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ORIGINAL PAPER

Use of selection with recurrent backcrossing and QTL mapping to identify loci contributing to southern leaf blight resistance in a highly resistant maize line

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Abstract B73 is a historically important maize line with excellent yield potential but high susceptibility to the foliar disease southern leaf blight (SLB). NC292 and NC330 are B73 near-isogenic lines (NILs) that are highly resistant to SLB. They were derived by repeated backcrossing of an elite source of SLB resistance (NC250P) to B73, with selection for SLB resistance among and within backcross families. The goal of this paper was to characterize the loci responsible for the increased SLB resistance of NC292 and NC330 and to determine how many of the SLB disease resistance quantitative trait loci (dQTL) were selected for in the development of NC292 and NC330. Genomic regions that differentiated NC292 and NC330 from B73 and which may contribute to NC292 and NC330s enhanced SLB resistance were identified. Ten NC250P-derived introgressions were identified in both the NC292 and NC330 genomes of which eight were shared between genomes. dQTL were mapped in two $F_{2:3}$ populations derived from lines very closely related to the original parents of NC292 and NC330—(B73*rhm1* × NC250A and NC250A × B73). Nine SLB dQTL were mapped in the combined populations using combined SLB disease data over all locations (SLB AllLocs). Of these, four dQTL precisely colocalized with NC250P introgressions in bins 2.05–2.06, 3.03, 6.01, and 9.02 and three were identified near NC250P introgressions in bins 1.09, 5.05–5.06, and 10.03. Therefore the breeding program used to develop NC292 and NC330 was highly effective in selecting for multiple SLB resistance alleles.

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Introduction

The rapidly growing demand for ethanol as a fuel source has increased the demand for maize (Aho 2007; Westhoff et al. 2007). This increased demand heightens the importance of maximizing and stabilizing yields by, among other things, increasing disease resistance. Most disease resistance used in maize (*Zea mays* L. ssp. *mays*) is quantitative rather than qualitative in nature. However, very little is known about the physiological or molecular genetic basis of quantitative disease resistance.

Southern leaf blight (SLB), causal agent *Cochliobolus heterostrophus* (Drechs.) Drechs. (anamorph = *Bipolaris maydis* (Nisikado) Shoemaker), is a widespread disease

with the potential to cause yield losses in hot and humid tropical and sub-tropical regions, such as the southeastern USA, parts of India, Africa, Latin America and Southern Europe. Prior to 1970 SLB had received little attention (Committee on Genetic Vulnerability of Major Crops 1972; Hooker 1972). However, in 1970, a SLB epidemic caused by race T of *C. heterostrophus* decimated Texas male-sterile cytoplasm (cms-T) maize which was widely used in hybrid seed production (Hooker et al. 1970a, b; Smith et al. 1970; Committee on Genetic Vulnerability of Major Crops 1972; Hooker 1972). An estimated loss of one billion dollars was attributed to the 15% drop in total production due to SLB race T (Tatum 1971; Committee on Genetic Vulnerability of Major Crops 1972; Hooker 1972). After the 1970 epidemic, cms-T maize was replaced by male-fertile, race T-resistant cytoplasm (Smith et al. 1970).

At least two races of *C. heterostrophus* have been identified, race O and race T. These races differ in symptoms produced, part of the plant infected, cytoplasm specificity, toxin production, reproduction rate on susceptible plants, and optimum temperature (Hooker et al. 1970a, b; Smith et al. 1970). Currently, race O is the predominant cause of SLB infection in the United States (Burnette and White 1985). Resistance to *C. heterostrophus* race O is quantitatively inherited and the gene action is primarily additive or partially dominant (Pate and Harvey 1954; Hooker et al. 1970a, b; Lim 1975; Lim and Hooker 1976; Thompson and Bergquist 1984; Burnette and White 1985; Holley and Goodman 1989). Under experimental conditions, yield losses as high as 38–46% has been observed in maize inoculated with *Cochliobolus heterostrophus* (Fisher et al. 1976; Byrnes et al. 1989). However, yield losses of this magnitude are exceptionally rare because hybrids with some level of quantitative resistance are used in the US and Southern Europe greatly reducing yield losses due to SLB.

The North Carolina State University (NCSU) maize breeding program has released many diverse lines with high levels of SLB resistance. NC250 and NC250A are two highly SLB resistant sister lines. These lines and their close common progenitor (henceforth termed NC250P) were used to develop and release a number of subsequent NCSU lines with high levels of resistance and improved agronomic characteristics including NC292 and NC330. NC292 and NC330 were developed by crossing SLB-susceptible maize line B73 with NC250P and repeatedly backcrossing the progeny to B73 (three times for NC292 and four times for NC330), selecting for SLB resistance among and within backcross families. NC292 and NC330 are therefore B73 near-isogenic lines (NILs), which are agronomically similar to B73, yet exhibit a level of SLB resistance similar to NC250. The specific genomic regions responsible for conferring SLB resistance in NC292 and NC330 are unknown.

The first objective of this study was undertaken to identify NC250P introgressions present in the genomes of NC292 and NC330. Such introgressions represent candidate genome regions for SLB resistance genes. The second objective of this study was to map disease resistance quantitative trait loci (dQTL) associated with SLB resistance in two related $F_{2:3}$ populations derived from B73*rhm1* (resistance to *Helminthopsorium maydis1*, the former name of *C. heterostrophus*) × NC250A and NC250A × B73 crosses.

The parents of NC292 and NC330 are very similar to, and share almost all of their alleles with, the parents of the $F_{2:3}$ populations used for dQTL mapping. Determining dQTL that colocalize with NC250P introgressions in the genomes of NC292 and NC330 provides evidence as to which of the introgressions are important in conferring resistance to SLB. Locating genomic regions important for SLB resistance provides a foundation for fine-mapping SLB resistance QTL and for identifying molecular markers useful in breeding for SLB resistance.

Materials and methods

Plant materials

NC250, a SLB resistant, yellow dent, inbred line, was developed from the cross (Nigeria Composite A-Rb × B37) × B37 by D. L. Thompson, a USDA-ARS scientist at NCSU in cooperation with R. R. Bergquist, a plant pathologist at Pfister Hybrid Corn Company (El Paso, IL). Inbred line B37 was developed at Iowa State University from Cycle 1 of recurrent selection of the Iowa Stiff Stalk Synthetic (BSSS; Russell et al. 1971; Hallauer 2000). NC250 shares a common BC_1F_2 family with NC250A and is 87.5% identical to NC250A by descent (Nelson, personal communication).

B73 was developed at Iowa State University out of Cycle 5 of BSSS (Russell 1972; Troyer 1999). Although B73 is susceptible to disease and insects, its yield potential is excellent (Baker 1984; Darrah and Zuber 1986). B73 has been widely used in development of public and proprietary inbred lines (Hallauer et al. 1983; Mikel and Dudley 2006). B73 and B37, both products of the BSSS, have an average marker-based genetic similarity estimate of 0.176 (Bernardo 1993).

NC292, a yellow dent maize with SLB tolerance, was developed from a cross between B73 and NC250P. The progeny were backcrossed three times to B73, and NC292 was subsequently derived by ear to row selfing. During the development of NC292, progeny were screened for SLB resistance among and within backcross families. The most resistant lines were used in the next cycle of backcrossing.

The resulting NC292 inbred line matures one to three days earlier than the susceptible parent, B73.

NC330, a yellow dent maize, shares a common BC_3F_1 family with NC292, but was subsequently backcrossed one additional cycle, again under selection for SLB resistance. The line was subsequently derived by ear to row selfing. NC330 has good standability but is slightly less SLB resistant than NC292.

Two $F_{2:3}$ populations, B73*rhm1* × NC250A and NC250A × B73, used in this study were also used in a gray leaf spot (GLS; caused by *Cercospora zeae-maydis* Tehon & E.Y. Daniels) dQTL study (Bubeck et al. 1993). The first population was developed by crossing B73*rhm1* with NC250A. The B73*rhm1* parent—received from R. R. Bergquist—is a B73 near-isogenic inbred containing the *rhm1* gene, a single recessive gene conferring seedling resistance to SLB (Smith and Hooker 1973; Bubeck 1991). The *rhm1* allele was isolated from an East African source and tested in Nigeria (Smith and Hooker 1973). Sources used to develop NC250 and NC250A originated from a source similar to that of the *rhm* gene (Thompson and Bergquist 1984). The second population, derived from the cross NC250A × B73, was genetically similar to the first population, but without the *rhm1* allele in the B73 genome (Bubeck et al. 1993). Random F_2 plants derived from the two crosses were self-fertilized to generate $F_{2:3}$ families; population sample sizes were 193 for the B73*rhm1* × NC250A population and 144 for the NC250A × B73 population. Since these two populations are nearly identical, they were combined here to construct a linkage map and QTL mapping was conducted separately for each individual population as well as for the two populations combined.

Field trials

B73*rhm1* × NC250A and NC250A × B73 $F_{2:3}$ families were each evaluated for SLB reaction in single randomized replications in each of two years, 1982 (FL1) and 1987 (FL2, B73*rhm1* × NC250A population only) or 1988 (FL3, NC250A × B73 population only) at Homestead, Florida. In 1982, plots were planted 15 plants per 3 m row with 0.91 m between rows. Five plants were planted per 1.5 m row, in the 1987 and 1988 nursery plots, with 0.91 m between rows. Both populations were also evaluated for SLB reaction in summer 2007 at Clayton, North Carolina (CL07). Seed for CL07 planting was available for 184 of the 193 initial B73*rhm1* × NC250A $F_{2:3}$ families, which were randomly subdivided into two sets of 92 families. Each set also included four replicates of the parent lines resulting in 100 plots per replication per set. NC250A × B73 $F_{2:3}$ population seed was available for 133 of the 144 initial families, and was randomly subdivided into two sets containing 66 and 67 families, respectively. The first set, with 66 families included three replicates of the parental lines. The second set, with 67 families included two sets of parental lines and a filler line. These designs resulted in 72 plots per replication per set. The experimental field design employed for each population was a sets-within-replications design with 72 or 100 entries per set and two replications. Plots were single rows 2 m in length with 0.97 m between rows and a 0.6 m alley at the end of each plot. Ten seeds per entry were planted in each plot and the rows were not thinned.

Fungal growth and inoculation

SLB infection in 1982 was from natural inoculum of *C. heterostrophus* race O. Experiments conducted in 1987 and 1988 were artificially inoculated with SLB grown on sorghum or oat grain and the susceptible maize inbred CM174 (from Illinois Foundation Seeds) was planted between ranges as a spreader. In 2007, inoculum used was prepared as previously described by Carson et al. (2004), and rows were inoculated at the four- to six-leaf stage by placing approximately 20 grains of *C. heterostrophus* race O, isolate 2-16Bm sorghum grain culture in the leaf whorl (Carson 1998; Carson et al. 2004) After inoculation, the field was irrigated to provide free moisture to initiate fungal growth.

Ratings

The SLB resistance levels of several parents (B73, NC250, NC250A, B37 and B73*Htrhm*) and two NILs (NC292, and NC330) were assessed in two replications at Clayton, North Carolina over three years (2004–2006) in inoculated field trials. Two replications of these lines were also observed for one year in Tifton, Georgia (2006) to compare SLB resistance levels. Ten seeds per entry were planted for each plot. Plots were not thinned and disease ratings were taken on a per plot basis using a 1–9 scale, in increments of 0.5. Ear height (from the base of the plant to the bottom of the highest ear) and plant height (from the base of the plant to the top of the tassel) were recorded on one representative plant per row.

B73*rhm1* × NC250A $F_{2:3}$ families in 1982 and 1987, and NC250A × B73 $F_{2:3}$ families in 1982 and 1988 were rated twice. All ratings in Florida during the 1980s were taken 12–45 days after flowering, on a per plot basis using a 1–9 scale, in increments of 1. Summer 2007 trials were rated similarly, with the exception that ratings were taken twice in increments of 0.5. The first disease rating was taken when the latest maturing plant reached anthesis; earliest maturing plants reached anthesis 29 days prior scoring. The second rating was scored seven days later. The two ratings were averaged for the CL07 location to obtain the SLB average disease rating (SLB avg). Days-to-anthesis (DTA)

was determined to be the number of days from planting to 50% pollen shed.

SLB disease ratings, ear and plant height, and DTA were collected on several parental lines (B73, NC250, NC250A, B37, and B73*Htrhm*) and two B73-derived NILs (NC292 and NC330). SLB ratings were collected using the 1–9 scale previously described in increments of 0.5 for a total of four to six times. Since more than two disease ratings were taken on these lines the SLB avg was calculated as previously described by Balint-Kurti et al. (2007). In this case SLB avg is equivalent to weighed mean disease.

DNA extraction

For SSR analyses, DNA was extracted using a modified CTAB extraction procedure (Saghai-Maroof et al. 1984). The tissue was extracted in a 96-well format using strips of eight 1.1 ml microtubes. Approximately 100 mg of leaf tissue was collected from young leaves from the two parents, B73 and NC250, and from sister lines NC292 and NC330. Tissue, frozen in liquid nitrogen, was ground using a Retsch® Mixer Mill MM301 Retsch GmbH & Co. (Haan, Germany) with a #4 stainless steel shot washed with 1× TE buffer. After tissue was ground, 600 µl of CTAB extraction buffer (Saghai-Maroof et al. 1984) was added to each sample and tubes were mixed by inverting several times. Samples were incubated at 65°C for 60 min and inverted approximately every 20 min. Four hundred microliters of Chloroform:isoamyl alcohol, 24:1 (v/v), was added, mixed by inverting approximately 20 times, and centrifuged at 3,473×*g* for 10 min. The aqueous phase was transferred to new round bottom strip tubes and 600 µl of cold isopropanol was added. Samples were inverted 15 times and placed for 1 h at −20°C. Samples were then centrifuged for 10 min at 3,473×*g*, isopropanol was decanted, and 600 µl of 75% ethanol was added. Before placing at −20°C for at least 1 h, samples were inverted five times. Samples were centrifuged for 15 min at 3,473×*g*, ethanol was decanted, and the samples were dried in a hybridization oven set at 60°C for 30 min or until all the ethanol had evaporated. DNA was resuspended in 200 µl of 1× TE and working solutions were prepared by making 1:5 dilutions with nuclease free water.

Molecular markers

The PCR reactions for the SSRs were prepared using the procedures described in Schuelke (2000) and Kirigwi et al. (2008), with modifications. Each reaction used 1.5 U of Taq DNA polymerase, final concentration of 1× PCR buffer (Promega, Madison, WI), 0.67 µM Betaine, Anhydrous, 2.5 mM $MgCl_2$, 0.52 mM dNTPs each, 0.025 µM Forward primer with M13(−21) universal sequence (18 bp), 0.1 µM Reverse primer, 0.1 µM fluorescent dye-labeled primer (Fam, VIC, PET, or NED) and 20 ng of template DNA in a 12 µl reaction. PCR amplification conditions used were described by Schuelke (2000) with slight modifications. The PCR amplification profile used consisted of 3 min at 95°C, followed by 30 cycles of 30 s at 95°C, 45 s at a temperature between 59 and 68°C (optimum annealing temperature for each primer pair), 45 s at 72°C, followed by 10 cycles of 30 s at 95°C, 45 s at 53°C, 45 s at 72°C, and a final extension step of 10 min at 72°C. Subsequently, 1ul of PCR product from each of four different reactions with different fluorescent dyes was added to 6.99 µl of Hi-Di formamide (Applied Biosytems, Foster City, CA) and 0.01 ul LIZ size standard (Applied Biosytems, Foster City, CA) and the PCR products were resolved using an ABI3130 genetic analyzer.

Markers used in genome scan of NILs

Two parents, B73 and NC250, and the sister lines, NC292 and NC330, were screened with 629 SSR primer pairs and 693 single nucleotide polymorphism (SNP) markers to identify polymorphic markers for the genome scan of NC292 and NC330 (see S1 for more information regarding SSR and SNP markers). NC250A was also screened with the SNPs and some of the SSRs. SNP genotype data was generated at Pioneer Hi-Bred International using standard SNP genotyping approaches involving PCR and/or probe based detection (Lyamichev et al. 1999; Fan et al. 2003). These markers are now publicly available. Of the initial 768 SNPs 75 assays failed, resulting in data available for 693 SNPs. Map positions and additional information for the SNP markers is available at the Panzea website http://www.panzea.org (cited 27 March, 2008).

Markers used for linkage map construction

Most of the marker data used for map construction and mapping in the B73 × NC250A $F_{2:3}$ populations was generated at Pioneer Hi-Bred International, Inc. (Johnston, IA). A total of 220 molecular markers were used in construction of linkage maps and to map QTL. One-hundred and six restriction fragment length polymorphism (RFLP) probes were mapped to 110 different loci for additional information regarding the RFLPs (Bubeck 1991; Bubeck et al. 1993). In addition to the RFLPs, 32 SSRs and 78 SNPs were used to construct the linkage map. Sequence identities of the 78 SNPs used for constructing linkage maps are proprietary.

Identification of NC250P-derived segments in NC292 and NC330

Markers polymorphic between B73 and NC250 were used to determine whether specific loci in NC292 and NC330

were derived from B73 or NC250. Since B73 and NC250 are somewhat related (a coancestry of 0.13), about 13% of the genome is expected to be identical between the two lines (Nelson, personal communication). Any region where at least three monomorphic SSR and/or SNP markers were located within a 30 IBM centimorgans (IcM) region on the IBM 2005 Neighbors map, corresponding to approximately 8 centimorgans (cM) on an F_2 map (Lee et al. 2002; Winkler et al. 2003; Falque 2005; Balint-Kurti et al. 2007), was assumed to be B73-derived.

Statistical analyses

Genotype least square means (LSM) for SLB average disease rating (SLB avg) were estimated across the three environments for the combined population analysis, as well as for each population individually. LSM were also estimated for DTA and SLB avg for the CL07 location using PROC MIXED in SAS Version 9.1.3 (SAS Institute, Cary, NC). LSM for SLB avg were calculated using PROC MIXED with line and population as fixed-effect factors. All other factors (location, sets, and replication) and all interactions were considered random effects. The PROC CORR procedure in SAS was used to calculate all phenotypic correlations.

The heritability for SLB disease resistance across all locations was estimated both for the combined populations and each population individually. To estimate heritability, line was considered a random-effect factor, together with location, sets, replication and all interactions in PROC MIXED (Holland et al. 2003). The random-effect covariance parameters were tested using the type III *F*-test (PROC MIXED, method = type 3).

Linkage mapping

JoinMap® 3.0 (Van Ooijen and Voorrips 2001) was used to estimate locus orders, identify linkage groups, and transform estimated recombination frequencies to centimorgans using the Kosambi mapping function. Linkage mapping was performed on the combined populations to produce a joint map, since the populations were derived from nearly identical parents. Segregation distortion was tested in JoinMap. However, since several regions differed from the marker order of the Intermated B73 × Mo17 (IBM) reference maps when constructed using JoinMap, MAPMAKER/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992) COMPARE command was used with each set of eight adjacent linked markers to finalize marker order. The final linkage map produced by MAPMAKER/EXP agreed closely with the order defined by the commonly used IBM2 2005 Neighbors (IBM 2005) reference map (http://www.maizegdb.org, cited 14 Sept. 2007).

dQTL detection and estimation

In the current study, detected dQTL are reported for each location separately (SLB avg) and with all locations combined (SLB AllLocs). SLB avg and SLB AllLocs analyses were performed for each population separately and for the combined population analysis.

QTL Cartographer version 2.5 (Wang et al. 2007) was used for dQTL mapping. Composite interval mapping (CIM) was used to create an initial model for each population separately and for the combined populations for the SLB AllLocs analysis. CIM was performed using a 0.5 cM walk speed, a window size of 10 cM, and the CIM Model 6 with forward and backward regression, using a probability of 0.05 to include or exclude a dQTL from the model. The threshold logarithm-of-odds (LOD) score that was used to declare genome-wide significance at $\alpha = 0.05$ was determined by performing 1,000 permutations. Using CIM, dQTL positions and effects were determined for each population separately and for the combined populations at each location.

Multiple interval mapping (MIM) was initiated from the CIM models using an LOD threshold of 2.6 and a minimum distance of 10 cM between dQTLs. The search for the best MIM model was completed in an iterative, stepwise manner, searching for new dQTL, testing for their significance after each cycle of searching for new dQTL, and optimizing dQTL positions when new dQTL were added to the model. New models were accepted if they decreased the Bayesian Information Content (BIC, Piepho and Gauch 2001). Use of the BIC criterion is the best choice if the experimental objectives are to deduce genetic parameters such as the number of dQTL (Zeng et al. 1999). Using the BIC gives preference to models with higher likelihoods, however, it also includes a penalty for each additional parameter that is added to the model to facilitate the prevention of overfitting the model (Robertson-Hoyt et al. 2006; Balint-Kurti et al. 2007; Vadasz et al. 2007). When no additional dQTL could be added to the model while decreasing the BIC, each pair of dQTL in the model was tested for epistatic interactions. An additional approach used to prevent overfitting QTL models was to exclude models in which the proportion of the total phenotypic variation accounted for by the dQTLs exceeded the entry mean heritability. If the model was overfit, the dQTL with the smallest effect was dropped out of the model and the effects of the remaining dQTL were re-estimated. When comparing the different traits, locations and populations, dQTL were considered to be overlapping when they were within a 20 cM distance (Visscher et al. 1996; Melchinger et al. 1998; Cardinal and Lee 2005).

Since QTL Cartographer treated the data as a selfed F_2 population (the population is actually a $F_{2:3}$ population which has undergone an additional selfing generation), the

Table 1 The maize lines NC292 and NC330, along with several of their progenitor lines (B73, B37, NC250 and NC250A) characterized for SLB resistance and agronomic traits using combined data from four locations in Clayton, North Carolina (2004, 2005, and 2006) and in Tifton, Georgia (2006)

Line	SLB avg[a]	DTA	Ear ht (cm)	Plant ht (cm)
B73	5.09^{A}	71.6^{B}	83^{A}	204^{A}
B37	3.99^{B}	71.1^{BC}	71^{ABC}	192^{AB}
B73*Htrhm*[b]	3.43^{C}	71.1^{BC}	78^{AB}	211^{A}
NC330	2.53^{D}	73.3^{AB}	70^{ABC}	193^{AB}
NC292	2.11^{DE}	68.4^{C}	62^{CD}	175^{BC}
NC250	1.99^{E}	71.8^{B}	54^{D}	162^{C}
NC250A	1.75^{E}	75.6^{A}	65^{BCD}	194^{AB}
LSD[c]	0.49	2.8	14	29

In addition, B73*Htrhm*, which is genetically very similar to B73 and B73*rhm1*, was characterized for SLB resistance and agronomic traits using combined data from these four locations. Agronomic traits characterized include days-to-anthesis (DTA, days after planting to 50% pollen shed), ear height (cm), and plant height (cm). Significant differences between lines were determined using the least significant difference (LSD) procedure using $\alpha = 0.05$. The superscripted capital letters to the right of the trait data show which lines are significantly different from the other lines for each trait

[a] SLB average disease rating (SLB avg) was calculated as previously described by Balint-Kurti et al. (2007). In this case SLB avg is equivalent to weighed mean disease

[b] B73 line with two single resistance genes, one to northern leaf blight (*Ht*), and one to southern leaf blight (*rhm*)

[c] Least significant difference

dominance effects estimated by QTL Cartographer were underestimated by half. Therefore, the dominance effects reported were multiplied by two. Dominance effects were reported when the LOD score was above 0.5 and the additive effects and dQTL interactions were reported when the LOD score was above 1.5. These threshold values were arbitrarily set to limit the number of QTL reported such that several QTL of very small effect identified in the final MIM analysis were not reported. In addition, these values were selected such that in most cases when an additive effect was reported the dominance and interaction effects were also reported, unless they were very small (i.e. lower than the set thresholds).

Results

SLB evaluation of parental and experimental lines

SLB disease screening field trials showed significant differences in SLB resistance between B37, B73, B73*Htrhm*, NC330, NC292, NC250, and NC250A (Table 1). NC250 and NC250A were significantly more resistant than NC330. There were no statistically significant differences between NC250, NC250A, and NC292, nor were NC292 and NC330 significantly different in their resistance to SLB. SLB resistance of NC250, NC292, and NC330 was visually striking compared to B73 in inoculated field experiments (Fig. 1). NC250A was significantly later maturing than NC250 and NC292 (Table 1).

Genome scan of NILs

Although NC292 and NC330 are nearly isogenic to B73, they are much more resistant than B73 to SLB (Fig. 1). Some of the genomic regions for which NC292 and NC330 differ from B73 (NC250P introgressions) must account for this increased resistance. For the genome scan, 1,322 SNP and SSR markers were screened. Of these, 275 SSR primer pairs and 218 SNPs were polymorphic between B73 and NC250. Ten NC250P-derived introgressions were identified in both the NC292 and NC330 genomes, of which eight were shared in both

Fig. 1 Late season southern leaf blight (SLB) symptoms in inoculated field trials. *i* B73, a SLB susceptible inbred line, *ii* NC250, a highly SLB resistant inbred line, *iii* and *iv* NC292 and NC330, two highly SLB resistant B73 near-isogenic lines

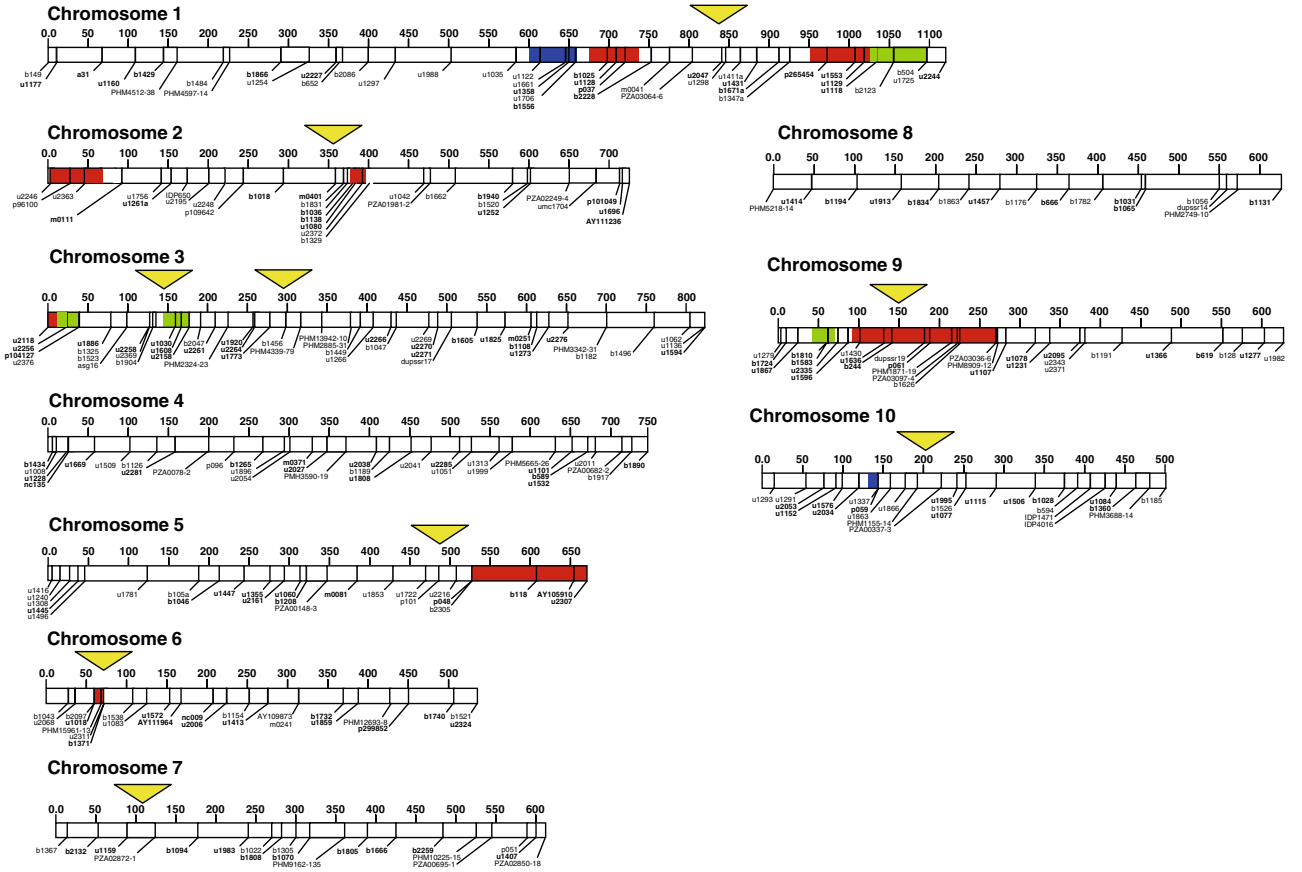

Fig. 2 Chromosome map of the 10 maize chromosomes showing NC250P introgressions and B73 background in the genomes of NC292 and NC330. The *white rectangles* represent B73 background. *Red rectangles* show genome regions with NC250P introgressions in both NC292 and NC330. *Green rectangles* represent NC250P introgressions present in NC292 and not present in NC330. *The blue rectangles* show NC250P introgressions that are present in NC330 and not present in NC292. *The yellow triangles* above the chromosomes represent regions where SLB dQTL have been identified. dQTL were identified using the combined analysis of the two crosses (B73*rhm1* × NC250A and NC250A × B73) over multiple environments (SLB AllLocs). SSR primer pairs in bold are core markers from the IBM2 2005 Neighbors map (http://www.maizegdb.org, cited 14 Sept. 2007). Marker names indicated with a '*b*' represent bnlg and those with '*u*' represent umc

genomes (Fig. 2 and S1). The two introgressions unique to NC292 were on chromosomes 3 and 9, and the two unique to NC330 were on chromosomes 1 and 10. Based on the marker analyses it was estimated that 89.7% and 91.3%, respectively, of the NC292 and NC330 genomes were derived from B73. For seven loci, alleles were identified in NC292 and/or NC330 which are not present in NC250, but are present in NC250A.

Five regions with extremely low levels of polymorphism were identified using SSR and SNP markers. These regions contained at least three monomorphic SSR and/or SNP markers within 30 IcM. They were identified as likely being identical by descent and, therefore, classified as B73 background. These regions were located between IBM 2005 IcM 579.50 and 760.90 on chromosome 3, IcM 349.80 and 411.3 on chromosome 4, and IcM 0.0 and 62.3 and IcM 285.80 and 385.34 on chromosome 9.

Disease and anthesis ratings

Initially, statistical analyses of SLB AllLocs were performed including a set effect in the model, but in all cases the variance component estimate due to sets was zero. Based on these results the set main effect was dropped out of the model. However, the set-by-replication interaction was significant and was, therefore, included in the final model (Table 2).

The population effect was not significant for SLB AllLocs. The location effect was significant for each population when analyzed separately but was not significant for the combined population analysis. For the combined population analysis line-within-population and location-by-line-within-population were significantly different from zero. When the populations were analyzed separately line and location-by-line were also significantly different from zero

Table 2 *F*-test of the fixed-effect, and the variance component estimates and standard errors (std err.) of the random effects in mixed-models analysis of southern leaf blight average disease rating for the combined populations of B73*rhm1* × NC250A and NC250A × B73 obtained in four environments—Florida 1982 (FL1), Florida 1987 (FL2), Florida 1988 (FL3), and Clayton, North Carolina 2007 (CL07) and for each population separately

Combined populations			B73*rhm1* × NC250A			NC250A × B73		
Fixed factor	*F*-value	*P*-value	Fixed factor	*F*-value	*P*-value	Fixed factor	*F*-value	*P*-value
Pop[a]	5.01	0.14						
Random factor	Variance component estimate (std err.)	*P*-value	Random factor	Variance component estimate (std err)	*P*-value	Random factor	Variance component estimate (std err)	*P*-value
Loc[b]	0.52 (0.68)	0.13	*Loc*	0.56 (0.58)	0.03	*Loc*	0.84 (0.94)	0.04
Loc x Pop	0.17 (0.18)	0.03						
Rep[c]*(Loc x Pop)*	0.01 (0.02)	0.29	*Rep(Loc)*	0.01 (0.01)	0.21	*Rep(Loc)*	0.013 (0.04)	0.39
Line(Pop)	0.39 (0.04)	<0.0001	*Line*	0.31 (0.05)	<0.0001	*Line*	0.50 (0.08)	<0.0001
Rep x Set(Loc x Pop)	0.01 (0.01)	0.0006	*Rep x Set(Loc)*	0	0.25	*Rep x Set(Loc)*	0.02 (0.04)	0.0006
Loc x Line(Pop)	0.26 (0.02)	<0.0001	*Loc x Line*	0.26 (0.03)	<0.0001	*Loc x Line*	0.26 (0.04)	<0.0001
Error	0.10 (0.01)		Error	0.09 (0.01)		Error	0.12 (0.02)	

[a] Population

[b] Location

[c] Replication

(Table 2). Because of the significant line by location interaction, individual analysis for each location was performed using CIM (see Supplementary Table 2), in addition to the combined locations analysis using CIM and MIM.

Pairwise Pearson correlation coefficients of SLB avg between replications were moderately high for the CL07 location data ($r = 0.59$; $P < 0.0001$). The correlation for the combined populations between FL1 and CL07 was highly significant ($P < 0.0001$, $r = 0.61$). The Pearson correlation coefficient between the SLB average and DTA at CL07 was not statistically significant ($r = -0.08$; $P = 0.15$).

The entry mean heritability of combined populations was 0.76 (standard error 0.02) for SLB disease resistance over all locations. The entry mean heritability over all locations was 0.72 (standard error 0.04) and 0.80 (standard error 0.03) for the B73*rhm1* × NC250A and NC250A × B73 populations, respectively.

Linkage mapping

Linkage maps were constructed using 220 molecular markers covering a total map length of 2,001.8 cM (Fig. 3). The average distance between mapped markers was 9.1 cM. The only marker for which the determined marker order was not in agreement with the IBM 2005 reference map was umc1430 on chromosome 9. The linkage map constructed in the current study positions umc1430 distal to umc113a, while the IBM 2005 map positions umc1430 proximal to umc113a.

QTL mapping

QTL mapping detected nine SLB dQTL for SLB AllLocs using the combined populations (Table 3). Of these dQTL, four precisely colocalized with NC250P introgressions identified from the genome scan of NC292 and NC330 in bins 2.05–2.06, 3.03, 6.01 and 9.02 (Fig. 2). SLB dQTL with the largest R^2 values for SLB AllLocs, were identified in chromosomal bins 3.03, 6.01, and 9.02. These represented three of the four dQTL which colocalized with NC250P introgressions identified from the NC292 and NC330 genome scan. Of the remaining five dQTL, the dQTL in bin 1.09 was located in the 210 IcM region between two NC250P introgressions (80 IcM or approximately 21 cM, from one NC250P introgression and 130 IcM or approximately 35 cM from the second), and the dQTL detected on chromosomes 5 (bins 5.05–5.06) and 10 (10.03) were in close proximity to NC250P introgressions. The only SLB AllLocs dQTL for the combined populations not in proximity to NC250P introgressions were located in bins 3.05 ($R^2 = 0.0$) and 7.01 ($R^2 = 1.9$). The dQTL in bin 3.05 was included due to a significant interaction between it and the dQTL in bin 3.03.

Analysis of the results using the B73*rhm1* × NC250A population identified only four dQTL, while seven dQTL were detected using the NC250A × B73 population. When the locations were analyzed individually, three SLB QTL were detected in all environments (S2). These dQTL are on chromosomes 2 (bin 2.04–2.06), 9 (bin 9.01–9.03), and 10

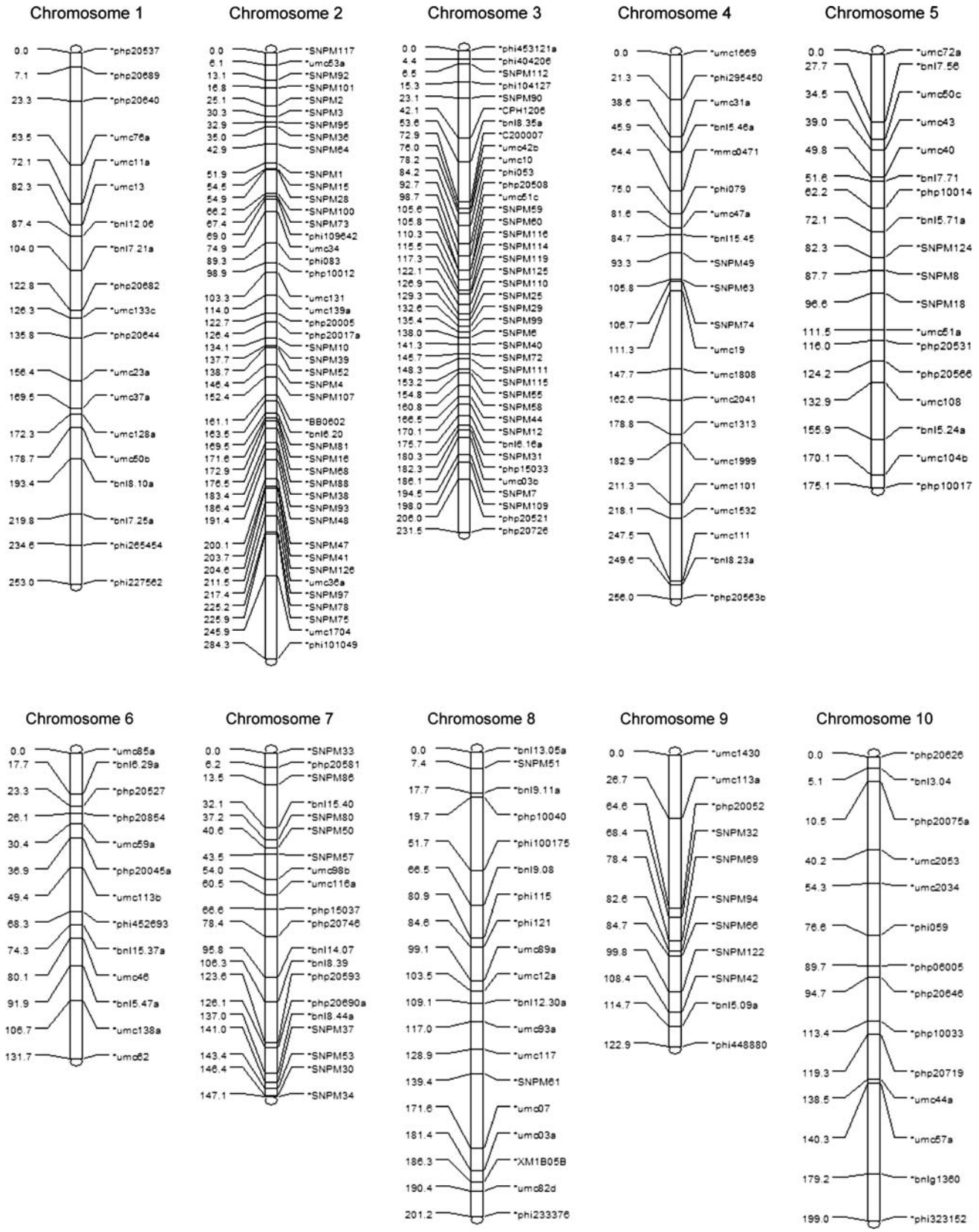

Fig. 3 Linkage map constructed using the combined populations of B73*rhm1* × NC250A and NC250A × B73

Table 3 Chromosomal location in centimorgans (cM) and parameters associated with disease resistance quantitative trait loci (dQTL) detected from the combined analyses of SLB data from multiple environments (SLB AllLocs)—Florida 1982 (FL1), Florida 1987 (FL2), Florida 1988 (FL3), and Clayton, North Carolina 2007 (CL07) from the populations (B73*rhm1* × NC250A and NC250A × B73) analyzed separately and combined

Bin, parameters[a]	Flanking markers[b]	B73*rhm1* × NC250A	NC250A × B73	Combined populations[c]
1.09	umc50b-bnl7.25a	191.7[d]	193.5	196.4
IBM 2005 IcM[e]		797.4	804.6	820.7
A		−0.31[f]	−0.40	−0.35
LOD		7.3[g]	4.8	9.2
R^2		10.5[h]	11.6	8.8
D				
LOD				
R^2				
2.05–2.06	umc131-php20005		117	103.3
IBM 2005 IcM			391.1	342.4
A			−0.31	−0.28
LOD			3.6	7.1
R^2			9.2	6.3
D				
LOD				
R^2				
3.03	CPH1206-bnl8.35a	48.1	45.1	48.1
IBM 2005 IcM		136.8	127.8	136.8
A		−0.37	−0.30	−0.36
LOD		9.4	3.1	8.3
R^2		12.9	6.5	7.6
D		0.57[i]	0.32	
LOD		2.8	0.6	
R^2		3.6	0.5	
3.05	phi053-php20508			88.2
IBM 2005 IcM				300.0
A				−0.06
LOD				0.2
R^2				0.0
D				0.18
LOD				0.2
R^2				1.0
5.05–5.06	SNPM18-umc51a	100.6		106.6
IBM 2005 IcM		464.2		480.4
A		−0.31		−0.43
LOD		5.2		11.2
R^2		10.9		11.2
D				
LOD				
R^2				
6.01	umc85a-bnl6.29a		7.0	0.0
IBM 2005 IcM			69.2	66.4
A			−0.51	−0.22
LOD			7.9	3.4

Table 3 continued

Bin, parameters[a]	Flanking markers[b]	B73*rhm1* × NC250A	NC250A × B73	Combined populations[c]
R^2			19.0	3.3
D			0.55	0.35
LOD			1.6	0.9
R^2			2.3	0.8
7.01	SNPM86-bnl15.40			13.5
IBM 2005 IcM				113.0
A				−0.16
LOD				2.4
R^2				1.9
D				
LOD				
R^2				
9.02	umc113a-php20052	40.7	47.7	45.7
IBM 2005 IcM		126.2	156.1	147.6
A		−0.50	−0.49	−0.39
LOD		12.1	3.4	8.3
R^2		26.8	16.8	10.4
D		−0.28		−0.37
LOD		0.6		0.8
R^2		1.3		0.2
10.03	php20646-php10033		103.7	101.7
IBM 2005 IcM			221.0	213.0
a			−0.12	−0.27
LOD			0.5	5.4
R^2			1.8	5.8
d			−0.52	
LOD			1.0	
R^2			2.7	

QTL interactions	Type of interactions[j]	B73*rhm1* × NC250A	NC250A × B73	Combined populations[c]
1.09 × 2.05–2.06	AA			0.23[k]
LOD				1.8
R^2				1.6
1.09 × 9.02	DD		0.53	
LOD			1.0	
R^2			1.2	
2.05–2.06 × 3.03	DA			0.27
LOD				1.5
R^2				1.4
3.03 × 3.05	AA			−0.39
LOD				2.0
R^2				5.3
3.03 × 9.02	DD		−0.96	
LOD			1.2	

Table 3 continued

QTL interactions	Type of interactions[j]	B73*rhm1* × NC250A	NC250A × B73	Combined populations[c]
R^2			8.4	
9.02 × 10.03	DA			−0.50
LOD				2.0
R^2				4.1

In addition to reported map position from the linkage map constructed from the combined populations of B73*rhm1* × NC250A and NC250A × B73 we also report the inferred map positions for the IBM2 2005 Neighbors map (http://www.maizegdb.org, cited 14 Sept. 2007). The maize $F_{2:3}$ population B73*rhm1* × NC250A consists of 193 lines. The NC250A × B73 population has 144 lines and the combined populations have 337 lines

[a] Chromosome bin location of dQTL peak on one of the 10 chromosomes of the maize genome. Bins divide the genetic map into 100 approximately equal segments. The segments are designated with the chromosome number followed by a two digit decimal (e.g., 1.00, 1.01, 1.02, etc.). The marker order determined for the population used in this experiment largely follows the marker order shown in the standard maize genetic map (the IBM map)

[b] Markers flanking the dQTL peak positions

[c] Analysis of the combined populations

[d] Chromosomal position of the predicted dQTL in centimorgans (cM)

[e] Approximate IBM 2005 Neighbors IcM (IBM centimorgans) (http://www.maizegdb.org, cited 14 Sept. 2007)

[f] Additive effect of the dQTL. A negative number indicates that the allele for resistance was derived from NC250A. A positive number indicates that the allele for resistance was derived from B73 or B73*rhm1*

[g] Logarithm-of-odds (LOD) value at the position of peak likelihood of the dQTL

[h] R^2 estimates the proportion of phenotypic variance (percentage) explained by the detected dQTL

[i] Dominance effect of the dQTL. A positive number indicates that susceptibility is dominant while a negative number indicates that resistance is dominant

[j] Type of dQTL interactions identified and determined by QTL Cartographer (AA is an additive by additive interaction, DD is a dominant by dominant interaction, and DA is a dominant by additive interaction

[k] Effect of the interaction. A positive number indicates that the two NC250P alleles together confer less resistance when they are present together than would be expected from the estimation of their individual effects, while a negative number indicates the opposite

(bin 10.02–10.04). The only dQTL identified both in the combined population analysis and in each population when analyzed separately were located in bins 1.09, 3.03 and 9.02. For all dQTL detected, the resistance allele was inherited from NC250A. Only a single significant dQTL was identified from analysis of the CL07 location. This was due to the high significance threshold for this location (probably due to extreme drought in 2007).

Discussion

The ear and plant height and flowering time data from the field trial screening of parents and NILs show that between the sister lines NC330 and NC292, NC330 is more B73-like and NC292 is more NC250-like for these agronomic traits (Table 1). Genetically, NC330 is also more like B73 than NC292, but the difference is relatively small. Based solely on pedigree the ratios of B73-derived DNA in the genomes of NC292 and NC330 were expected to be 94.6 and 97.3%, respectively. Our data indicated that NC292 and NC330 are 89.7 and 91.3% B73-derived, respectively. We can largely attribute this difference to the selection of some NC250P introgressions, important in conferring SLB resistance, during development of NC292 and NC330. Not all of the NC250P introgressions in NC292 and NC330 necessarily confer SLB resistance, however.

Four of the nine dQTL identified from the SLB AllLocs in the combined population analysis colocalized with NC250P introgressions, including the three largest effect dQTL identified in the current study, providing strong evidence that these regions are important in conferring SLB resistance (Fig. 2; Table 3). Three of the other dQTL were identified very near other NC250P introgressions. It is possible that these dQTL correspond to the introgressions they are near (i.e. the genes that underlie the dQTL are contained within the introgressions), but that the inaccuracies associated with QTL mapping led to imprecise localization. These findings demonstrate that the selection performed during the development of NC292 and NC330 was highly effective in selecting most or all of the largest effect dQTL and that conventional breeding and phenotypic selection are powerful tools for developing SLB resistant maize.

The NC250P introgressions on chromosomes 2 (bins 2.00–2.01) and 3 (bins 3.00–3.01) were possibly introgressed by chance (i.e. they were not selected for) during the development of NC292 and NC330 since SLB dQTL were not identified near these introgressed regions. Conversely,

it is possible that SLB resistance genes are carried by these introgressions, but their effects were too small to be detected in the QTL analysis.

The dQTL detected on chromosome 1 (bin 1.09) in both populations (analyzed separately) and in the combined analysis is also a large effect dQTL. It does not colocalize with an NC250P introgression, but is flanked by two nearby NC250P introgressions. Therefore, it is likely that in this case the single detected dQTL represents the combined resistance effects conferred by the two linked NC250P introgressions with a peak QTL effect estimated between the two underlying resistance genes. If this is the case, population size was probably a limiting factor in our ability to delineate the two dQTL in this region. Balint-Kurti et al. (2006) identified two SLB dQTL in bins 1.05–1.09 in the B104 × NC300 population which may be in similar regions as the two NC250 introgressions that were identified in the current study. This can be further addressed by generating NILs with single introgressions and evaluating the effects of the individual introgressions on SLB resistance.

It would be expected that NC250P-derived SLB resistance genes in NC292 and NC330 must have been completely or partially dominant with respect to the B73 alleles based on the breeding strategy employed to produce NC292 and NC330. Selection for SLB resistance was performed among and within backcross families, so recessive resistance alleles would often not have had any effect on phenotype and therefore would not have been selected for phenotypically. Our QTL results bear this out; all the dQTL that colocalized with NC250P introgressions had substantial additive effects and some also had substantial dominance effects. One caveat for the estimation dominance and epistatic effects is that only 40 plants for each $F_{2:3}$ family were evaluated. Among these families, the ones corresponding to the heterozygous F_2 plants, carried only 50% heterozygotes. So the estimation of dominance effects on average relied on only 20 plants per family over the different locations, years, and inoculation methods, and may therefore be somewhat inexact.

In the NC250A × B73 population, the largest effect dQTL was identified on chromosome 6 (IBM 2005 IcM of 69.2). This is very near the location of the *rhm1* SLB resistance gene previously reported by Smith and Hooker (1973) at IBM 2005 IcM of 66.06; this dQTL was not detected in the B73*rhm1* × NC250A population, presumably because the NC250A allele at this locus provided a similar level of SLB resistance as the *rhm1* gene. Therefore NC250A/P contains either the *rhm1* gene itself, a different allele of *rhm1* with a similar effect on SLB resistance, or a different SLB resistance gene that is tightly linked to the *rhm1* gene. However resistance may be conferred by two tightly linked recessive genes (Chang and Peterson 1995).

The results of the current study show that *rhm1* has an effect on adult plant resistance. Adult plant disease resistance scores for the B73*rhm1* × NC250A population were lower than those for the NC250A × B73 population, providing evidence regarding the effectiveness of *rhm1* in conferring SLB resistance in adult plants. This may in part contradict the idea that SLB resistance conferred by *rhm1* is effective only prior to silking (Thompson and Bergquist 1982).

The two dQTL identified in this study with the largest R^2 values were also identified as large effect dQTL in previous SLB QTL mapping studies (Balint-Kurti et al. 2006, 2007). The current study identified a large effect dQTL on chromosome 3 (bin 3.03, Table 2, S2). This is consistent with the largest effect dQTL identified by the Balint-Kurti et al. (2007) SLB QTL mapping study based on the intermated B73 × Mo17 population. The resistance allele that study was inherited from Mo17. Four additional studies have reported SLB resistance QTL in this same region using different populations (Bubeck 1991; Jiang et al. 1999; Balint-Kurti et al. 2006, 2008). Bubeck (1991) identified dQTL in the same region on chromosome 3 of an ADENT × B73*rhm1* cross where the resistant allele was derived from B73. ADENT was an S_5 line derived from the cross [Amarillo Dentado 2 × (A632 × B14A)] × Amarillo Dentado 2. Amarillo Dentado 2 is a synthetic population from the International Maize and Wheat Improvement Center (CIMMYT, Mexico). In a cross between lowland and highland tropical maize inbreeds Jiang et al. (1999) reported an SLB dQTL on chromosome 3 (bins 3.01–3.04); resistance was inherited from the lowland inbred parent. Balint-Kurti et al. (2006) identified an SLB dQTL in this region on chromosome 3 in a B104 (Stiff Stalk) × NC300 (tropical) cross with resistance conferred by the NC300 allele. Lastly, Balint-Kurti et al. (2008) reported a dQTL from a B73 × H99 cross in bin 3.04 that is very near and likely overlaps the dQTL found in bin 3.03 of the current study. The resistance was derived from H99. In addition, a SLB dQTL on chromosome 9 (bins 9.01–9.03) was identified in the same region as another large effect dQTL that was identified by Balint-Kurti et al. (2006) in a B104 × NC300 cross; resistance was inherited from NC300.

In addition to identifying dQTL that colocalize with NC250P introgressions, several of the SLB dQTL identified in the current study also colocalize with GLS dQTL that were previously reported using the same mapping populations (Bubeck et al. 1993). The SLB dQTL identified on chromosome 1 (bins 1.08–1.10), chromosome 2 (bins 2.04–2.06), chromosome 5 (bins 5.05–5.06), and chromosome 7 (bins 7.01–7.04) all colocalize with GLS dQTL detected by Bubeck et al. (1993). This suggests that genes responsible for conditioning SLB resistance may also provide resis-

tance to GLS. Another possible explanation for the colocalization of some SLB and GLS dQTL is that different clustered or tightly linked genes may be conferring resistance.

The results reported here allowed identification of specific regions of the genome are responsible for conferring the level of SLB resistance found in lines NC292 and NC330. Chromosomal regions (bins 3.03, 6.01 and 9.02) where NC250P introgressions in NC292 and NC330 and SLB QTL colocalized are excellent candidates for finer-resolution genetic analysis. A long-term goal of this research is identification and functional characterization of genes responsible for SLB resistance, which provide valuable insight into the mechanisms of QDR in maize and other species.

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