Increasing Complexity of Resistance in Host Populations Through Intermating to Manage Rust of Pearl Millet

H. Tapsoba and J. P. Wilson

First author: Department of Plant Pathology, University of Georgia, Athens 30602; and second author: USDA-ARS Forage & Turf Research Unit, University of Georgia Coastal Plain Experiment Station, Tifton 31793-0748.
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ABSTRACT

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Pearl millet inbreds Tift 23DB, Tift 85DB, PS748BC, and Tift 89D₂ were used to develop three categories of host mixtures (physical mixtures, random-mated populations, and mixtures of two-way and three-way crosses) representing different levels of complexity of resistance through increased heterogeneity within populations and through stacking of resistance genes within the heterogeneous populations. The potential of these mixtures to reduce rust epidemics was evaluated in the field. Area under the disease progress curves (AUDPCs) of all physical mixtures were less

than the mean of the components in 1995 and were less than the mean of the components for five of the six mixtures in 1997. In 1996, AUDPCs of the physical mixtures were consistently greater than the mean of their components. AUDPCs of the random-mated mixtures and the mixtures of crosses were consistently less than the mean of the components in 1996 and 1997, with reductions ranging from 12 to 71%. Dry matter yield (DMY) of physical mixtures relative to the mean DMY of the components was inconsistent, ranging from 18% less to 50% more than the mean of the components. The random-mated populations and the mixtures of crosses yielded 18 to 40% more DMY than the mean yield of the pure stands of their components.

Faced with the “boom and bust” cycles (6) characteristic of the use of monogenic host resistance in pure line cultivars, diversification strategies such as cultivar mixtures and multilines have been advocated as alternative approaches to plant disease control (1,4,5, 13). Use of cultivar mixtures and multilines incorporates principles from studies of natural plant populations and communities. By mimicking some aspects of natural systems, it has been postulated that more stable and longer-lasting plant disease management can be achieved. The disease-buffering capacity of natural systems may be partially regained by planting cultivar mixtures and multilines (2).

Rust, caused by *Puccinia substriata* Ellis & Barth. var. *indica* Ramachar & Cummins, has become the most devastating disease of pearl millet (*Pennisetum glaucum* (L.) R. Br.) in the southeastern United States. Since its first report in the United States in the 1950s (16), pearl millet rust has increasingly become a seriously limiting factor for forage and grain production.

Efforts to develop rust-resistant pearl millet hybrids have been impeded by variability for virulence in the pathogen population in the southeastern United States. Virulence to *Rr*₁ (28) and the subsequent identification of many pathotypes (26) have indicated the potential response to control through the use of major gene resistance. In light of the usual consequences of using highly effective monogenic resistance, an alternative is needed. If “boom and bust” disease cycles (6) are to be avoided for pearl millet in the southeastern United States, there is a need to assess different approaches to reduce rust severity and minimize forage and grain yield losses.

Genetic heterogeneity in three-way hybrids suppressed rust epidemics (30); therefore, selected multilines or mixtures are likely to be an effective disease management method. Because pearl mil-

let is an outcrossing species, a modified approach to the standard multiline or mixture has been proposed. In the dynamic multiline population (originally called the population multiline approach) (29), resistant backcross derivatives are allowed to intermate, creating increased complexity for genetic resistance in the host population. The advantages of the dynamic multiline population are that it integrates favorable attributes of the multiline and gene-stacking approaches to resistance breeding. In a standard multiline, the number of potential genotypes in the host population equals the number of component inbreds. In the dynamic multiline population, sufficient intermating should increase the frequency of plants possessing more than one resistance gene, which is characteristic of the gene-stacking strategy. However, genotypic heterogeneity within the population is also increased, characteristic of the multiline strategy. Assuming no linkage among resistance loci, the theoretical number of genotypes (*G*) in a randomly mated population in Hardy-Weinberg equilibrium can be described by $G = 3^X$, in which *X* = the number of resistance genes. If all resistance alleles are assumed to be completely dominant in expression, the number of theoretical phenotypes (*P*) can be described by $P = 2^X$.

The current study was undertaken to investigate the possibility of using modified genotype mixtures to manage pearl millet rust. Host populations that represent different levels of genotypic complexity for rust resistance were created and evaluated in the field for their ability to reduce rust severity. Complexity within each population varied not only for the potential number of resistance genes, but also for the distribution and combination of those resistance genes in the population. It was hypothesized that the increased genetic heterogeneity for rust resistance resulting from intermating or its subsequent enhancement through stacking of the resistance genes within heterogeneous populations should significantly suppress disease with an accompanying yield advantage.

MATERIALS AND METHODS

Pearl millet inbreds Tift 23DB, Tift 85DB, PS748BC, and Tift 89D₂ were used to develop the experimental populations. Tift 23DB

Corresponding author: J. P. Wilson
E-mail address: jwilson@tifton.cpes.peachnet.edu

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is not known to possess any resistance genes and was the recurrent parent in the development of the other three inbreds. Tift 85DB carries the *Rr*₁ rust resistance gene (12) transferred from PS 34, a *P. glaucum* subsp. *monodii* accession from Senegal. PS748BC is a backcross derivative of PS 748, a different accession of *P. glaucum* subsp. *monodii* from Senegal. The inheritance of resistance in this inbred is not known. Tift 89D₂, a parental line released in 1993 (11), has resistance derived from pearl millet 'Se Fa' from Senegal. It has at least one resistance gene that is independent from *Rr*₁ (18). When mixtures of these inbreds are referenced in this text, Tift 23DB, Tift 85DB, PS748BC, and Tift 89D₂ are designated as 23DB, 85DB, 748BC, and 89D₂, respectively.

Four types of populations were created from the inbreds: pure stands, physical mixtures, random-mated populations, and mixtures of crosses (Table 1). The four types of populations represent different levels of genotypic complexity with regard to the potential number of rust resistance genes and the distribution and combinations of those genes within each population. The pure stands represent genetic uniformity. All individuals in each pure stand carried the same resistance genes, if any. All plants were resistant or susceptible to the same races of the rust pathogen.

The physical mixtures represent the first level of genetic diversification. Populations in that category carried rust resistance genes from two or more components. Although rust resistance genes from two or more sources were included in the population, those genes remained independent, being carried only by the individual plants of the components. In that sense, the spatial distribution of any particular resistance gene coincided with the distribution of the individual component from which that gene came, as in a conventional multiline.

The random-mated populations introduced greater complexity into the populations by allowing redistribution of the resistance genes among the plants in the population. The random mating would increase the genotypic heterogeneity in the population and introduce a level of gene stacking into the population.

Four random-mated populations were developed through a series of inoculations and open-pollinations in the greenhouse. An initial population, M₀, was obtained by mixing equal numbers of seeds of Tift 23DB, Tift 85DB, PS748BC, and Tift 89D₂. To create the host population, M_n (*n* = 1 to 4), about 1,000 plants of the preceding population (M_{*n*-1}) were grown in pots (five to eight seedlings per 10-cm-diameter pot) in the greenhouse and allowed to outcross. To facilitate cross-pollination, a fan placed 30 cm above the plants at anthesis was operated for 15 s every 5 min by an electric timer. At maturity, all panicles were harvested and the seeds were collected to constitute the M_{*n*} population.

To determine if changes in resistance gene frequencies could result from selection pressure by rust infection, pathogen races PS93-5 and PS93-6 were used in inoculations during development of the random-mated mixtures. Although they do not appear to be important components of the pathogen population, these races were selected because the reactions of the inbreds to these races differed (26). Tift 23DB is susceptible to both races. Tift 85DB is susceptible to race PS93-5 and resistant to race PS93-6. PS748BC is resistant to race PS93-5 but susceptible to race PS93-6. Tift 89D₂ is resistant to both races. During each intermating cycle, plants were inoculated with an equal mixture by weight of urediniospores of PS93-5 and PS93-6, for a total inoculum concentration of 3.5 × 10⁴ urediniospores per ml. Seedlings were inoculated at 3 weeks and at 6 weeks after emergence. Susceptible plants were not removed; only selection pressure through reduced reproductive fitness of infected plants occurred. Over four generations of open-pollination in the presence of disease, it was hypothesized that the frequency of plants carrying multiple resistance genes would significantly increase.

Changes in the frequencies of plants resistant to PS93-5, PS93-6, or both in the four random-mated populations were evaluated in the greenhouse. For each race, five samples of each random-mated

population, each averaging 850 seeds, were planted and seedlings were screened for resistance to one of the two races. The number of resistant and susceptible plants were counted 7 to 10 days after inoculation. Susceptible plants were removed, and plants resistant to one race were inoculated with the other race to determine proportions of plants resistant to both races.

The fourth type of host population, the mixtures of crosses, was created by systematically crossing two or three resistant lines to produce heterogeneous host populations consisting predominantly of individuals with multiple resistance genes per plant. The mixtures of two-way crosses were obtained by blending equal numbers of seeds of the six possible F₁ hybrids (reciprocal crosses were included) of Tift 85DB, PS748BC, and Tift 89D₂. All F₁s had resistance genes from two parents. The mixture of three-way crosses was a similar blend of all possible combinations of the same three pearl millet inbreds, except that F₁ hybrids produced by crossing two of the inbreds were pollinated by and used to pollinate plants of the third inbred. Equal numbers of seeds from the six resulting three-way hybrids (reciprocal crosses were included) were blended. Thus, in this population, 25% of individuals would have resistance genes from all three parents, 50% would have resistance genes from two parents, and 25% would have resistance genes from only one parent.

Field evaluations of the host populations were conducted in two early-planted and three late-planted tests. Early-planted evaluations in rust-free conditions, conducted to assess heterosis for yield in mixtures derived by intermating, were planted on 21 June 1996 and 16 June 1997. Late-planted evaluations in the presence of rust were planted on 1 August 1995, 7 August 1996, and 4 August 1997. Due to availability of seed of the populations, the 1995 test included 10 of the 15 treatments, and the early 1996 experiment included 13 treatments. The late 1996 experiment and both 1997 experiments included 15 treatments.

Plots consisted of four 6-m-long rows spaced 0.90 m apart. Treatments were arranged in a randomized complete block design with four replications. In the 1995 test, blocks were separated from each other and surrounded by two rows of sorghum, a nonhost of *P. substriata* var. *indica*. Within each block, the experimental plots were separated by plots of sorghum. In 1996, the pearl millet forage hybrid 'Tifleaf 1' was used in border rows in order to separate the blocks and treatment plots. In 1997, border rows consisted of the rust-susceptible pearl millet grain hybrid 'HGM 100'. In all evaluations, plots were fertilized at planting with 250 kg of NPK (10-10-10) per ha and about 4 weeks later with 100 kg of ammonium nitrate per ha.

TABLE 1. Composition of pearl millet treatments evaluated in the field

Population type	Tift 23DB	Tift 85DB	PS748BC	Tift 89D ₂
Pure stands	100.0 ^x	...	...	...
	...	100.0	...	...
	...	...	100.0	...
	...	...	...	100.0
Physical mixtures	...	50.0	50.0	...
	...	50.0	...	50.0
	...	...	50.0	50.0
	...	33.3	33.3	33.3
	25.0	25.0	25.0	25.0
Random-mated ^y	1st generation random-mated			
	2nd generation random-mated			
	3rd generation random-mated			
	4th generation random-mated			
Mixtures of crosses ^z	Mixture of two-way crosses			
	Mixture of three-way crosses			

^x Percent inbred in that treatment. ... = Inbred not present.

^y 1st, 2nd, 3rd, and 4th generations, respectively, of random-mated populations from an equiproportional mixture of Tift 23DB, Tift 85DB, PS748BC, and Tift 89D₂.

^z Mixture of all possible two-way and three-way crosses of Tift 85DB, PS748BC, and Tift 89D₂.

The rust epidemic was initiated in 1995 by transplanting into the middle of each experimental plot two Tift 23DB plants that had been inoculated 3 weeks earlier in the greenhouse with a bulk urediniospore inoculum collected in the field in 1994. For the late 1996 and 1997 tests, the rust epidemic was initiated by inoculating the border rows with the same 1994 field bulk inoculum. The border plants were sprayed early in the evening with an aqueous suspension of urediniospores (approximately 3×10^4 spores per ml). In all late tests, the epidemic was initiated soon after natural rust infections were observed in local nurseries.

Starting at approximately 2 weeks before anthesis, rust severity ratings (percent diseased leaf area) of the two middle rows were visually estimated at weekly intervals for 7 weeks using standard area diagrams (21). The last disease rating was taken at the dough stage of grain development. The area under the disease progress curve (AUDPC) was determined for each plot.

Green forage yield of each plot was determined. For the 1995 test, each plot was sampled on 16 August by manually harvesting two 1-m-long random sections in the two middle rows. For the 1996 and 1997 evaluations, the two middle rows of each plot were individually harvested with a forage chopper. Early tests were harvested 8 August 1996 and 8 August 1997. Late tests were harvested 17 October 1996 and 16 October 1997. A 300- to 500-g sample of green forage from each harvested plot section was oven-dried at 60°C. Weights determined before and after drying were used to calculate dry matter yield (DMY).

TABLE 2. Forage dry matter yield (in kg/ha) of pearl millet inbreds and mixtures evaluated in rust-free conditions at Tifton, GA

Treatment	1996	1997
Tift 23DB	966 bc ^w	823 a
Tift 85DB	993 b	845 a
PS748BC	954 c	1,043 a
Tift 89D ₂	1,366 a	1,171 a
85DB+748BC	908 c	761 a
85DB+89D ₂	895 c	844 a
748BC+89D ₂	1,024 b	863 a
85DB+748BC+89D ₂	979 b	899 a
23DB+85DB+748BC+89D ₂ M ₀	908 c	922 a
Random-mated M ₁ ^x	628 d	952 a
Random-mated M ₂	915 c	888 a
Random-mated M ₃	980 b	887 a
Random-mated M ₄	... ^y	877 a
Mixture of two-way crosses ^z	1,036 b	1,000 a
Mixture of three-way crosses	...	968 a

^w Means within a column followed by the same letter are not significantly different based on Fisher's least significant difference test ($P = 0.05$). Mean separations are based upon transformed data. Untransformed means are presented.

^x 1st, 2nd, 3rd, and 4th generations, respectively, of random-mated populations from an equiproportional mixture of Tift 23DB, Tift 85DB, PS748BC, and Tift 89D₂.

^y Mixture not evaluated in 1996.

^z Mixture of all possible two-way and three-way crosses of Tift 85DB, PS748BC, and Tift 89D₂.

TABLE 3. Frequencies of plants resistant to two races of *Puccinia substriata* var. *indica* in four populations^a

Treatment	PS93-5	PS93-6	PS93-5 and PS93-6
Initial mixture M ₀	50.7 ± 0.5	50.5 ± 0.5	25.2 ± 0.3
1st random-mated M ₁	58.6 ± 0.5	61.6 ± 0.5	31.3 ± 0.5
2nd random-mated M ₂	61.5 ± 0.5	66.2 ± 0.4	35.4 ± 0.5
3rd random-mated M ₃	66.5 ± 0.4	68.6 ± 0.5	37.4 ± 0.4
4th random-mated M ₄	68.6 ± 0.5	71.6 ± 0.4	41.6 ± 0.5
LSD($P=0.05$)	0.4	0.4	0.3

^a Populations derived from an equiproportional mixture of pearl millet inbreds Tift 23DB, Tift 85DB, PS748BC, and Tift 89D₂ and advanced through four cycles of random-mating.

Data collected in all five field evaluations were analyzed using SAS General Linear Model procedures (SAS Institute Inc., Cary, NC). To achieve normal distributions for analyses of variance and to reduce the relationship between treatment means and variances, data for AUDPC were $(x + 1)^{1/2}$ -transformed and a $\log_{10}(x + 1)$ transformation was applied to the DMY data. Treatment means were differentiated using Fisher's least significant difference test. Relative AUDPCs and DMYs of the physical mixtures and intermated populations were determined by comparison of the observed population value to the average value of the component inbreds used to produce either the physical or intermated populations.

RESULTS

Heterosis for yield was not apparent among the nondiseased treatments. Early-planted yield tests evaluated in the absence of rust infection did not show consistent yield increases among the random-mated mixtures and the mixtures of crosses in either 1996 or 1997 (Table 2). Tift 89D₂ was the highest-yielding treatment in both years, but statistically higher only in 1996.

There were continuous, significant increases in the frequencies of plants resistant to PS93-5 or PS93-6 among the four random-mated populations inoculated in the greenhouse (Table 3). The proportion of plants resistant to PS93-5 and PS93-6 increased from 50% in the initial physical mixture (M₀) to 69 and 72% in the fourth random-mated population. There was also a significant increase in the proportion of plants simultaneously resistant to both races of the pathogen from one generation to the next, but the observed frequencies were less than expected based upon the frequencies of plants with resistance to either race.

In disease trials during the 3 years, final rust severity (Y) was linearly related to AUDPC (X) ($r = 0.79$) by the equation $Y = 0.038 X$. The pure stand of Tift 23DB was the most susceptible treatment and Tift 89D₂ was the most resistant (Table 4). In each evaluation, the physical mixtures, the random-mated populations, and the mixtures of crosses were all more resistant than the pure stands of Tift 23DB and Tift 85DB. The binary mixture 748BC+89D₂, the two most resistant inbreds, was the most resistant physical mixture. A significant increase in resistance occurred by the M₂ generation, but after that, there was no trend to increased resis-

TABLE 4. Area under the disease progress curve of pearl millet inbreds and mixtures evaluated in rust epidemics at Tifton, GA

Treatment	1995	1996	1997
Tift 23DB	2,996 a ^w	2,503 a	2,955 a
Tift 85DB	2,711 b	2,097 b	2,555 b
PS748BC	326 f	747 i	657 e
Tift 89D ₂	53 h	159 k	95 h
85DB+748BC	970 d	1,660 c	1,389 c
85DB+89D ₂	1,299 c	1,258 de	660 e
748BC+89D ₂	128 g	566 j	518 fg
85DB+748BC+89D ₂	340 f	1,525 c	843 d
23DB+85DB+748BC+89D ₂ M ₀	516 e	1,448 cd	899 d
Random-mated M ₁ ^x	434 e	1,042 fg	922 d
Random-mated M ₂	... ^y	933 gh	444 g
Random-mated M ₃	...	1,216 ef	599 ef
Random-mated M ₄	...	807 hi	648 e
Mixture of two-way crosses ^z	...	944 gh	562 ef
Mixture of three-way crosses	...	926 gh	660 e

^w Means within a column followed by the same letter are not significantly different based on Fisher's least significant difference test ($P = 0.05$). Mean separations are based upon transformed data. Untransformed means are presented.

^x 1st, 2nd, 3rd, and 4th generations, respectively, of random-mated populations from an equiproportional mixture of Tift 23DB, Tift 85DB, PS748BC, and Tift 89D₂.

^y Mixture not evaluated in 1995.

^z Mixture of all possible two-way and three-way crosses of Tift 85DB, PS748BC, and Tift 89D₂.

tance in the field with advancing generation despite the increasing resistance in the random-mated populations in greenhouse evaluations. The mixtures of crosses were not different from one another for AUDPCs in 1996 and 1997.

Comparisons of disease development in the mixtures relative to the mean AUDPC of the individual components of each mixture in pure stands were made (Table 5). AUDPCs of the physical mixtures were reduced by 6 to 67% in 1995. In 1996, however, all physical mixtures had AUDPCs 5 to 52% higher than the mean AUDPC of the pure stands of their components. In 1997, 748BC+89D₂ was the only physical mixture with a higher AUDPC (38%) than the average AUDPC of its components. In all 3 years, the AUDPCs of the random-mated populations and the mixtures of crosses were consistently lower than the mean AUDPC of the four pure stands.

Treatments differed for DMY in 1995 (Table 6). The pure stands of Tift 23DB yielded less than any other treatment. The pure stand of Tift 89D₂, the mixture 748BC+89D₂, and the first random-mated population were the highest-yielding treatments.

In the 1996 evaluation, the pure stands of Tift 23DB and Tift 85DB and the mixture 85DB+748BC were the lowest-yielding treatments, whereas the pure stand of Tift 89D₂ and the mixture of two-way crosses were the highest yielding. There were no differences among PS748BC, the mixture 748BC+89D₂, the ternary mixture 85DB+748BC+89D₂, the equiproportional mixture of the four inbreds, the random-mated populations, or the mixtures of two-way and three-way crosses.

Pure stands of Tift 23DB and Tift 85DB were the lowest-yielding treatments in 1997 (Table 6). There were few differences among any of the mixtures, but physical mixtures with Tift 85DB tended to be lower yielding.

Comparisons of the physical mixtures, the random-mated populations, and the mixtures of crosses to the mean DMY of the pure stands of their components were made (Table 7). DMYs of the physical mixtures relative to the mean DMY of the components were inconsistent in all 3 years, ranging from 18% less to 50% more than the mean yield of their components. The physical mixtures 85DB+89D₂ and 748BC+89D₂ consistently yielded less than the average yield of the pure stands of their components. All random-mated populations yielded 18 to 40% more than the mean yield of the four pure stands. The two mixtures of crosses yielded 19 to 39% more than the mean yield of the pure stands of their components.

TABLE 5. Area under the disease progress curve of physical mixtures, random-mated populations, and mixtures of two-way and three-way crosses of pearl millet relative to the component means^v evaluated in rust epidemics at Tifton, GA

Treatment	1995	1996	1997
85DB+748BC	-36 b ^w	17 c	-14 b
85DB+89D ₂	-6 a	12 c	-50 cd
748BC+89D ₂	-32 b	25 b	38 a
85DB+748BC+89D ₂	-67 c	52 a	-24 b
23DB+85DB+748BC+89D ₂ M ₀	-66 c	5 c	-43 c
Random-mated M ₁ ^x	-71 c	-24 d	-41 c
Random-mated M ₂	... ^y	-32 de	-72 e
Random-mated M ₃	...	-12 d	-62 d
Random-mated M ₄	...	-41 e	-59 d
Mixture of two-way crosses ^z	...	-31 de	-64 d
Mixture of three-way crosses	...	-33 de	-58 d

^v The mean area under the disease progress curve values of the parental inbreds used to comprise the random-mated and mixtures of two-way and three-way crosses were used for comparisons.

^w Means within a column followed by the same letter are not significantly different based on Fisher's least significant difference test ($P = 0.05$). Mean separations are based upon transformed data. Untransformed means are presented.

^x 1st, 2nd, 3rd, and 4th generations, respectively, of random-mated populations from an equiproportional mixture of Tift 23DB, Tift 85DB, PS748BC, and Tift 89D₂.

^y Mixture not evaluated in 1995.

^z Mixture of all possible two-way and three-way crosses of Tift 85DB, PS748BC, and Tift 89D₂.

DISCUSSION

Any resistance gene that confers resistance to part of the pathogen population has some value if used in an appropriate breeding and deployment strategy. Although much less effective than that of PS748BC and Tift 89D₂, the resistance of the "defeated" *Rr₁* gene in Tift 85DB was still detectable in all 3 years, and thus, contributed resistance to some portion of the pathogen population.

In many of the mixtures, the resulting AUDPC was lower than that of the mean of the components. The physical mixtures provided a measure of protection, but the intermated populations were consistently more effective, when the performance was compared with the mean AUDPC of the components. Although none of the

TABLE 6. Forage dry matter yield (in kg/ha) of pearl millet inbreds and mixtures evaluated in rust epidemics at Tifton, GA

Treatment	1995	1996	1997
Tift 23DB	226 e ^w	234 c	209 f
Tift 85DB	319 d	270 c	244 ef
PS748BC	412 bc	527 ab	516 abc
Tift 89D ₂	538 a	689 a	729 a
85DB+748BC	397 bcd	357 c	407 cde
85DB+89D ₂	351 cd	404 b	406 cde
748BC+89D ₂	459 ab	533 ab	531 abc
85DB+748BC+89D ₂	371 bcd	567 ab	523 abc
23DB+85DB+748BC+89D ₂ M ₀	339 cd	644 a	602 abc
Random-mated M ₁ ^x	441 ab	548 ab	503 bc
Random-mated M ₂	... ^y	559 ab	514 abc
Random-mated M ₃	...	601 ab	558 abc
Random-mated M ₄	...	578 ab	529 abc
Mixture of two-way crosses ^z	...	685 a	618 ab
Mixture of three-way crosses	...	588 ab	690 ab

^w Means within a column followed by the same letter are not significantly different based on Fisher's least significant difference test ($P = 0.05$). Mean separations are based upon transformed data. Untransformed means are presented.

^x 1st, 2nd, 3rd, and 4th generations, respectively, of random-mated populations from an equiproportional mixture of Tift 23DB, Tift 85DB, PS748BC, and Tift 89D₂.

^y Mixture not evaluated in 1995.

^z Mixture of all possible two-way and three-way crosses of Tift 85DB, PS748BC, and Tift 89D₂.

TABLE 7. Forage dry matter yield of physical mixtures, random-mated populations, and mixtures of two-way and three-way crosses of pearl millet relative to the component means^v evaluated in rust epidemics at Tifton, GA

Treatment	1995	1996	1997
85DB+748BC	9 b ^w	-10 d	7 d
85DB+89D ₂	-18 d	-16 d	-17 e
748BC+89D ₂	-3 c	-12 d	-15 e
85DB+748BC+89D ₂	-12 d	14 c	5 d
23DB+85DB+748BC+89D ₂ M ₀	-9 d	50 a	42 a
Random-mated M ₁ ^x	18 a	27 bc	18 c
Random-mated M ₂	... ^y	30 b	21 b
Random-mated M ₃	...	40 b	32 ab
Random-mated M ₄	...	34 b	25 b
Mixture of two-way crosses ^z	...	38 b	25 b
Mixture of three-way crosses	...	19 c	39 a

^v The mean dry matter yield values of the parental inbreds used to comprise the random-mated and mixtures of two-way and three-way crosses were used for comparisons.

^w Means within a column followed by the same letter are not significantly different based on Fisher's least significant difference test ($P = 0.05$). Mean separations are based upon transformed data. Untransformed means are presented.

^x 1st, 2nd, 3rd, and 4th generations, respectively, of random-mated populations from an equiproportional mixture of Tift 23DB, Tift 85DB, PS748BC, and Tift 89D₂.

^y Mixture not evaluated in 1995.

^z Mixture of all possible two-way and three-way crosses of Tift 85DB, PS748BC, and Tift 89D₂.

physical mixtures, random-mated populations, or mixtures of crosses had rust severities or AUDPCs as low as the pure stand of Tift 89D₂. AUDPCs of those three categories of mixtures were significantly better than the pure stands of Tift 85DB and often comparable to PS748BC.

Although the AUDPCs of the random-mated M₂ to M₄ populations were consistently less than that of M₀, the significant increase of the proportion of plants resistant to PS93-5 and PS93-6 observed in the greenhouse inoculations was not predictive of the reaction of the random-mated populations in the field. This can be attributed to the fact that although mild selection pressure was placed on the intermating populations developed in the greenhouse by inoculation with isolates PS93-5 and PS93-6, differences in the reactions of the four inbreds to other isolates of the pathogen are more likely to account for the reactions in the field. The race composition of the pathogen population in the southeastern United States is not known, but PS93-5 and PS93-6 are not likely to be among the prevalent races (26). Resistance to either race in the greenhouse is not indicative of the reaction to the population in the field. In the greenhouse evaluations, however, selection pressure imposed by the pathogen can shift resistance gene frequencies. The result from the greenhouse experiment is similar to the increased frequency of disease resistance observed in barley composite cross II populations (19,22). After 45 generations of intermating in field conditions, the frequencies of resistance and of multiple resistances to *Rhynchosporium secalis*, *Blumeria graminis* f. sp. *hordei*, and *Drechslera teres* were greater in advanced generations of the population. It is likely that if pearl millet populations genetically diverse for resistance genes were grown in the field in the presence of rust, an increase of resistance would be observed in advanced populations.

The three categories of mixed populations had a forage DMY advantage over their most susceptible components. The physical mixtures containing Tift 23DB or Tift 85DB yielded 10 to 180% more than the pure stands of those two inbreds. The four random-mated populations consistently yielded at least 150% more than the pure stand of Tift 23DB. The mixtures also were often comparable in yield to the highest-yielding component.

Kølster et al. (14) observed that, in barley multiline cultivars, powdery mildew levels were 50 to 80% lower than the mean level for the components in pure stands. Wolfe (31) reported reductions of up to 80% for the same disease. Reductions in our study were lower and more similar to levels observed in a study of spot blotch of wheat (caused by *Bipolaris sorokiniana*) (23) in which the AUDPC was significantly reduced by 9 to 57% in the cultivar mixtures compared with the average of their components in monoculture.

Besides their capacity to significantly reduce disease severity, cultivar mixtures and multiline cultivars also yield at least as well as the mean yield of their components, and sometimes exceed the highest-yielding component. It is uncommon for a cultivar mixture or a multiline cultivar to yield less than the mean of its components, and rare for it to be as low or lower than its lowest-yielding component (24,27,31). Newton and Thomas (20) reported that the yields of mixtures of barley cultivars with partial resistance to *B. graminis* f. sp. *hordei* were significantly greater than the mean yields of their components in pure stands. Mahmood et al. (17) found that the grain yields of two-component wheat cultivar mixtures equaled or exceeded the mean yields of their components by as much as 11 to 26%. In a two-season evaluation of two wheat multiline cultivars in India, Singh et al. (25) observed that the multiline cultivars consistently produced significantly higher yields (1,830 to 2,060 kg/ha) than the pure stand varieties (730 to 1,470 kg/ha).

Relative DMY of our mixtures was often greater in intermated populations, whereas the relative DMY of physical mixtures was often less than the mean of the components. Redistribution and stacking of resistance genes and increased heterogeneity for resistance as a result of intermating, and not heterosis for forage yield, are the likely reasons for the benefit.

The factors proposed to explain the value of cultivar mixtures and multiline cultivars include the direct effect of the resistance genes and the indirect effects of mixing the components. The indirect effects of mixing; fewer numbers of susceptible plants in the mixtures, increased distances between susceptible plants, interference by resistant plants with inoculum spread between susceptible plants, and induced resistance caused by avirulent inoculum (2,3, 7–10,15,24,31); are functions of the heterogeneity for resistance in the host population. An advantage of intermated populations for foliar disease control is the exponential increase in resistance phenotypes with increasing numbers of resistance genes.

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