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ORIGINAL PAPER

Mapping resistance to Southern rust in a tropical by temperate maize recombinant inbred topcross population

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Abstract Southern rust, caused by *Puccinia polysora* Underw, is a foliar disease that can severely reduce grain yield in maize (*Zea mays* L.). Major resistance genes exist, but their effectiveness can be limited in areas where *P. polysora* is multi-racial. General resistance could be achieved by combining quantitative and race-specific resistances. This would be desirable if the resistance alleles maintained resistance across environments while not increasing plant maturity. Recombinant inbred (RI) lines were derived from a cross between NC300, a temperate-adapted all-tropical line, and B104, an Iowa Stiff Stalk Synthetic line. The RI lines were topcrossed to the tester FR615 × FR697. The 143 topcrosses were scored for Southern rust in four environments. Time to flowering was measured in two environments. The RI lines were genotyped at 113 simple sequence repeat markers and quantitative trait loci (QTL) were mapped for both traits. The entry mean heritability estimate for Southern rust resistance was 0.93. A multiple interval mapping model, including four QTL, accounted for 88% of the variation among average disease ratings. A major QTL located on the short arm of chromosome 10, explained 83% of the phenotypic variation, with the NC300 allele carrying the resistance. Significant ($P < 0.001$), but relatively minor, topcross-by-environment interaction occurred for Southern rust, and resulted from the interaction of the major QTL with the environment. Maturity and Southern rust rating were slightly correlated, but QTL for the two traits did not co-localize. Resistance was simply inherited in this population and the major QTL is likely a dominant resistant gene that is independent of plant maturity.

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Introduction

Southern rust, caused by *Puccinia polysora* Underw, has been a major problem for corn production in Africa (Agarwal et al. 2001) and Asia (Chen et al. 2004). In the southern United States, significant Southern rust infections have occurred approximately once in five years. When epiphytotics have occurred they were often serious, causing yield losses of up to 45% (Raid et al. 1988; Rodriguez-Ardon 1980). The periodic nature of Southern rust epiphytotics has made breeding for resistance challenging, and the severity of the disease when it does occur can be attributed, in part, to the limited resistance of the U.S. maize crop (Futrell 1975; Futrell et al. 1975).

Several races of *P. polysora*, distinguished by the reactions they incite on different maize lines, have been reported. Three races, EA1, EA2 and EA3 were found in East Africa (Ryland and Storey 1955; Storey and Ryland 1954; Storey and Howland 1961). Six further

races (PP. 3, PP. 4, PP. 5, PP. 6, PP. 7 and PP. 8) were identified from North and Central American isolates and were shown to be distinct from the East African races (Robert 1962). A tenth *P. Polysora* race (PP. 9) was discovered by Ullstrup (1965).

At least three unique, major, race-specific Southern rust resistance genes have been discovered. Major genes, *Rpp1* and *Rpp2* were identified by Storey and Howland (1957) and confer resistance to *P. polysora* races EA1 and EA2, respectively. These genes were shown to be loosely linked to each other (Storey and Howland 1959), but their genomic locations have not been determined. A major resistance gene, *Rpp9*, conferring resistance to *P. polysora* race PP. 9 was identified from Boesman Yellow Flint (Ullstrup 1965). It was shown to be closely linked to the *Rp1* gene for resistance to common maize rust (causal agent *Puccinia sorghi* Schw.) on the short arm of chromosome 10. Another major gene that also confers resistance to race PP. 9 was identified (Futrell 1975), but its linkage and allelic relationships with *Rpp9* were not established.

Major genes for resistance to Southern rust on the short arm of chromosome 10 have been reported in at least four different subsequent studies, using different sets of maize germplasm (Scott et al. 1984; Holland et al. 1998; Liu et al. 2003; Chen et al. 2004). The major genes reported in these studies were closely linked to *Rpp9* in each case, but linkage or allelic relationships and racial specificity of these genes were not determined. This knowledge is of importance as major resistance (race-specific) genes commonly fail in the tropics, where multiple races of *P. polysora* exist (Carlos and Ferreira 2002). The loss of valuable maize and fungal stocks from the closing of both Hooker and Ullstrup programs in combination with the tight linkages of dominant resistance genes on chromosome 10 has made obtaining such information very difficult.

General resistance could be achieved by combining quantitative and race-specific resistances. This would be desirable if the resistance alleles maintained resistance across environments while not increasing plant maturity. Quantitative trait loci (QTL) for Southern rust resistance have been mapped on chromosomes 3 and 4 (Holland et al. 1998), 3, 4 and 9 (Jiang et al. 1999) and 9 (Brunelli et al. 2002), but none co-localized across studies (Wisser et al. 2006). Most of these experiments, unfortunately, did not use complete genome coverage in mapping resistance QTL, and some QTL may have not been detected. In addition, the effectiveness of the resistance provided by these QTL to hybrids was not addressed.

In these Southern-rust-resistance mapping studies, the interaction between resistance genes and the environment was not extensively investigated because the phenotypic distributions of populations were often assumed to result from simple modes of inheritance. Only Holland et al. (1998) evaluated Southern rust resistance in multiple environments, and they reported significant, but relatively minor, genotype-by-environment interaction. However, they did not investigate QTL-by-environment interaction. Understanding the interaction between QTL and the environment is important in determining the consistency of QTL effects over environments (Bubeck et al. 1993). Such knowledge can further assist in choosing candidate QTL for marker assisted selection (MAS) by preventing erroneous decisions resulting from often overestimating the percent of phenotypic variation explained by QTL (Bubeck et al. 1993; Beavis et al. 1998).

Southern rust, and other foliar diseases of maize, such as gray leaf spot and anthracnose (caused by *Cercospora zeae-maydis* Tehon and E. Y. Daniels and *Colletotrichum graminicola* (Ces.) G. W. Wils, respectively), are generally late season diseases in North Carolina, with most disease development occurring post-anthesis (White 1999). Although significant correlation between Southern rust and maturity has not been reported, there is concern that disease ratings could be correlated with maturity, as demonstrated in studies that mapped resistance to other diseases and maturity QTL in the same populations (Bubeck et al. 1993; Carson et al. 2004; Clements et al. 2000; Jiang et al. 1999; Jung et al. 1994). These studies collectively demonstrated that disease resistance and flowering time were slightly correlated, that QTL for each trait would sometimes map to similar genomic regions, and that such regions usually increased both disease resistance and maturity.

The infrequent occurrence of Southern rust in the United States has resulted in inconsistent selection environments, which has led to difficulties in selecting and maintaining Southern rust resistance in U.S. maize breeding lines. In the absence of selection pressure, stochastic processes govern gene frequencies in breeding populations (Wright 1952). Such processes can often result in losing alleles, especially those with minor effects on resistance, from populations, as has occurred with common rust resistance genes (Davis et al. 1990). In this case, it might be more effective to use marker-assisted selection for loci linked to major and partial-resistance QTL, despite the questionable durability of major race-specific resistance alleles.

The first objective of this study was to localize and estimate the effects of minor and major sources of Southern rust resistance loci using DNA markers with thorough genome coverage in a tropical by temperate

RI topcross population for potential use in developing resistant hybrid varieties via MAS. The second objective was to determine the impact of genotype-by-environment interaction on the expression of Southern rust resistance genes. The final objective was to determine the relationship between Southern rust resistance and time to flowering. This knowledge is important as differences in maturity can be confounded with foliar disease resistance measurements and later maturity can lead to increased production costs (Hawbaker et al. 1997).

Materials and methods

One hundred and forty-three $S_{4:5}$ recombinant inbred (RI) lines were developed from single seed descent from a cross between NC300, an all-tropical, temperate-adapted line, and B104, an Iowa Stiff-Stalk Synthetic line. The inbreeding coefficient of the RI lines was expected to be $F = 0.97$ (Cockerham 1983). Each RI line was topcrossed to the C103 (Lancaster) type tester FR615 × FR697. Topcrosses of the RI lines had much more uniform maturity than the RI lines themselves and made scoring of Southern rust resistance possible. The 143 topcrosses were randomly subdivided into two sets and the experimental design deployed was a replication-within-sets design.

Topcrosses and commercial checks are referred to as entries. Set 1 consisted of 81 entries including 67 topcrosses and twelve commercial hybrid checks. Set 2 consisted of 90 entries including 76 topcrosses and 12 commercial hybrid checks. Both sets shared the same commercial checks (DK689, DK697, DK743, G8288, LH132 × LH51, LH195 × LH256, LH200 × LH262, NK91-R9, P31G98, P32K61, P3394, TR7322 × HC33), as well as parental topcrosses between NC300 and B104 to the tester, FR615 × FR697. Sets were grown at four North Carolina locations in 2003. Locations included Clayton, Jackson Springs, Salisbury, and Plymouth, N.C. Lattice designs for each set (9 × 9 and 10 × 9, respectively) were used to assign entries to experimental units at the Clayton, Jackson Springs, and Plymouth locations. A randomized complete block design (RCBD) was used for each set at Salisbury.

Three replicates were grown at the Clayton, Jackson Springs, and Plymouth locations, whereas, two replicates were grown at Salisbury. Experimental units consisted of two 4.86-m length-rows containing a total of 44 plants at all locations except Salisbury. An experimental unit at the Salisbury location was a single 4.86-m row with 20 plants. A 1-m alley was allocated at the end of each plot at all locations. Inter-row spacing was 0.91 m at the Salisbury location and 0.97 m elsewhere. Plots were over-planted to obtain a target plant density of 43,000 plants ha^{-1} (44 plants per plot) at all locations except Salisbury, where plant density was 54,147 plants ha^{-1} (20 plants per plot).

Response variables measured on plots included Southern rust rating and anthesis date. Natural inoculum was relied upon, and a late season visual Southern rust rating was taken at all locations approximately two weeks prior to harvest. *P. polysora* has several tropical alternative hosts, but comes into North Carolina as urediospores, probably in many cases from a very restricted origin that then leads to secondary inoculation (Ullstrup 1977; Shurtleff 1986). Southern rust is apparently not multi-racial in North Carolina and rarely impacts grain yield, as the disease tends to occur late in the growing season.

Ratings were recorded on a plot basis (i.e., the visual average of all plants in a plot) using a nine-point scale, with one designated as fully susceptible and nine as fully resistant (Holland et al. 1998). Ratings were based upon the percent leaf area of a plot affected by pustules and impact of the disease on late season plant health. Anthesis date, measured as days from planting until 50% of the pollen in a plot shed, was recorded at the Clayton location in 2002 and 2003. (The same sets using corresponding experimental designs as the Clayton 2003 location were also grown at Clayton in 2002, but Southern rust was not present.)

Genotyping and linkage map construction

Genotypic information for 113 simple sequence repeat markers has been reported previously for the RI lines (Robertson-Hoyt et al. 2006). Briefly, a linkage map was constructed with a length of 1,993 cM and an average distance between markers of 17.64 cM. Eight percent of the genotypic data was missing, half of which involved heterozygous loci. Twelve percent of the markers displayed significant ($P \leq 0.01$) segregation distortion, which is typical in maize mapping populations (Lu et al. 2002). Marker-locus ordering was in agreement with the consensus genetic maps of maize (http://www.maizegdb.org).

Statistical analysis for phenotypic data

Within environment analyses were performed for each response variable and set-by-environment combination. Analyses included fitting spatial and conventional mixed models. The conventional model corresponded to the appropriate analysis associated with the experimental design used (i.e., a lattice or RCBD). Spatial models

included trend, trend-plus-correlated-errors, and correlated-errors analyses. Entry was treated as a fixed effect in all analyses. Trend effects were modeled as first through fourth degree polynomial terms for rows and columns in the trend and trend-plus-correlated-errors analyses (Brownie et al. 1993). A spatial power function was specified to model local effects for the trend-plus-correlated errors and correlated-errors analyses.

Only significant ($P \leq 0.01$) global effects were retained in the spatial models (Tamura et al. 1988), and the analysis with the largest *F*-value for entry main effects was considered the preferred model for each set-by-environment combination (Brownie and Gumpertz 1997). Within-environment spatial analyses were performed because accounting for spatial variation can often improve entry mean estimation (Brownie et al. 1993; Brownie and Gumpertz 1997; and Gilmour et al. 1997) and QTL mapping (Moreau et al. 1999; Smith et al. 2002).

A combined analysis across environments was then performed for each set. Entry least square means from the preferred model for each set-by-environment combination served as the response variable in the combined analyses. Combined analyses were performed using PROC MIXED in SAS version 8.2 (Littell et al. 1996; SAS Institute 1999), considering environment to be a random factor and entry to be a fixed factor.

A limitation of spatial analytical approaches is the difficulty in testing for the presence of genotype-by-environment interaction, because different models are fit for each environment (Qiao et al. 2004) and genotype-by-environment interaction is the residual term in the combined analysis. To test the significance of the entry-by-environment interaction term, a data set lacking the commercial checks was constructed. Using this subset of data, a model was fitted in PROC GLM that included set, environment, set-by-environment interaction, replication-nested-within-set-by-environment interaction, entry-by-environment-nested-within set as random factors while entry-nested-within set was considered fixed. An appropriate *F* test was performed to test the significance of the entry-by-environment-nested-within set factor.

Entry mean heritabilities were estimated for each response variable following Holland et al. (2003). The model included random sources of variation due to environment, set, set-by-environment interaction, replication-nested-within-set-by-environment interaction, entry-nested-within set, and entry-by-environment-nested-within-set interaction. Approximate standard errors were estimated by the delta method.

Entry means from the combined analyses were adjusted for set effects, using set means as the adjustment (Schutz and Cockerham 1962). Set-adjusted entry mean comparisons were performed which involved constructing pooled error terms to calculate least significant differences. The set-adjusted entry means from the across environment analyses served as response variables in subsequent analyses involving QTL mapping.

Spearman rank correlation coefficients for Southern rust ratings were estimated with PROC CORR in SAS version 8.2 (SAS Institute 1999) for all pair-wise combinations of set-adjusted entry means from the preferred within-environment analyses. In addition, a Spearman rank correlation coefficient was estimated between set-adjusted entry means from the combined analyses for both Southern rust and flowering date.

Composite and multiple interval mapping (MIM) were performed in Windows QTL-Cartographer version 2.5 (Wang et al. 2001–2004) for each response variable following Robertson-Hoyt et al. (2006). Composite interval mapping (CIM) was used initially to map QTL for all phenotypic data sets (PDS). Both backward and forward selection procedures were specified to perform the permutation testing and cofactor selection. The threshold for factors to enter and remain in the model was 0.01 and a window size of 10 cM was selected for the genome scans.

QTL positions from CIM pertaining to the across-environment analyses were designated in an initial model for MIM. The MIM models were created and tested in an iterative fashion and the Bayesian information criterion (BIC) was used for model selection (Piepho and Gauch 2001). After identifying QTL additive-topcross-main effects, additive-by-additive topcross epistactic interaction effects were tested among all pair-wise combinations of QTL. Epistatic interactions were retained in the model if the BIC was reduced. After identifying the best model, QTL effects were simultaneously estimated using the "summary" option. Genetic variability explained by QTL for each response variable was calculated as the total phenotypic variation explained by QTL divided by the entry mean heritability estimate.

Marker-by-environment interactions on Southern rust scores were tested by ANOVA in PROC GLM in SAS version 8.2 (SAS Institute 1999). Markers closest to QTL positions identified by MIM were included in the multiple factor ANOVA. The model included marker-nested-within-set and environment-by-marker-nested-within-set as fixed and random factors, respectively, for each marker. Additional random factors were set, environment, set-by-environment, and replication-nested-within-set-by- environment. The error variance of the model included pooled variation due to

higher order interactions among markers and environment as well as residual variations.

Results

Set-adjusted RI topcross ratings from the combined analysis displayed a bimodal distribution (Fig. 1). This suggested a single major gene might be responsible for most of the variation. The entry mean heritability estimate for Southern rust in this population was 0.93 (standard error 0.01). The severity of the Southern rust epiphytotic in 2003 is evident by the low average rating of the commercial checks (Table 1). The NC300 topcross was rated 7.8 whereas the B104 topcross was rated 3.5. The range of the RI topcross ratings was greater than the range of the parental topcrosses, although no significant transgressive segregates ($P = 0.05$) were observed (Table 1). The mean rating of the RI topcrosses did not differ significantly ($P = 0.05$) from the parental topcross average. One hundred RI topcrosses were rated significantly higher ($P = 0.05$) than the mean of the commercial checks (data not shown).

Significant ($P < 0.001$) entry-by-environment interaction was observed for Southern rust (Table 2). Despite the significant interaction, Spearman rank correlation coefficients among set-adjusted-entry means obtained from the within environment analyses were high, with the lowest pair-wise correlation being 0.8 (Table 3). Further, the entry main effect was highly significant ($P < 0.0001$), as the entry mean square was nearly 17 times larger than the entry-by-environment mean square (Table 2). For this reason, MIM was only performed on entry means from the combined analyses.

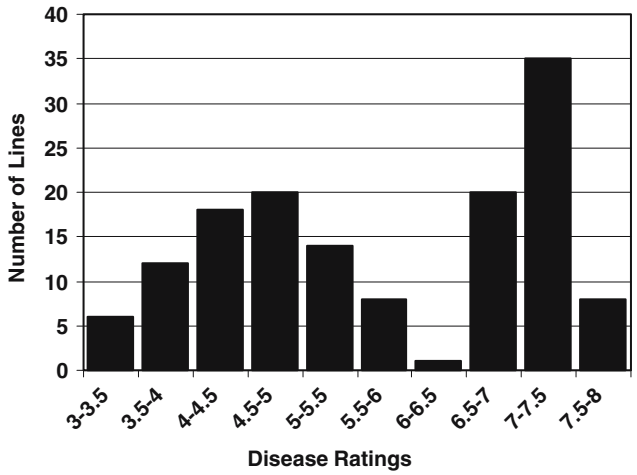

Fig. 1 A histogram of set-adjusted Southern rust rating entry means combined over four environments in 2003 for a population of 143 NC300/B104 maize recombinant inbred lines topcrossed with FR615 × FR697. Ratings were made on a 1–9 scale with '1' being susceptible and '9' fully resistant

Table 1 The five most resistant and five most susceptible NC300/B104 RI topcrosses to FR615 × FR697, and the average resistance ratings of the commercial checks (check mean), parental topcrosses (parental topcross mean), and RI topcrosses (RI topcross mean) to Southern rust (rust) combined across four environments in 2003

RI topcrosses	Rust[a]
2054 × (FR615 × FR697)	7.84
1976 × (FR615 × FR697)	7.76
2070 × (FR615 × FR697)	770
1972 × (FR615 × FR697)	7.68
2012 × (FR615 × FR697)	7.62
1968 × (FR615 × FR697)	3.51
2044 × (FR615 × FR697)	3.30
2004 × (FR615 × FR697)	3.29
2021 × (FR615 × FR697)	3.29
2039 × (FR615 × FR697)	3.26
Parental topcrosses	
NC300 × (FR615 × FR697)	7.76
B104 × (FR615 × FR697)	3.46
Check mean	3.99
RI topcross mean	5.73
Parental topcross mean	5.61
$LSD1^{b}_{\alpha = 0.05}$	0.95
$LSD2^{c}_{\alpha = 0.05}$	0.82
$LSD3^{d}_{\alpha = 0.05}$	0.69
$LSD4^{e}_{\alpha = 0.05}$	0.34

[a] Ratings are on a 1–9 scale, with a '1' denoting susceptibility and a '9' designating full resistance

[b] Appropriate for comparing RI topcrosses

[c] Appropriate for comparing RI topcrosses to a parental topcross

[d] Appropriate for comparing RI topcrosses to the check mean

[e] Appropriate for comparing the mean RI topcross rating to the parental topcross mean

Table 2 The combined ANOVA across four environments for Southern rust rating in 2003, of a population of 143 NC300/B104 maize recombinant inbred lines topcrossed with FR615 × FR697, using a replication nested-within-sets design

Source[a]	DF	MS	*F* value	*P* value
Entry (set)	141	21.72	16.61	<0.0001
Environment × entry (set)	423	1.31	2.16	<0.0001
Error	984	0.61		

$R^2 = 0.90$, CV = 13.71

[a] Sources of variation due to environment, set, environment-by-set, and replication nested within environment-by-set are not presented in the ANOVA

A major Southern rust QTL was mapped by CIM on the short arm of chromosome 10. Map position was 6.01 cM for the major QTL and was positioned between markers UMC1380 and BNLG1451 (bins 10.0 and 10.1, respectively). The NC300 allele increased resistance with an additive effect of 1.3 and explained

Table 3 Spearman rank correlation coefficients among all pairwise combinations of within-environment, set-adjusted, least square Southern rust resistance entry means from a population of 143 NC300/B104 maize recombinant inbred lines topcrossed with FR615 × FR697 and scored in four environments in 2003

	Clayton	Plymouth	Salisbury	Jackson Springs
Clayton	–	0.85*	0.81*	0.82*
Plymouth	–	–	0.83*	0.80*
Salisbury	–	–	–	0.81*
Jackson Springs	–	–	–	–

*Significantly different from zero at the 0.001 level

82% of the phenotypic variation. It was the major cause of the bimodal distribution in Fig. 1. The NC300 allele increased resistance and explained at least half of the phenotypic variation within each test environment (data not shown).

Four Southern rust QTL were identified from MIM for the combined analysis (Table 4). The MIM model explained 88 and 94% of the phenotypic and genotypic variation, respectively. The NC300 allele increased resistance for three of the four QTL. The QTL located on the short arm of chromosome 10, also identified by CIM, had the largest effect and accounted for 83 and 89% of the phenotypic and genotypic variation, respectively, for the MIM model. Estimates of effect, position, and explained phenotypic variation for this QTL were similar to the results of CIM. The remaining QTL individually explained less than 2%, and collectively accounted for 5%, of the phenotypic variation. Additive-by-additive-topcross epistatic interactions were not identified among these QTL, and segregation distortion did not occur at any flanking markers.

A significant ($P < 0.001$) marker-by-environment interaction occurred for marker UMC1380, which is linked to the major resistance QTL (Table 5). Changes of magnitude of the marker effects, rather than changes in sign, led to the significant interaction (data not shown). Significant marker-by-environment interactions did not occur for the other markers included in the multiple-marker-by-environment model.

The Spearman rank correlation coefficient between set-adjusted entry means from the combined analyses for anthesis date and Southern rust rating was 0.26 ($P = 0.002$). Although anthesis date and Southern rust rating were slightly correlated, significant genomic regions for the two traits did not over-lap (data not shown).

Discussion

The Southern rust entry mean heritability estimate, although potentially biased upwardly by additive-topcross-by-year and additive-by-additive-topcross epistatic interaction variance components, was high and comparable in magnitude to Holland et al. (1998). The

Table 5 Summary of the multiple-marker-by-environment (env) ANOVA for Southern rust resistance measured on topcrosses of 143 NC300/B104 maize recombinant inbred lines with FR615 × FR697

Source[a]	DF	Type III SS	MS	*F* value	*P* value
Umc1380/set	2	1086.31	543.16	32.94	0.001
Bnlg589/set	2	125.96	62.98	58.52	<0.001
Umc1360/set	2	43.80	21.90	16.08	0.004
Bnlg1401/set	2	31.62	15.81	8.10	0.020
Umc1380 × env/set	6	98.94	16.49	12.22	<0.001
Bnlg589 × env/set	6	6.46	1.08	0.80	0.572
Umc1360 × env/set	6	8.17	1.36	1.01	0.418
Bnlg1401 × env/set	6	11.71	1.95	1.45	0.193
Error[b]	1057	1426.26	1.35		

R^2 =0.68, CV = 20.53

[a] Sources of variation due to set, environment, environment-by-set, and replication nested within environment-by-set are not presented in the ANOVA

[b] The error variance of the model includes pooled variation due to higher order interactions among markers and environment in addition to residual variation

Table 4 Estimates of chromosome positions, left and right flanking markers and their corresponding positions, additive effects of the NC300 allele, percent of explained phenotypic variation (R^2), and the percent of explained genotypic variation (G%) for Southern rust quantitative trait loci detected by multiple interval mapping using combined mean disease scores over four environments in 2003

Chromosome	Position (cM)	Left[a]	Position (cM)	Right[b]	Position (cM)	Effect	R^2	G%
4	205.05	Bnlg589	203.1	Umc1503	206.4	0.16	1.6	2.0
8	51.72	Umc1360	51.7	Umc1034	70.3	0.16	2.0	2.0
9	31.42	Bnlg1401	31.3	Phi022	43.1	−0.14	1.5	2.0
10	6.01	Umc1380	0.0	Bnlg1451	16.2	1.27	82.7	89.0

Total R^2 = 87.8, likelihood = −128.23, Bayesian information criterion = 286.20

[a] Marker linked to the QTL position on the left side of the linkage map

[b] Marker linked to the QTL position on the right side of the linkage map

high upper bound for heritability for this population suggests that inheritance for resistance was simple in nature. The identification of a major QTL explaining most of the phenotypic variation and the nearly 1:1 segregation ratio demonstrated by the bimodal distribution (Fig. 1) of the RI topcross ratings support this statement. Further, no additive-by-additive topcross epistatic interactions were identified, which is concurrent with the phenotypic data, as the average rating of the RI topcrosses was equivalent to the parental topcross average.

Significant, but relatively minor, genotype-by-environment interaction was observed, as in Holland et al. (1998). Despite the significant genotype-by-environment interaction, very similar groupings of susceptible and resistant RI topcrossess were observed within each environment. The significant genotype-by-environment interaction in this study resulted from changes in the magnitude of the effect of the major gene on chromosome 10, as interactions between partial resistant QTL and the environment were not significant. Although the major gene interacted significantly with the environment, the segregation of the gene was clearly responsible for explaining at least half of the phenotypic variation within each test environment. The interaction likely resulted from leaves being more senesced when ratings were taken for plots at the two earlier-planted locations (Jackson Springs and Clayton). The overall ranking of marker genotypes for all marker loci linked to QTL did not change across environments, which agrees with the consistent ranking of entries across the environments. The absence of cross-over-interactions implies that the same race(s) was present at all environments.

The major gene identified in this study maps directly to a cluster of rust resistance genes previously identified on the short arm of chromosome 10 (Ullstrup 1965; Scott et al. 1984; Holland et al. 1998; Liu et al. 2003; Chen et al. 2004). Hulbert and Bennetzen (1991) also established the existence of common rust resistance genes, *Rp1* and *Rp5* in this region. Three QTL that confer partial resistance were also mapped. Their usefulness may be limited as these QTL each explained very small proportions of the phenotypic variation, and none co-localized to previously mapped partial resistance QTL (Bailey et al. 1987; Zummo 1988; Holland et al. 1998; Jiang et al. 1999; Brunelli et al. 2002).

The effectiveness of these major resistance (probably race-specific) genes can be limited in the tropics where multiple races of *P. polysora* exist (Carlos and Ferreira 2002). In these areas, general resistance is required, but the qualitative or quantitative nature of this resistance remains unclear. For example, inbred line Ki14 from Suwan-1 is one of the more generally resistant lines in the tropics (Kim et al. 1988), but its general resistance appears to result from a major resistant gene (Moon et al. 1999). In this study, the resistant ratings were taken in Hawaii, where Southern rust is multi-racial, on Ki14/B73 RI lines, which were bimodal-normally distributed. Therefore, Ki14 must either have multiple resistant alleles at several tightly linked loci and/or alleles that confer resistance to multiple races. Since partial resistance QTL appear to be of minor importance and non-repeatable across populations, it seems that emphasis should be placed on developing improved haplotypes for the chromsome10 region.

As with previous studies, the allelic relationships and pathogen specificity between the previously described genes and the major gene herein remains unknown. Resistant inbreds 1416-1 and 1497-2 from Holland et al. (1998) could share resistance alleles with NC300 as all have double-cross tropical hybrids PX105A and PX306B in their pedigrees (Goodman 1992). Holland et al. (1998) were unable to establish allelism between the QTL from inbred 1416-1 and *Rpp9*, as both sources of resistance were susceptible in a Mexican test environment. NC300 has maintained its resistance throughout several epiphytotics in North Carolina and certainly does provide resistance to at least one race.

The identified partial-resistant QTL and the major resistant gene were independent of plant maturity, which differs from results of similar studies conducted on different diseases (Bubeck et al. 1993; Carson et al. 2004; Clements et al. 2000; Jiang et al. 1999; Jung et al. 1994). This is surprising since Southern rust tends to be a late-season disease in North Carolina and earlier materials tend to escape. The major resistance gene from NC300 should not increase maturity if introgressed into U.S. materials.

Marker assisted selection has been successfully deployed for traits that are simply inherited, and is justified for such traits that are either too difficult or expensive to phenotype (Holland 2004). The infrequent occurrence of the Southern rust pathogen in the U.S. has resulted in inconsistent selection environments, which has contributed to the poor Southern rust resistance of U.S. commercial hybrids (Futrell 1975; Futrell et al. 1975; and Table 1). Progress in delineating pathogen specificities and allelic relationships among the several resistance genes that have been identified on the short arm of chromosome 10 is first needed before applying MAS, and that seems unlikely until pertinent race-specific fungal and maize inbred stocks are replenished. Once obtaining such stocks, studies

need to be conducted to identify racial specificities of the different resistance genes, which in some cases would require breaking tight linkages of mostly dominant resistant loci which collectively appear to account for general resistance, such as in Ki14. Upon obtaining such information, improved haplotypes could be constructed by combining favorable resistant alleles at the various loci from the different donor lines. The donor lines of the component alleles would be almost certainly unrelated to U.S. materials and breeding with markers could be used to introgress such regions into U.S. materials as linkage disequilibrium between marker and target alleles for future crosses would be expected to be maintained (Cregan et al. 1999; Holland 2004).

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