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Inheritance of resistance to southern corn rust in tropical-by-corn-belt maize populations

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Abstract The inheritance of resistance to southern rust (caused by *Puccinia polysora* Underw.) was investigated in two $F_{2:3}$ populations derived from crossing two temperate-adapted, 100% tropical maize (*Zea mays* L.) inbred lines (1416-1 and 1497-2) to a susceptible Corn Belt Dent hybrid, B73*Ht* × Mo17*Ht*. The inbred lines possess high levels of resistance to southern rust and may be unique sources of resistance genes. Heritability for resistance was estimated as 30% and 50% in the two populations from regression of $F_{2:3}$ family mean scores on F_2 parent scores, and as 65% and 75% from variances among $F_{2:3}$ families on a single-plot basis. RFLP loci on three chromosomal regions previously known to possess genes for resistance to either southern rust or common rust (*P. sorghi* Schw.) were used to localize genes affecting resistance to southern rust in selected genotypes of both populations, and to estimate their genetic effects. A single locus on 10S, *bnl3.04*, was associated with 82–83% of the variation among field resistance scores of selected $F_{2:3}$ families in the two populations. Loci on chromosomes 3 (*umc26*) and 4 (*umc31*) were significantly associated with resistance in the 1497-2 population, each accounting for 13–15% of the phenotypic variation for $F_{2:3}$ field scores. Multiple-marker locus models, including loci from chromosomes 3, 4, and 10 and their epistatic interactions, accounted for 96–99% of the variation in $F_{2:3}$ field scores. Similar results were obtained for resistance measured by counting pustules on juvenile plants in the greenhouse. An attempt was made to determine if the major gene for resistance from 1416-1 was allelic to *Rpp9*, which is also located on 10S. Testcross families from the cross (1416-1 × B37*Rpp9*) × B14A*Ht* were evaluated for resistance to southern rust in Mexico. Neither source of resistance was completely effective in this environment, preventing determination of allelism of the two genes; however, both sources of resistance had better partial resistance to southern rust than did B14A*Ht*.

Key words *Puccinia polysora* · RFLP markers · Partial resistance

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Introduction

Southern corn rust, caused by *Puccinia polysora*, has enormous destructive potential on susceptible maize hosts. Severe epiphytotics were observed in Africa beginning in 1949 and lasting several years. Yield losses were estimated at up to 50% (Rhind et al. 1952). Futrell (1975) reported that southern corn rust reached epiphytotic levels in the Mississippi Valley in 1972–1974. The severity of the disease was attributed, in part, to the nearly uniform susceptibility of the U.S. maize crop to southern rust (Futrell 1975; Futrell et al. 1975). Raid et al. (1988) and Rodriguez-Ardon et al. (1980) reported that southern rust caused yield losses of up to 39% to 45% on susceptible maize hybrids. Rodriguez-Ardon

et al. (1980) suggested that most, if not all, commercial U.S. hybrids were susceptible to southern rust. Recently, a few commercial hybrids with race-specific resistance to southern rust were released (David Smith, DEKALB Genetics Corporation, personal communication).

Ullstrup (1965) identified a single, dominant gene, *Rpp9,* from a South African plant introduction (PI 186208) that conferred resistance to *P. polysora* race PP. 9, isolated in Indiana. Futrell et al. (1975) identified another source of single-gene resistance to race PP. 9, but the relationship of this gene to *Rpp9* was not investigated. The possibility exists that the two genes are identical, particularly since both were obtained from South African germplasm. Scott et al. (1984) studied the inheritance of resistances found in lines from four populations, including both exotic and adapted U.S. germplasm. One- or two-gene models with varying degrees of dominance were used to explain the resistances found. Tests of allelism with *Rpp9* indicated that each line carried a resistance gene either at, or closely linked to, the *Rpp9* locus.

Robinson (1976) suggested that genes conferring race-specific resistance were not effective in preventing the spread of southern rust disease in Africa. He suggested that natural and farmer selection for quantitatively inherited general resistance was ultimately responsible for reducing southern rust disease to tolerable levels. Several studies have indicated that partial resistance to southern rust exists in some maize genotypes. Zummo (1988) found variability among corn genotypes for characters representing components of partial resistance: pustule incidence, size, tumescence, and sporulation. Bailey et al. (1987) found that genotypes varied greatly for the area under the disease progress curve (AUDPC) when infected with southern rust. They suggested that AUDPC was a useful measure of the slow-rusting trait, which could be a mechanism for horizontal resistance.

Increased levels of general resistance against southern rust are desirable for U.S. maize. Given the elite nature of commercial hybrid maize grown in the U.S., however, it is unlikely that quantitatively inherited forms of resistance to southern rust derived from exotic germplasm sources can be easily incorporated into commercial Corn Belt maize. Natural epiphytotics are so rare in many parts of the U.S. that general resistance genes could easily be lost in the absence of selection pressure, as has been observed for common rust resistance (Davis 1990). If elite inbred lines possessing general resistance are not available for use directly in Corn Belt breeding programs, introgression of simply inherited, but possibly race-specific, resistances might be acceptable, with the knowledge that they are not invulnerable to constantly evolving pathogen populations.

The objectives of this study were to, first, estimate heritability for southern rust resistance in two populations developed from crosses between temperate adapted, 100% tropical inbreds and a Corn Belt Dent hybrid. Second, restriction fragment length polymorphism (RFLP) markers were used to localize and estimate the effects of genes conferring resistance to southern rust in the two populations. Localization of genes controlling resistance to southern rust via DNA markers could allow introgression of these genes into elite materials, even in areas where the disease is not common, via marker-assisted backcrossing (Young and Tanksley 1989; Hillel et al. 1990; Hospital et al. 1992). Finally, an attempt was made to determine if one of the newly identified genes is allelic to *Rpp9*.

Materials and methods

Population development

Two temperate-adapted, 100% tropical maize inbred lines developed from a second cycle of pedigree selection from crosses made among tropical hybrids were found to possess high levels of resistance to southern corn rust in a previous experiment (Uhr 1991). Inbred 1416-1 was developed from lines derived from Agroceres brand 155, and Pioneer brands X105A and X306B. The racial background of these hybrids includes Azteca, Tuxpan Yellow Dent, Tuxpeño, ETO, Cuban Flint, Chandelle, Tusón, and Coastal Tropical Flint (Uhr and Goodman 1995). Inbred 1497-2 was developed from lines derived from Pioneer brand hybrids X105A, X306B, and public hybrids H5 and H101 from El Salvador. The racial background of these hybrids includes Cuban Flint, Tuxpeño, Tusón, ETO, Chandelle, and Coastal Tropical Flint (Uhr and Goodman 1995).

Two populations, referred to as the 1416-1 and 1497-2 populations, were developed to study the inheritance of resistance to southern rust conferred by these inbred lines. Each population was developed by crossing one resistant inbred line as a male parent to the susceptible hybrid B73*Ht* × Mo17*Ht*, and self-pollinating a single F_1 from each cross to produce 140 F_2 plants per population. Each F_2 plant was self-pollinated to produce 140 $F_{2:3}$ families per population. A hybrid parent was used in order to facilitate seed production of the F_1 generation. Thus within a population, some genomic regions were segregating for B73 alleles and alleles from the tropical parent, while other regions were segregating for Mo17 allele and alleles for the tropical parent.

Field evaluations

Individual F_2 plants grown in a Florida winter nursery in 1991 were scored for resistance to southern rust using a nine-point relative scale, where a score of one indicated a plant with leaves completely covered with southern rust pustules, and a score of nine indicated a plant entirely free of pustules. Natural inoculum was relied upon, and plants were rated between anthesis and maturity.

Each population was evaluated in a randomized complete block experiment replicated twice in Clayton, N.C., in summer 1991. Each experiment included 140 $F_{2:3}$ families; the resistant inbred parent line; the susceptible parent (B73*Ht* × Mo17*Ht*); a bulk of remnant F_1 individuals from the respective cross; and two susceptible commercial hybrid check entries, Pioneer 3369A, and Northrup King N8727. Plots consisted of one 4.86-m row, including an approximately 1-m alley at the ends of each plot. Inter-row spacing was 0.97 m. Plots were sown with 25 kernels plot^{-1} on May 15 1991. Natural inoculum was relied upon and plots were scored on either August 24 or 29 (depending upon the experiment), between anthesis and maturity,

using the nine-point scale. Scores for each plot represented mean values for individuals within the plot.

Based upon data from these two environments, $F_{2:3}$ families representing the most-resistant and most-susceptible phenotypic extremes were selected for further phenotypic and genotypic evaluation. Thirty-two resistant and 13 susceptible families were chosen from the 1416-1 population. Thirty-three resistant and 20 susceptible families were chosen from the 1497-2 population.

Two experiments, representing the selected families from each population, were planted in Clayton, N.C. on June 13 1994. Each experiment consisted of 44 or 52 $F_{2:3}$ families (from the 1416-1 or 1497-2 population, respectively); the resistant inbred parent line; the susceptible parent, B73*Ht* × Mo17*Ht*; a bulk of remnant F_1 individuals from the respective cross; the common rust-resistant, southern rust-susceptible inbred line B14A*Ht*; the southern rust-resistant, common rust-susceptible inbred line B37*Rpp9*; and three susceptible commercial check hybrids, Pioneer brands 3369A and 3165, and Northrup King brand N8727. B14A*Ht* carries the *Rp1-d* gene for common rust resistance, originally obtained from Cuzco maize (W. A. Russell, personal communication). Experiments consisted of two replications of randomized complete-block designs. Plot size, plot spacing, and seeding rate were the same as in 1991.

Epiphytotic conditions were initiated in these experiments by inoculating early planted border rows (B73*Ht* × Mo17*Ht* or Mo17*Ht* × Oh43E) with a solution of 10^7 to 10^8 uredospores l^{-1} in water with 2–4 drops of Tween-20 (polyoxyethylene-sorbitan monolaurate; Sigma Chemical CO., St. Louis, M.O.) per l. Uredospores were collected from greenhouse-grown B73*Ht* × Mo17*Ht* plants which had been inoculated with descendants of an isolate of *P. polysora* originally from Texas. Three weeks after inoculation of the border rows, uredospores were collected from the border rows to produce spore solutions as before. A single concentration of spores was used for each experiment. Each plant in the experiments was inoculated by injecting 0.5 ml of spore solution into the whorl with a micropipettor, 24 or 25 days after planting, depending upon the experiment. At anthesis, 21 or 22 days after inoculation (depending upon the experiment), plots were rated for rust resistance using the nine-point scale.

Greenhouse evaluations

Greenhouse evaluations for rust resistance were performed during the winter of 1993–1994. Each population was represented by two replications of a randomized complete block experiment containing: the selected $F_{2:3}$ families; the resistant inbred parent; the susceptible hybrid parent, B73*Ht* × Mo17*Ht*; a bulk of remnant F_1 individuals from the respective cross; B14A*Ht*; B37*Rpp9*; and three commercial hybrids, Pioneer brands 3369A and 3165, and Northrup King brand N8727. Six plants per experimental unit were sown in Metromix growing medium (Grace Sierra Co., Milpitas, Calif.) in 10-cm-deep trays. Each tray had four experimental units plus a check row of four plants of the susceptible hybrid 3369A grown in the center of each tray.

Approximately 3 weeks after planting, at the 4–5 leaf stage, each plant within a replication, including the four 3369A check plants per flat, was inoculated with a uniform solution of approximately 10^7–10^8 uredospores l^{-1} water, 2–4 drops of Tween-20 l^{-1}. Uredospores were collected from spreader plants of hybrid 3369A previously inoculated with the Texas isolate of *P. polysora*. Inoculations were performed by injecting approximately 1 ml of spore solution into the whorl of each plant, just below the growing point, using a size BD-20 hypodermic needle and syringe. Approximately 2–3 weeks after inoculation, all plants within a replicate were scored by counting the total number of uredospore-producing pustules on each plant. Because the residuals from analysis of the counts were not normally and independently distributed, the data were transformed by taking the natural logarithm of one plus the number of pustules per plant. Tests of significance, coefficients of variation, and r^2 values reported for the greenhouse data were based on analysis of the transformed data. Estimates of additive effects are reported in the original units.

Estimation of heritability

Estimates of the heritability of rust resistance in each population were made by regressing the 140 mean $F_{2:3}$ family scores from the 1991 field experiment on their F_2 parent scores from Florida, 1991. For this study, the inbreeding coefficient of the F_2 populations was assumed to be zero by analogy to a randomly mating population, and also because the F_2 populations were considered the reference populations to which the heritability estimates are applicable. For the case of $F = 0$ in the F_2 generation where two equally frequent alleles exist, the single-locus covariance is $Cov_{F2/F3} = \sigma^2_A + (1/2)\sigma^2_D + \sigma^2_{AA}$ (Nyquist 1991). This differs from the formulation given by Frey and Horner (1957), where $Cov_{F2/F3}$ was equated to $\sigma^2_A + (1/4)\sigma^2_D$, ignoring epistasis. Smith and Kinman (1965) suggested a correction factor to account for inbreeding in such estimates, but Nyquist (1991) noted that it was not correct. The slope of the regression of F_3 offspring on F_2 parents is therefore equal to $[\sigma^2_A + (1/2)\sigma^2_D + \sigma^2_{AA}]/\sigma^2_P$, where σ^2_P is the phenotypic variance among F_2 plants. Thus, this estimate is not truly a narrow-sense heritability, but is biased upward by dominant and epistatic genetic variances.

A second estimate of heritability for southern rust scores was made in each population based on the ratio of genotypic variation among $F_{2:3}$ families to their phenotypic variation in the 1991 field experiment. The numerator of this estimate equals $\sigma^2_A + (1/4)\sigma^2_D + \sigma^2_{AA}$ (Nyquist 1991) but is biased upward by genotype-by-environment interaction because the evaluations were performed in only a single environment. Phenotypic variances were estimated both on a single-plot basis and on the basis of a family mean (the average of two plots), allowing estimation of heritability both on a single-plot basis and on a family mean basis. These phenotypic variances also differ from the variance among individual plants used to compute the heritability based on offspring-parent regression.

Heritability of southern rust scores on a family basis across environments could not be estimated because a random sample of $F_{2:3}$ families from each population was grown in only one environment (North Carolina, 1991). Selected families were grown in both 1991 and 1994 in North Carolina, however, and the data from these families allowed an estimation of the repeatability of scores across environments and genotype-by-year interaction for scores. Because the extremes of each population were grown in these environments, the repeatability estimates do no reflect heritability in the reference populations, but comparison of repeatabilities of the selected families in 1991 with the heritability estimate based on the unselected populations in 1991 provides an estimate of the bias of the repeatability estimates.

Genotypic evaluation

Genes for common rust (*P. sorghi*) resistance exist on at least three chromosomes in maize: chromosomes 3 (Wilkinson and Hooker 1968), 4 (Wilkinson and Hooker 1968; Hooker 1985), and 10 (Hooker 1985). Because of the apparent clustering of both common and southern rust resistance genes on chromosome 10 (Wilkinson and Hooker 1968), it was decided to test RFLP markers previously mapped to regions known to contain common and/or southern rust-resistance genes for linkage to genes conferring southern rust resistance in the populations studied here (Table 1). Five loci mapping to chromosome 3 in the vicinity of Rp_3 were used: *umc10*, *umc102*, *umc26*, *umc60*, and *bnl5.37*. In the 1416-1 population, *umc102* and *umc60* were not polymorphic. Three loci mapping to the vicinity of Rp_4 on 4S were used: *umc123*, *umc31*, and *bnl5.37*. Five loci on 10S were used: *bnl3.04*, *bnl10.17*, *npi285*, *isu167*, and *umc130*.

Table 1 Map positions of the RFLP loci used, based on data from two previous studies (Matz. et al. 1994; Senior et al., 1996)

BNL map[a]			B73 × Mo17 Map[b]		
Chromosome	Locus	Map distance cM	Chromosome	Locus	Map distance cM
3	*umc10*		3	*umc10*	
		11.8			2.0
3	*umc102*		3	*umc102*	
		13.9			8.1
3	*umc60*		3	*umc26*	
		8.1			9.6
3	*bnl5.37*		3	*bnl5.37*	
					24.5
			3	*umc60*	
4	*umc123*		4	*umc123*	
		32.7			36.6
4	*umc31*		4	*umc31*	
		8.1			11.0
4	*bnl5.46*		4	*bnl5.46*	
10	*bnl10.17*		10	*npi285*	
		10.2			15.1
10	*bnl3.04*		10	*isu167*	
		10.5			9.9
10	*npi285*		10	*umc130*	
		15.8			
10	*umc130*				

[a] Matz et al. (1994)
[b] Senior et al. (1996)

DNA was extracted (Saghai Maroof et al. 1984) from a bulk of ground, lyopholized tissue from approximately ten plants for each selected $F_{2:3}$ family. Genomic DNA was digested with the restriction enzymes *Bam*HI, *Eco*RI, or *Hin*dIII. Ten micrograms of digested DNA per family were loaded on 0.8 $g\,l^{-1}$ agarose gels. Electrophoresis was carried out in 100 mM TRIS-acetate EDTA, pH 8.1, at 25–30 V for 16–18 h. Southern transfers were made by capillary transfer to MSI nylon membranes. Isolated maize DNA probes were random prime-labeled (Feinberg and Vogelstein 1983) and hybridized to maize DNA bound to membranes overnight at 65°C. Blots were washed in 2 × SSPE (1 × SSPE = 0.25 mM EDTA, 150 mM NaCl, 11.5 mM NaH_2PO_4), 1 $g\,l^{-1}$ SDS (sodium dodecyl sulfide) at room temperature for 20 min; 1 × SSPE, 1 $g\,l^{-1}$ SDS at 60–65°C for 15 min; and 0.1 × SSPE, 1 $g\,l^{-1}$ SDS at 60–65°C for 10 min, or as needed. Blots were then wrapped in plastic and exposed to X-ray film overnight-to-7 days to produce autoradiograms.

Analyses of variance (ANOVA) were calculated for each marker locus in each population for three measures of rust resistance: individual F_2 scores, $F_{2:3}$ family mean field scores, and transformed $F_{2:3}$ family mean greenhouse pustule counts (Edwards et al. 1987). $F_{2:3}$ family mean field scores were calculated from the experiments in 1991 and 1994. The presence of additive and dominant gene action at each marker was tested using single degree-of-freedom contrasts (Edwards et al. 1987).

Multiple marker-locus models were analyzed to estimate simultaneous effects of the three chromosomal regions. For each chromosome, the locus explaining the largest proportion of phenotypic variance was chosen to represent the chromosomal region. Thus, markers used in the multiple-locus models were genetically independent, and epistatic interactions among different chromosomal regions were tested by including interaction effects in the models. All possible combinations of the three loci and their epistatic interactions were evaluated for each trait and population. The model with the highest adjusted R^2 in which all factors were significant at $P = 0.05$ was chosen as the best model for a trait. Adjusted R^2 statistics were used to reduce bias in the estimates of phenotypic variance associated with marker loci (Charcosset and Gallais 1996).

Allelism test

We attempted to test allelism of the major resistance gene from 1416-1 and *Rpp9* by crossing 1416-1 to B37*Rpp9*. This F_1 was crossed to the common rust-resistant, southern rust-susceptible inbred tester, B14A*Ht*, to form testcross progeny. One hundred and seventyone testcross progeny were self-pollinated to form testcross progeny families. Recombination between the two resistance genes in the F_1 would be expected to produce gametes with susceptible alleles at both genes half of the time. These gametes, in combination with gametes from B14A*Ht*, would produce uniformly susceptible testcross progeny families. All other gametes from the F_1 would be expected to produce testcross progeny families that segregate 3 : 1 resistant to susceptible, or nearly so.

Six seeds of each testcross family and of each parental inbred line, 1416-1, B37*Rpp9* and B14A*Ht*, were planted in Poza Rica, Mexico, on August 16 1996. This location is regularly infested with *P. polysora*, but not *P. sorghi*, during this season. A period of extremely hot weather occurred soon after germination. All plants exhibited a susceptible phenotype. Therefore, each plant was rated on a 5-point quantitative scale for southern rust reaction phenotype, where 1 is completely immune, and 5 is extremely susceptible. Scores were made on October 23 1996, approximately 2–3 weeks after flowering.

Results and discussion

Heritability of resistance to southern corn rust

The distribution of mean southern rust scores from $F_{2:3}$ families was strongly skewed toward the resistant phenotype in both populations (Figs. 1 and 2). This could be interpreted as an effect of dominant gene action for resistance, which was also indicated by the

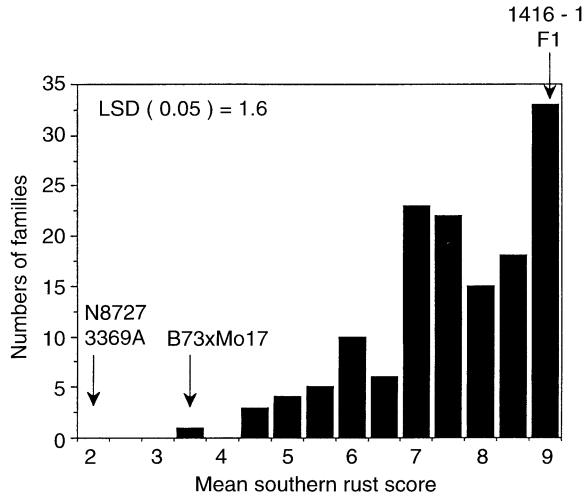

Fig. 1 Distribution of mean $F_{2:3}$ family southern rust scores in the (B73*Ht* × Mo17*Ht*) × 1416-1 population, North Carolina, 1991

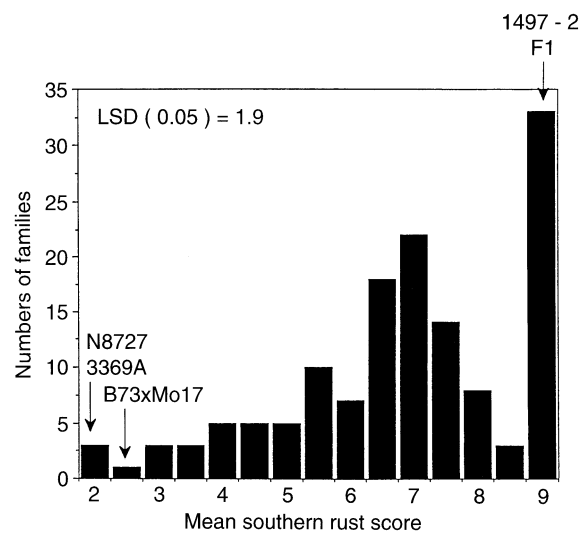

Fig. 2 Distribution of mean $F_{2:3}$ family southern rust scores in the (B73*Ht* × Mo17*Ht*) × 1497-2 population, North Carolina, 1991

resistant phenotype of the F_1 remnant bulk. No reasonable grouping of the distributions into dominant vs susceptible classes was found that would fit a single-gene model, as tested by chi-square statistics. Therefore, the study of inheritance of resistance as a quantitative trait in these populations seemed reasonable.

The slopes of regression of $F_{2:3}$ mean rust scores on F_2 parent scores were estimated to be 0.30 ± 0.04 in the 1416-1 population and 0.50 ± 0.05 in the 1497-2 population (Table 2). These heritability estimates were free from biases arising from covariances among parent and offspring genotype-by-environment interactions because parent and offspring generations were measured in separate, independent environments (Casler 1982). These estimates would be biased upward by any dominance or additive-by-additive epistatic genetic variances present in the populations. Therefore, these estimates should be considered as upper limits of heritabilities for resistance as measured on individual plants in these populations.

Environmental influences may strongly affect the expression of resistance, and this would limit the heritability of the trait on a single-plant basis. Replicated progeny testing of $F_{2:3}$ families allowed good estimation of the level of rust resistance of entries, as indicated by the repeatable performances of resistant and susceptible check entries across experiments (Table 3), and by the high repeatabilities of resistance among selected families on a family mean basis, both in the field and in the greenhouse (Table 2). Heritabilities for southern rust scores estimated based on the variances among $F_{2:3}$ families (single-plot basis) in the 1991 experiment were 25 and 35 percentage points higher than those estimated from offspring-parent regression (Table 2). Two factors could contribute to these higher estimates: (1) variation due to genotype-by-environment interactions, which are included in the $F_{2:3}$ family variance estimate, but excluded from the offspring-parent regression estimate; and (2) reduced phenotypic variance of scores based on ratings of a sample of progeny within a plot compared to ratings of individual plants. Variance due to genotype-by-environment interactions was estimated based on the evaluations of selected $F_{2:3}$ families in both 1991 and 1994. Genotype-by-environment interaction variance was significant only in the selected families of the 1497 population, but was of small magnitude compared to the genotypic variance. This can be confirmed by noting that the repeatability estimates of scores on the selected families of the 1497 population based on a single environment or replicated environments were almost identical (Table 2). While this does not rule out the possibility that genotype-by-environment interactions are important for scores made in Florida and North Carolina, it does suggest that improved precision of scores based on progeny rows was a major factor in increasing the heritability of the trait.

Repeatabilities for southern rust scores based on evaluations of selected families were higher than corresponding heritabilities estimated from unselected populations. This is expected because variance among extreme families selected from a population will be greater than variance among the whole population. The extent of the bias introduced to the repeatability estimates can be gauged by comparing heritability on a plot basis computed from unselected families in 1991 and repeatabilities calculated from subsets of the same data corresponding to the selected families. In this case, the repeatability estimates from the two populations were 16 and 23 percentage points higher than their corresponding heritability estimates (Table 2).

Table 2 Heritabilities and repeatabilities of southern rust scores estimated from unselected and selected families, respectively

Basis of estimate	Environments	Estimate	
		1416 pop	1497 pop
Field scores			
Heritability			
$F_{2:3}$ mean regression on F_2 parent, individual-plant basis	FL and NC 1991	30%	50%
$F_{2:3}$ family variance, single-plot basis	NC 1991	65%	75%
$F_{2:3}$ family variance, family mean basis	NC 1991	79%	86%
Repeatability			
Selected $F_{2:3}$ family variance, single-plot basis	NC 1991	88%	91%
Selected $F_{2:3}$ family variance, family mean basis	NC 1991	93%	95%
Selected $F_{2:3}$ family variance, single-plot basis	NC 1991, 1994	88%	90%
Selected $F_{2:3}$ family variance, family mean basis	NC 1991, 1994	97%	96%
Greenhouse pustule counts			
Repeatability			
Selected $F_{2:3}$ family variance, individual-plant basis	Greenhouse	66%	73%
Selected $F_{2:3}$ family variance, family mean basis	Greenhouse	95%	86%

Table 3 Check entry means for southern rust-resistance experiments

Entry	Field						Greenhouse	
	Southern rust score						No. of pustules	
	1416-1 pop			1497-2 pop			1416-1	1497-2
	1991	1994	Mean	1991	1994	Mean		
1416-1	9.0	9.0	9.0	9.0	9.0	9.0	0	0
1497-2	9.0	9.0	9.0	9.0	9.0	9.0	0	0
B73*Ht* × Mo17*Ht*	3.5	3.0	3.3	2.5	2.5	2.5	81	96
F_1 remnants	9.0	9.0	9.0	9.0	9.0	9.0	0	1
N8727	2.0	3.5	2.8	2.0	2.0	2.0	24	44
3369A	2.0	2.5	2.3	2.0	3.0	2.5	43	32
3165	–	3.0	–	–	3.0	–	56	30
B37*Rpp9*	–	9.0	–	–	9.0	–	0	0
B14A*Ht*	–	1.5	–	–	1.5	–	5	6
LSD (0.05)	1.6	1.4	1.1	1.9	0.6	1.3	14	18
CV (%)	10.7	9.3	9.0	14.0	4.6	9.4	86	56

Identification of genomic regions possessing resistance genes

RFLP analysis indicated that a gene or genes with major effects is/are located on the short arm of chromosome 10 of both resistant tropical inbred parents (Tables 4 and 5). Based upon single-marker ANOVA, the major gene(s) for resistance appear to be most closely linked to *bnl3.04* on chromosome 10. Among all markers, *bnl3.04* explained the highest proportion of variance for rust resistance in both populations, both in the field and greenhouse studies. Variation in genotypes at *bnl3.04* accounted for 82–83% of the phenotypic variation for southern rust scores among selected $F_{2:3}$ families in the two populations.

Genes with minor effects on rust resistance were detected on chromosomes 3 and 4. Loci *umc26* (chromosome 3) and *umc31* (chromosome 4) each accounted for 13–15% of the variation in resistance expressed by $F_{2:3}$ families of the 1497-2 population in the field (Tables 4 and 5). In the 1416-1 population, chromosome 4 appeared to have no effect, while *umc26* was significant at $P = 0.06$, suggesting that a minor gene could exist in that population also. Fewer families were genotyped in the 1416-1 population compared to the 1497-2 population. Therefore, there was less statistical power to detect minor genes in the 1416-1 population. Increased sampling error in small samples of progeny may also contribute to inability to detect minor genes. Locus *umc26* was not associated with resistance measured on F_2 individuals or on $F_{2:3}$ families in the greenhouse at $P = 0.10$ in the 1416-1 population (Table 4). *umc26* was significant for all traits, while *umc31* was significant only at $P = 0.06$–0.07 in F_2 individuals and in the greenhouse in the 1497-2 population (Table 5). Higher coefficients of variation were obtained from the

Table 4 RFLP loci-associated significant ($P \leq 0.10$) effects on rust resistance in the (B73*Ht* × Mo17*Ht*) × 1416-1 population

Chromosome	Locus	F_2 Field score			$F_{2:3}$ Field score			$F_{2:3}$ Greenhouse pustule count		
		a[a]	r^2	*P*-value	a	r^2	*P*-value	a	r^2	*P*-value
3	*umc26*	NS[b]			− 0.8	0.10	0.06	NS		
10	*bnl10.17*	1.9	0.49	< 0.001	2.2	0.78	< 0.001	–9.8	0.73	< 0.001
10	*bnl3.04*	2.0	0.52	< 0.001	2.2	0.82	< 0.001	–9.8	0.81	< 0.001
10	*npi285*	1.8	0.44	< 0.001	2.2	0.79	< 0.001	–8.5	0.75	< 0.001
10	*isu167*	1.6	0.32	0.001	1.6	0.37	< 0.001	–3.2	0.28	0.002
10	*umc130*	1.3	0.19	0.01	1.5	0.32	< 0.001	–3.6	0.26	0.003

[a] a = additive effect of marker = (mean of families homozygous for 1416-1 allele minus mean of families homozygous for allele from B73 × Mo17)/2
[b] NS – effect not significant at $P = 0.10$

Table 5 RFLP loci associated with significant ($P \leq 0.10$) effects on rust resistance in (B73*Ht* × Mo17*Ht*) × 1497-2 population

Chromosome	Locus	F_2 Field score			$F_{2:3}$ Field score			$F_{2:3}$ Greenhouse pustule count		
		a[a]	r^2	*P*-value	a	r^2	*P*-value	a	r^2	*P*-value
3	*umc10*	− 1.1	0.06	0.10	− 1.2	0.09	0.06	NS[b]		
3	*umc26*	− 1.4	0.11	0.03	− 1.6	0.13	0.01	2.6	0.12	0.02
3	*umc102*	− 1.3	0.09	0.04	NS			NS		
3	*bnl5.37*	− 1.1	0.08	0.10	− 1.3	0.11	0.04	1.8	0.11	0.04
4	*umc31*	− 0.9	0.07	0.06	− 1.0	0.15	0.03	2.6	0.12	0.07
4	*bnl5.46*	− 1.0	0.07	0.07	− 1.1	0.10	0.04	2.4	0.09	0.07
10	*bnl10.17*	1.5	0.28	< 0.001	2.1	0.43	< 0.001	− 10.1	0.54	< 0.001
10	*bnl3.04*	2.1	0.49	< 0.001	2.6	0.83	< 0.001	− 12.5	0.83	< 0.001
10	*npi285*	2.0	0.44	< 0.001	2.5	0.61	< 0.001	− 10.4	0.53	< 0.001
10	*isu167*	1.6	0.23	< 0.001	2.6	0.59	< 0.001	− 8.3	0.45	< 0.001
10	*umc130*	1.4	0.20	0.002	2.1	0.40	< 0.001	− 6.9	0.33	< 0.001

[a] a = additive effect of marker = (mean of families homozygous for 1497-2 allele minus mean of families homozygous for allele from B73 × Mo17)/2
[b] NS – effect not significant at $P = 0.10$

greenhouse pustule counts compared to the $F_{2:3}$ field scores. Error variances could not be calculated for the unreplicated individual F_2 scores, but one would expect replicated progeny tests to provide more precise estimates of resistance than scores of individual plants. The lower precision of greenhouse pustule counts and individual F_2 field scores compared to $F_{2:3}$ ratings probably accounted for the reduced statistical significance of the effects of *umc26* and *umc31* on these traits.

The alleles providing increased resistance (higher field scores or lower greenhouse pustule counts) near the marker loci on chromosomes 3 and 4 with significant effects were from the susceptible parent (B73*Ht* × Mo17*Ht*). In both populations, the alleles from Mo17 rather than B73 were segregating at *umc26* and *umc31*. This suggests that alleles from Mo17 at genes near *umc26* and *umc31* may confer partial resistance to southern rust. Bailey et al. (1987) reported that Mo17 has significantly superior slow-rusting resistance compared to B73.

There was good agreement among greenhouse and field ratings for the relative effects of markers (Tables 4 and 5). This suggests that the genes involved in reducing pustule number on young, greenhouse-grown plants were the same as those that affected resistance scores in the field. Greenhouse pustule counts in the 1497-2 population appeared to be influenced by a gene having major effects (chromosome 10) as well as genes with minor effects (chromosomes 3 and 4, Table 5).

The estimates of additive effects associated with markers based on selective genotyping are biased upward with respect to the complete F_2 reference population (Lander and Botstein 1989; Darvasi and Soller, 1992). The selective genotyping provided greater efficiency for the detection of QTLs, but over-estimated their F_2 population effects. The correction suggested by Darvasi and Soller (1992) was derived assuming a normal distribution, which was violated in these data (Figs. 1 and 2). Therefore, no attempt was made to correct the bias, but it should be noted that while these effect estimates are valid for the set of genotypes selected, they do not represent gene effects in the reference population of unselected $F_{2:3}$ families derived from these crosses. It was already noted that the repeatabilities of

selected families were 16–23 percentage points higher than the heritabilites of unselected families due to this same selection bias.

Despite the fact that the skewness in the $F_{2:3}$ population distribution suggested dominant gene action for resistance, we found little evidence for significant (at $P = 0.05$) marker locus-associated dominance. *umc26* was significantly associated with a dominant effect in F_2 individuals, and *bnl10.17* and *npi285* were associated with a dominant effect on $F_{2:3}$ field and greenhouse scores in the 1416-1 population only. Dominance is expected to be more difficult to detect from $F_{2:3}$ family means compared to F_2 individual values because selfing one generation reduces heterozygosity, and therefore the expression of dominance, to half of its value in the F_2 generation.

Multiple marker-locus models for resistance

Having detected additive effects on different chromosomes, it was of interest to estimate the combined effects of significant markers, and also to test for interactions among markers, which might suggest epistasis. A gene conferring complete resistance to southern rust would partly mask the effects of minor genes conferring partial resistance in an epistatic fashion. A single locus was chosen from each of chromosomes 3, 4, and 10 to develop multiple-locus models including epistasis for each trait. *umc26*, *umc31*, and *bnl3.04* were chosen to develop models for each population because they had the most consistently large effects among loci from each of the three chromosomes. The best model for each trait (except for F_2 individuals in the 1497-2 population) included at least one epistatic interaction in addition to the main effects of loci on at least two different chromosomes (Tables 6 and 7). Surprisingly, *umc31* was included in the best models for $F_{2:3}$ field and greenhouse scores in the 1416-1 population, although it was not significant individually. The best model for $F_{2:3}$ field scores in the 1497-2 population included all three markers and all two-way interactions and explained 99% of the phenotypic variation. These results suggest that minor genes affecting partial rust resistance exist on chromosomes 3 and 4 and that they interact epistatically with the major gene on 10S.

The predictive ability of the models can be gauged by comparing them to the repeatabilities of selected families on a family mean basis, to which they correspond. The best multiple-locus models in the 1416-1 population were associated with 96% of the phenotypic variation among field scores, which had a corresponding repeatability of 97%, and with 92% of the phenotypic variation among greenhouse pustule counts, which had a repeatability of 95% (Table 2 and 5). In the 1497-2 population, the best models accounted for 99% of the phenotypic variation among field scores, which had a repeatability of 96%, and for 85% of the phenotypic variation for greenhouse pustule counts, which had a repeatability of 86%. Thus, the multiple-locus models were able to account for almost as much phenotypic variation for resistance among selected lines as could the genotypic variance. In the case of field scores in the 1497-2 population, however, the best multiple-locus model accounted for slightly more than the genotypic variation, indicating that the model also accounted for some error variation, resulting in an overestimate of its predictive ability. Some upward bias in predictive ability due to overfitting of models developed from small data sets is not surprising. In this case, the difference between repeatability and model adjusted R^2 was only three percentage points, however, suggesting that the problem was probably not severe. Finally, the predictive ability of the models was biased due to selection of extreme genotypes in the same manner as were the single-locus models and the repeatability estimates.

Allelism test

The consistency of the effect of the 10S region across populations, generations, and environments suggests that both 1416-1 and 1497-2 possess an allele with major effects on resistance to southern rust located between RFLP loci *bnl*3.04 and *npi*285 on chromosome 10. *bnl3.04* and *npi285* flank the *Rp1* locus (Hulbert and Bennetzen 1991), which has been estimated to be 1.6 cM from the *Rpp9* locus (Ullstrup 1965). Although, to our knowledge, *Rpp9* has not been directly mapped with RFLP markers, it is most likely also located within the region defined by *bnl3.04* and *npi285*. Therefore, it is possible that the major genes for southern rust resistance identified in the tropical inbreds studied here are identical to or allelic to *Rpp9*.

The test of allelism of the major resistance gene from 1416-1 and *Rpp9* proved inconclusive because both parental sources of resistance, 1416-1 and B37*Rpp9*, lacked complete resistance to southern rust under the conditions of the experiment. It is not clear whether this was due to the unusually hot conditions under which they were grown or to the presence of a race of *P. polysora* which possesses virulence on both sources of resistance. Inactivation of rust-resistance genes in wheat (*Triticum aestivum* L.) by high temperatures has been reported (Kaul and Shaner 1989). Only a single plant of each resistant parental line survived to be scored. Using a five-point scale, in which 1 = no disease symptoms and 5 = maximum expression of susceptibility, 1416-1 was scored as 2.5 and B37*Rpp9* was scored as 3.5. In contrast, B14A*Ht*, had a mean score of 4.5. The experiment was designed to test discrete segregation ratios within testcross progeny families and, therefore, no valid estimate of experimental error is available to test comparisons among these scores. The scores of the six B14A*Ht* plants ranged from 4 to 5, however, and these were planted immediately adjacent

Table 6 Best multiple-locus models for the (B73*Ht* × Mo17*Ht*) × 1416-1 population. Best model for a trait was that model for which R^2 was highest and all factors in the model were significant at $P = 0.05$

F_2 Field score			$F_{2:3}$ Field score			$F_{2:3}$ Greenhouse pustule count		
Factor	Type III SS	*P*-value	Factor	Type III SS	*P*-value	Factor	Type III SS	*P*-value
bnl3.04	75.8	0.0001	*bnl3.04*	125.1	0.0001	*bnl3.04*	67.4	0.0001
umc26	30.0	0.0025	*umc31*	16.3	0.0001	*umc31*	6.6	0.0001
*bnl3.04***umc26*	22.2	0.0099	*bnl3.04***umc31*	19.8	0.0001	*bnl3.04***umc31*	8.5	0.0001
MODEL ADJ. $R^2 = 0.61$			MODEL ADJ. $R^2 = 0.96$			MODEL ADJ. $R^2 = 0.92$		

Table 7 Best multiple-locus models for the (B73*Ht* × Mo17*Ht*) × 1497-2 population. Best model for a trait was that model for which R^2 was highest and all factors in the model were significant at $P = 0.05$

F_2 Field score			$F_{2:3}$ Field score			$F_{2:3}$ Greenhouse pustule count		
Factor	Type III SS	*P*-value	Factor	Type III SS	*P*-value	Factor	Type III SS	*P*-value
bnl3.04	180.0	0.0001	*bnl3.04*	135.7	0.0001	*bnl3.04*	56.9	0.0001
			umc26	1.9	0.0004	*umc26*	4.6	0.0062
			umc31	4.0	0.0001	*bnl3.04***umc26*	6.1	0.0045
			*bnl3.04***umc26*	2.2	0.0005			
			*bnl3.04***umc31*	6.8	0.0001			
			*umc26***umc31*	1.1	0.0182			
MODEL $R^2 = 0.49$			MODEL ADJ. $R^2 = 0.99$			MODEL ADJ. $R^2 = 0.85$		

to the plots containing 1416-1 and B37*Rpp9*, indicating a rather clear difference between B14AHt and the two parental sources of resistance. Furthermore, the 171 testcross family mean scores ranged from 2.0 to 4.4, with a mean of 3.1, suggesting that both *Rpp9* and the gene from 1416-1 confer partial resistance to southern rust in Mexico, outside of their adaptive range.

Conclusions

Our results suggest that resistance to southern corn rust in these populations is under relatively simple genetic control, with a major gene or genes on chromosome 10S and minor genes on chromosomes 3 and 4, together with their epistatic interactions, explaining much of the phenotypic variation for resistance. The possibility exists that other regions of the genome possess genes with minor effects on resistance in these populations, but our experiments do not address this issue. The heritabilities of the trait in the two populations range from 30% and 50% on a single-plant basis to 65% and 75% on a progeny plot basis to 79% and 86% on a progeny mean basis in the environments studied. The dramatic increase in heritability achieved by replicated progeny testing suggests that much of the phenotypic variation for southern rust observed on a single-plant basis is attributable to random micro-environmental error effects that can be controlled by progeny testing and replication. The genomic regions studied affected resistance in a consistent manner in adult plants in the field and in juvenile plants in the greenhouse. We were unable to determine whether or not a major resistance gene detected on chromosome 10S was allelic to *Rpp9*.

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