (Table 2). There was no correlation between percent seed set and percent florets with sori (r = 0.03, P = 0.780).

The landraces were ranked according to resistance to each pathogen, and the most resistant 20% of the landraces in each group were compared to identify landraces with resistance to more than one pathogen. Accessions 122 and 162 were resistant to Pyricularia leaf spot and rust, accession 192 was resistant to Pyricularia leaf spot and smut, and accessions 133 and 224 were resistant to rust and smut (Table 3). No landrace was ranked in the most resistant 20% for all three evaluations. Resistance to Pyricularia leaf spot and resistance to rust were negatively correlated (r = -0.21, P = 0.030), and there was a correlation between Pyricularia leaf spot and smut resistance (r = 0.04, P = 0.713) or between rust and smut resistance (r = 0.07, P = 0.487).

**DISCUSSION**

The _S_ values were examined in these experiments because of the limited quantity of _S_ seed available. Only a few seeds were used to increase the landraces; the rest were put into long-term storage. The correlation between the observed _S_ evaluations and the actual _S_ performance
Rust resistance was less common but could be identified. These sources of resistance will allow us to test for the existence of races in *P. s. var. indica.* There is evidence for physiologic specialization within the fungus population in India (8). This question has not been addressed in the United States because until recently (6) no effective genes for resistance to populations of *P. s. var. indica* in the United States had been identified. The expression of mesothetic reactions in many of the landraces suggests there indeed may be differences in physiologic specialization. Because the plants were inoculated with a bulk culture, it is possible that some of the plants had genes for resistance that were not effective against all the virulence in the collection, resulting in resistant and susceptible infection types. Alternatively, the mesothetic reactions may be due to environment-sensitive genes that confer a mesothetic reaction, as do some genes for resistance to rust fungi in small grains (3).

*B. setariae* normally causes the most damage to pearl millet plants in the seedling stage and as the plants mature (16). The plants in our study had reached the stage where differences in susceptibility might be distinguished, but no differences were noted among the plants under these conditions. If differences do exist, they would be subtle, since resistance is almost universal and susceptibility is the exception. A high level of susceptibility has been found in progeny segregating from a cross between *P. glaucum* with a weedy subspecies, *P. glaucum* subsp. *monodii.* The complex genetic control of susceptibility (17) and the severe selection pressure against susceptible plants that fail to produce viable seed in the field, as observed in the United States, probably account for the absence of susceptibility in these populations and the infrequent occurrence of extreme susceptibility in pearl millet.

Identification of genetic resistance to *M. penicillarea* can be confounded by self-fertility. Pollination up to 72 hr after infection significantly reduces smut severities (18), yet differences in severities are found among inoculated cyttoplasmic male sterile inbreds and among their maintainer lines, indicating that heritable resistance can be identified. Because of this fact, and because we found no correlation between seed set and smut infection, landraces in this evaluation with the lowest smut severities within accessions showing comparable seed set may be likely sources of resistance.

We believe that sequential inoculations is an expedient approach to screening a large collection of pearl millet landraces and that the final result is a reasonably representative evaluation of the S. bulks. The inverse correlation between the frequency of resistance to Pyricularia leaf spot and rust suggests that the removal of *Pyricularia*-susceptible plants while thinning stands before the rust evaluation may have resulted in removal of rust-resistant plants. Because of the way that pearl millet is maintained by growers in Burkina Faso, these landraces represent regionally adapted, nonintermating gene pools. Positive or negative associations between unrelated traits such as, in this case, resistance to different pathogens is probably due more to differences in allelic frequencies that confer regional adaptation than to genetic linkage within the genome. Within all species, the probability is greater that genes for resistance to different pathogens are independent rather than linked. In addition, genetic linkages are more likely to be broken within a cross-pollinated species such as pearl millet than in a self-pollinating crop. The likelihood exists that each inoculation shifted the allelic frequencies of the sample for subsequent inoculations, but if linkages within the genome are loose or nonexistent, the importance of the shifts may have been reduced through the use of replicated inoculations.

In these experiments, the practical results obtained from screening the germ plasm may compensate for the theoretical problems associated with sequential inoculations. Disease resistance is a useful descriptor to include in a germ plasm database. Of the pathogens with which these accessions were screened, *P. grisea, P. s. var. indica,* and *M. penicillarea* appear to be useful descriptors for differentiating between some of the landraces and identifying possible duplications within the collection. The Burkina Faso landrace accessions are likely to yield valuable genes for pearl millet improvement as well as provide genes for resistance that can be used to characterize the diversity of virulence in the populations of the pathogens that affect the crop.

**ACKNOWLEDGMENTS**
We thank Dar Snyder, Director of the University of Georgia International Development, for assistance in organizing the collection of landraces from Burkina Faso, and Robert Branch for his thorough technical assistance.

**LITERATURE CITED**

**Table 2.** Analysis of variance of percent seed set and percent florets infected by *Moesziomyces penicillarea* in S1 bulks of pearl millet landraces from Burkina Faso

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
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<tr>
<td>Landrace</td>
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<td>530.65**</td>
<td>41.77**</td>
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<tr>
<td>Replication</td>
<td>3</td>
<td>2225.16**</td>
<td>12.94</td>
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<td>Error</td>
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<td>200.41</td>
<td>25.01</td>
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\*\* \* \* = Significant effects at *P = 0.01.*

**Table 3.** Burkina Faso landrace accessions with multiple disease resistance, as determined from inoculations in the greenhouse

<table>
<thead>
<tr>
<th>Landrace</th>
<th>Leaf spot*</th>
<th>Rust*</th>
<th>Smut*</th>
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<tr>
<td>122</td>
<td>91.8</td>
<td>24.0</td>
<td>8.1</td>
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<td>133</td>
<td>69.5</td>
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<tr>
<td>224</td>
<td>58.1</td>
<td>21.7</td>
<td>2.7</td>
</tr>
</tbody>
</table>

\*\*\* Average percentage of plants resistant to *Pyricularia grisea.*

\* Average percentage of plants resistant to *Puccinia striiformis* var. *indica.*

\*\*\* Average percentage of florets with sori of *Moesziomyces penicillarea.*
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