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ORIGINAL ARTICLE

A remorin gene is implicated in quantitative disease resistance in maize

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Abstract

Key message **Quantitative disease resistance is used by plant breeders to improve host resistance. We demonstrate a role for a maize remorin (*ZmREM6.3*) in quantitative resistance against northern leaf blight using high-resolution fine mapping, expression analysis, and mutants. This is the first evidence of a role for remorins in plant-fungal interactions**.

Abstract Quantitative disease resistance (QDR) is important for the development of crop cultivars and is particularly useful when loci also confer multiple disease resistance. Despite its widespread use, the underlying mechanisms of QDR remain largely unknown. In this study, we fine-mapped a known quantitative trait locus (QTL) conditioning disease resistance on chromosome 1 of maize. This locus confers resistance to three foliar diseases: northern leaf blight (NLB), caused by the fungus *Setosphaeria turcica*; Stewart's wilt, caused by the bacterium *Pantoea stewartii*; and common rust, caused by the fungus *Puccinia sorghi*. The Stewart's wilt QTL was confined to a 5.26-Mb interval, while the rust QTL was reduced to an overlapping 2.56-Mb region. We show tight linkage between the NLB QTL locus and the loci conferring resistance to Stewart's wilt and common rust. Pleiotropy cannot be excluded for the Stewart's wilt and the common rust QTL, as they were fine-mapped to overlapping regions. Four positional candidate genes within the 243-kb NLB interval were examined with expression and mutant analysis: a gene with homology to an F-box gene, a remorin gene (*ZmREM6.3*), a chaperonin gene, and an uncharacterized gene. The F-box gene and *ZmREM6.3* were more highly expressed in the resistant line. Transposon tagging mutants were tested for the chaperonin and *ZmREM6.3,* and the remorin mutant was found to be more susceptible to NLB. The putative F-box is a strong candidate, but mutants were not available to test this gene. Multiple lines of evidence strongly suggest a role for *ZmREM6.3* in quantitative disease resistance.

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Introduction

Quantitative disease resistance (QDR) is frequently employed by plant breeders to protect crops from pathogen attack, yet the underlying genes and mechanisms remain largely a matter of conjecture. A synthesis study of 50 quantitative trait loci (QTL) studies conducted using biparental populations found that 89 % of the maize genetic map was covered by disease QTL (Wisser et al. 2006). QTL resolution has subsequently been improved using a large, multi-parental maize population, confirming the highly complex genetic architecture for QDR. For southern leaf blight (SLB) and northern leaf blight (NLB), 32 and 29

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independent loci, respectively, were implicated in quantitative resistance (Kump et al. 2011; Poland et al. 2011). Each of those QTL may be complex and conditioned by multiple genes (Jamann et al. 2014). Through genome-wide association studies, hundreds of candidate genes have emerged (Poland et al. 2011; Chia et al. 2012; Van Inghelandt et al. 2012; Schaefer and Bernardo 2013; Wallace et al. 2014a). These genes must be validated because of the high false positive rate in GWAS.

A number of mechanisms have been postulated to be involved in QDR, including avoidance, perception, and signaling (Poland et al. 2009). Although many genomic regions have been associated with incomplete resistance, these regions typically encompass hundreds of genes (Wisser et al. 2005). Examples of genes that have been demonstrated to play a role in QDR include an ABC transporter, a kinase-START, a cluster of germin-like proteins, a proline-rich protein, and copy number variation of an amino acid transporter, an α-SNAP protein, and a WI12 protein (Fu et al. 2009; Fukuoka et al. 2009; Krattinger et al. 2009; Manosalva et al. 2009; Cook et al. 2012). These genes implicate a broad range of mechanisms (St Clair 2010). Cloning of additional loci will elucidate the mechanisms that underlie QDR and provide further insight into their diversity.

QDR can vary in specificity, from providing protection against one race of a pathogen, to providing protection against diverse microbes (St Clair 2010). Many regions of the genome have been associated with resistance to multiple diseases, but the low resolution of these mapping studies has not allowed linkage to be distinguished from pleiotropy (Wisser et al. 2006; St Clair 2010). Mechanisms by which single genes could influence multiple diseases can be envisaged; for example, proteins at highly connected nodes of the proteome are targets of effectors from various pathogens, as are those related to plant hormones (Mukhtar et al. 2011). Only in a few instances, however, has a gene been demonstrated to confer pleiotropic resistance (Jamann et al. 2014; Nurmberg et al. 2007; Todesco et al. 2010). The mechanism of resistance may provide some insight into the specificity of resistance conditioned by a gene. For example, regulatory genes, such as those related to hormones, have been shown to provide protection against diverse pathogens (Todesco et al. 2010). In other cases, the mechanisms of pleiotropic resistance remain obscure, as in the case of the putative ABC transporter encoded by *Lr34*, which confers durable resistance to two rusts and powdery mildew (Krattinger et al. 2009).

A QTL on the short arm of chromosome 1 conditioning resistance to NLB, hereafter referred to as $qNLB1.02_{B73}$, has been identified as a pleiotropic locus in maize with effects on a number of traits including flowering time and resistance to multiple diseases. The region has been identified as providing protection against a wide range of diseases caused by an array of microbial diversity including the foliar fungal diseases NLB, SLB, common rust, the foliar bacterial disease Stewart's wilt, as well as ear and stalk rots caused by multiple fungal pathogens (Wisser et al. 2006; Chung et al. 2010b).

NLB, a foliar disease of maize caused by the fungus *Setosphaeria turcica* (anamorph = *Exserohilum turcicum*), is endemic in many regions of the world (Adipala et al. 1993; Fininsa and Yuen 2001; Levy and Pataky 1992). Yield losses of up to 63 % have been attributed to severe NLB epidemics (Perkins 1987; Raymundo 1981). Stewart's wilt, a seed-borne and insect-vector transmitted disease caused by the bacterium *Pantoea stewartii*, is important in part because of zero-tolerance phytosanitary requirements (Esker and Nutter 2002; Khan et al. 1996). Although these two pathogens are classified into different kingdoms, they have very similar pathogenic lifestyles colonizing living tissue, spreading through vascular tissue causing wilted lesions by plugging xylem vessels, and ultimately causing necrotic lesions (Chung et al. 2010b; Jennings and Ullstrup 1957; Roper 2011). The third disease against which this locus provides resistance is common rust, caused by the fungus *Puccinia sorghi*. Common rust requires host cells to be living and can decrease yields by up to 49 % (Groth et al. 1983). The MDR locus $qNLB1.02_{B73}$ thus conditions resistance against pathogens that are both taxonomically diverse and that have very different pathogenic lifestyles.

Joint linkage mapping for NLB identified a QTL at 17.6 Mb in the maize nested association mapping (NAM) population (Poland et al. 2011), which co-localizes with $qNLB1.02_{B73}$. The joint linkage mapping revealed that the inbred line 'Tx303' carried a unique allele for susceptibility to NLB among the 26 founders of the NAM population (Poland et al. 2011). A relationship between flowering time and disease severity has been noted in maize, in which lines that flower later tend to be more resistant (Wisser et al. 2011). The overall correlation was apparently due to confounding population structure, as $qNLB1.02_{B73}$ was the only NLB QTL in the NAM for which the allele effects for disease correlated with the effects for flowering time, indicating a potential role for pleiotropy at the locus (Poland et al. 2011). In NAM, lines carrying the resistance allele at $qNLB1.02_{B73}$ flowered earlier, a relationship opposite to that previously observed.

This QTL has been shown to restrict post-penetration fungal colonization. In order to dissect the resistance conditioned by this locus, Chung et al. (2010b) used histopathology on introgression lines to infer the mechanism of resistance and found that the QTL acted to slow *S. turcica* pathogenesis between the initial penetration phase and vascular invasion. Introgression lines contrasting for NLB severity also contrasted for common rust and Stewart's wilt

severity, and 'B73' was the resistance donor at this locus for all three diseases while Tx303 was the susceptibility donor, even though Tx303 is the more resistant parent (Chung et al. 2010b).

In order to understand the genetic relationship(s) underlying resistance at this locus and to identify genes involved, we used high-resolution fine mapping, complemented by association mapping, expression analysis, and mutant analysis. We were able to separate regions conferring resistance to common rust and Stewart's wilt from resistance to NLB, but were unable to differentiate the common rust and Stewart's wilt intervals. By triangulating with fine mapping, expression analysis, and mutant evaluations, we provide strong evidence suggesting that a remorin (REM) plays a role in quantitative disease resistance.

Materials and methods

Plant materials

The *qNLB1.02*$_{B73}$ QTL at 1.02 for NLB, Stewart's wilt and common rust had been identified and confirmed previously (Chung et al. 2010b). The fine-mapping population was derived from the TBBC3 (Tx303 x B73 Backcross 3) population, a population of chromosomal segment introgression lines composed of Tx303 introgressions in a B73 background (Szalma et al. 2007). TBBC family 42_10E (Chung et al. 2010b) was crossed to B73 and seeds from 22 heterozygous F_2 individuals were planted at Cornell's Robert Musgrave Research Farm in Aurora, NY in 2009. A total of 3328 plants were screened for recombinants in 2009, and an additional 1631 plants were screened in 2011. Recombinants ($n = 1239$) were identified between PZA02393.2 and chr1|29925693, representing the flanking markers for *qNLB1.02*$_{B73}$ in 2009. Recombinants ($n = 230$) were identified between SYN38630 and chr1|27735302 in 2011. Recombinants were self-pollinated and homozygous recombinants selected. Homozygous recombinants were evaluated for NLB in Aurora, NY in 2010, 2011, 2012, and 2013 and for Stewart's wilt and common rust in Aurora, NY in 2010, 2011, and 2012.

Disease evaluations

Northern leaf blight

A single race 1 isolate, StNY001 (Chung et al. 2010a; Leonard et al. 1989), collected in Freeville, NY in 1983, was used for all disease trials. Field NLB disease trials were carried out at Cornell's Robert Musgrave Research Farm in Aurora, NY from 2010 to 2013. For NLB fine-mapping trials, there were two replications in 2010, and three replications in 2011, 2012, and 2013. Inoculations were conducted as previously described (Chung et al. 2010b). Briefly, both spore suspension and solid inoculum were used to ensure infection in all weather conditions in the field, while only spore suspension was used for greenhouse trials. For the spore suspension, *S. turcica* isolates were cultured on lactose-casein agar for 2–3 weeks at 25 °C with a 12/12 h light/dark cycle. A spore suspension was prepared by flooding plates with 5 mL ddH_20 and using a glass rod to dislodge conidia. The resulting suspension was filtered through two layers of sterilized cheesecloth. Conidial concentration was adjusted to 4×10^3 per mL using a hemocytometer, and the suspension was brought to a final concentration in 0.02 % Tween 20 solution. Spore suspension (0.5 mL) was introduced into the whorl of 5–6 leaf stage plants for both field and greenhouse inoculations. Field inoculations were supplemented with solid inoculum consisting of autoclaved sorghum grains cultured with *S. turcica* for 2–3 weeks at 25 °C with a 12 h light/dark cycle (Chung et al. 2010a).

Plants in the field were visually scored for diseased leaf area (DLA) three times after flowering. Disease was scored on a per-row basis using a scale from 0 to 100 with single integer units where 0 describes a non-diseased row and 100 represents a completely diseased row, and each integer indicates one percent diseased leaf area. NLB is a polycyclic disease and there are multiple pathogen generations in a given field season. Using the three ratings, the area under the disease progress curve (AUDPC) was calculated (Chung et al. 2010a; Wilcoxson et al. 1974). For greenhouse trials, the percentage of leaf area that was necrotic from primary infections (PrimDLA) was scored with the same scale of 0-100.

Stewart's wilt

Stewart's wilt trials were carried out at Cornell's Robert Musgrave Research Farm in Aurora, NY in 2010, 2011, and 2012 and an incomplete block design was employed. In 2010, 2011, and 2012, there were two replications included for Stewart's wilt trials. Inoculations were carried out as described by Chung et al. (2010a). Briefly, nutrient broth was inoculated with a stock culture of *Pantoea stewartii* isolate PsNY003 and grown at room temperature on a shaker for approximately 2–3 days. Cells were diluted to a final concentration of about 10^7 CFU/mL and suspended in a 0.1 M NaCl solution (Suparyono and Pataky 1989). Pin-prick inoculations were conducted on 5- to 6-leaf stage plants (Chung et al. 2010a). Disease was visually scored on a row basis at 2–3 weeks post inoculation using a scale from 0 to 100 with single integer intervals representing one percentage of the diseased leaf area scale.

Common rust

Rust field trials were carried out at Cornell's Robert Musgrave Research Farm in Aurora, NY in 2010, 2011, and 2012 using an incomplete block design. In 2010, 2011, and 2012, there were three replications for common rust trials. Urediniospores of *Puccinia sorghi* were collected in Aurora, NY in 2007. Rust inoculations were carried out as previously described (Chung et al. 2010b). Briefly, about 1 month before field inoculations, spores were increased by inoculating seedlings of susceptible maize germplasm (sweet corn) at the 3–4 leaf stage in the greenhouse. About 200–300 mg of urediniospores were suspended in 100 ml of Sortrol oil (Chevron Phillips Chemical Company, Borger, TX, USA) and sprayed onto leaves using a spray gun. Plants were kept at >80 % humidity overnight. Urediniospores were collected about 3 weeks later by agitating infected sweet corn leaves in water and filtering the spore suspension through four layers of cheesecloth. Field trials were inoculated with 1.0 mL of spore suspension (2.0×10^5 urediniospores in 0.02 % Tween 20) in the whorl. Disease was visually scored on a per-row basis three times, beginning ~3 weeks after inoculation with ratings every ~10 days. A scale of 0–9 with 0.5 increments was used, where 0 indicated no disease and 9 indicated 100 % diseased leaf area. The AUDPC was calculated as previously described (Chung et al. 2010a).

Genotyping assays

DNA extraction

High-throughput DNA extractions followed by high-throughput SNP assays were used to identify recombinants, and high-quality CTAB DNA extractions were performed for all other applications. The high-throughput extraction was performed using ExNAmp (Sigma-Aldrich, St. Louis, MO, USA). Leaf tissue (1 mm^2) was plated into 0.2-mL PCR tubes, and 8 μL of extraction buffer was added to each sample. Samples were then incubated at 95 °C for 10 min, and 8 μL of dilution buffer was added to the samples. The DNA was diluted 1:100 for KASP genotyping assays (LGC Genomics, Beverly, MA, USA). The high-quality CTAB extractions were performed as previously described (Chung et al. 2010b; Doyle and Dickson 1987).

Single nucleotide polymorphism (SNP) genotyping

Two approaches were used to identify polymorphic markers between the inbred lines B73 and Tx303. Available SNPs from the Maize Diversity Project were queried at http://www.panzea.org (Canaran et al. 2008). SNPs polymorphic between B73 and Tx303 were selected from the following resources: the original NAM genetic map marker set (McMullen et al. 2009), the first-generation haplotype map of maize (Gore et al. 2009), and the second-generation haplotype map of maize (Chia et al. 2012). Additional polymorphisms between B73 and the near-isogenic line (NIL) TBBC_42 were identified using an Illumina MaizeSNP50 Beadchip (Illumina, San Diego, CA, USA) run at the David H. Murdock Research Institute, Kannapolis, NC, USA. Allele-specific PCR was utilized to perform all SNP genotyping using KASP (LGC Genomics, Beverly, MA, USA) as described by Jamann et al. (2014). Briefly, 100 ul of 1:100-diluted ExNAmp DNA was dried down in a KASP plate and a 4 uL KASP reaction performed. Results were read using an Applied Biosystems (Foster City, CA) 7900 HT (Life Technologies) and analyzed using SDS v2.1 (Life Technologies). All markers used for this study are shown in Table S1. Sequencing of the fine-mapping region including the reference gap was conducted using the protocol in Chung et al. (2010a) and the primers found in Table S2.

QTL mapping

For NLB, best linear unbiased prediction (BLUPs) were calculated for each year by fitting a mixed model with 'AUDPC' as the response, and 'replication', 'block' nested within 'replication' and 'line' as random factors using the lme4 package in R (De Boeck et al. 2011; R Core Development Team 2013). For both Stewart's wilt and common rust, data for all 3 years were analyzed together using 'AUDPC' or 'DLA' as the response, 'year', and 'replication' nested within 'year', 'block' nested within 'replication' and 'year', and 'line' as random factors. Stewart's wilt was rated once per year, so single DLA ratings were used as the response in the model. Missing genotypes were imputed based on the nearest flanking marker's genotype. Lines with fewer than 15 markers and markers with fewer than 150 lines genotyped after imputation were removed from the analysis. BLUPs were then used for single marker regression using the qtl package in R (Broman et al. 2003). Analysis was conducted on a per-year basis for NLB and a combined basis for Stewart's wilt and common rust. Bayes confidence intervals (95 %) were calculated using R/qtl (Broman et al. 2003). Association mapping using the 282-line maize diversity panel (Flint-Garcia et al. 2005) was conducted as described previously (Jamann et al. 2014).

Race testing

To determine the resistance spectrum of the QTL, races 0, 1, 23, and 23 N (Chung et al. 2010a; Leonard et al. 1989) were used in greenhouse inoculation experiments, represented by isolates St10a, StNY001, St86A, and St28A, respectively (Chung et al. 2010a). Plants carrying either

the Tx303 or B73 allele from a single family of F_2 42_10E NILs were used for race testing. Greenhouse trials were conducted at Cornell University in Ithaca, NY. A complete block design was used. The percentage of leaf area that was necrotic from primary infections (PrimDLA) was scored with 1 % increments. Statistical analysis for race testing was conducted in JMP 9.0 (SAS, Cary, NC, USA). A mixed model was constructed with 'PrimDLA' as the response, and 'replication' as a random factor, and 'genotype' as a fixed factor. A Student's *t* test was performed to test for significant differences between lines.

Identification of candidate genes

The maize reference genome sequence based on B73 (AGP_ V2) (Schnable et al. 2009) was consulted to find all annotated genes in the fine-mapping interval spanning from ss228821000 (25,156,553 bp) to ss228821424 (25,399,986 bp). All annotated genes from the working gene set were considered candidates and can be found in the Table S3.

Expression analysis

To test for expression differences, two NILs were selected from a single family of F_4 plants: one carrying the B73 allele and one carrying the Tx303 allele. Tissue (80 mg/sample) was collected from field-grown mature leaf tissue in 2011 three days after inoculation with *S. turcica* isolate NY0001 and placed in liquid nitrogen. Three biological replications were included. Frozen tissue was pulverized and total RNA was extracted using a Qiagen Plant Easy RNA Kit (Valencia, CA, USA). cDNA was synthesized using Invitrogen SuperScript III First strand (Grand Island, NY, USA). TaqMan primers and probes were designed using Primer Express (Life Technologies, Grand Island, NY, USA) or selected from available validated assays from Life Technologies (Grand Island, NY, USA). Validated assays Zm04021391_m1, Zm04048055_g1, and Zm04040368_g1 were used for the chaperonin, *ZmREM6.3*, and ubiquitin control, respectively. Three technical replications were included. Primers and probes for other genes tested are listed in Table S2. qRT-PCR was performed with TaqMan gene expression master mix using standard conditions (Life Technologies, Grand Island, NY, USA) and analyzed on a ViiA7 (Life Technologies) using the relative quantification and comparing expression of the four candidate genes to that of ubiquitin by the comparative C_T method.

Mutant analysis

Three UniformMu lines (UfMu-06505, UfMu-06509 and UfMu-07948) lines were tested for NLB phenotypes. F_4 UniformMu seed obtained from the maize stock center was grown out and self-pollinated. F_5 plants were evaluated for NLB, as previously described, and days to anthesis (DTA) in Aurora, NY in 2013 (UfMu-06505, UfMu-06509 and UfMu-07948), Aurora, NY in 2014 (UfMu-06505 and UfMu-06509) and Clayton, NC in 2014 (UfMu-06509). Days to anthesis was scored as the number of days until 50 % of the plants were shedding pollen. A PCR protocol adapted from Settles et al. (2004) was used to confirm the presence or absence of insertions. Two markers flanking the insertion were designed with Primer 3 (Untergasser et al. 2012) and oligonucleotides were obtained from IDT (Coralville, IA). The primers are shown in Table S2. Each reaction contained ~100 ng CTAB-extracted DNA, 20 mM Tris ± HCl pH 8.4, 50 mM KCl, 2 mM $MgCl_2$, 5 % DMSO, 200 mM of each dNTP, 1 mM gene-specific primer, 100 nM TIR6 primer and 1 U of Taq polymerase. Thermocycling conditions were as follows: 94 °C for 1 min; 8–10 cycles of 94 °C for 25 s, 62 °C for 30 s, and 72 °C for 1 min; 27 cycles of 94 °C for 25 s, 56 °C for 30 s, and 72 °C for 1 min; and 72 °C for 5 min. Products were then visualized on an agarose gel. The data were analyzed with a mixed linear model in JMP 11.0 (SAS, Cary, NC) where 'AUDPC' or 'DTA' was the response and 'replication' nested within 'environment' and 'environment' were fitted as random factors, and 'genotype' was fitted as a fixed factor.

Phylogenetic analysis

Maize genes to include in the phylogenetic analysis were identified by querying maize genes for PFAM domain PF03763 (Remorin_C). A total of 34 genes were identified with the domain. Remorin sequences from Raffaele et al. (2007) were also included. MUSCLE was used for the multiple sequence alignment of the amino acid sequences (Edgar 2004). PHYLIP was used to construct the phylogenetic tree (Felsenstein 1989). Bootstrapping was preformed using SEQBOOT with 1000 replications. Next, PROTPARS was used and the dataset was jumbled 10 times. A consensus tree was constructed using CONSENSE. PROML was used to calculate the branch lengths. ETE was used to draw the final tree (Huerta-Cepas et al. 2010).

Results

Mapping of *qNLB1.02*$_{B73}$

A number of QTL studies have mapped a QTL for NLB resistance to the short arm of chromosome 1 (Wisser et al. 2006). A previous study showed that there was a QTL for NLB resistance at 1.01/1.02 based on the evaluation of the

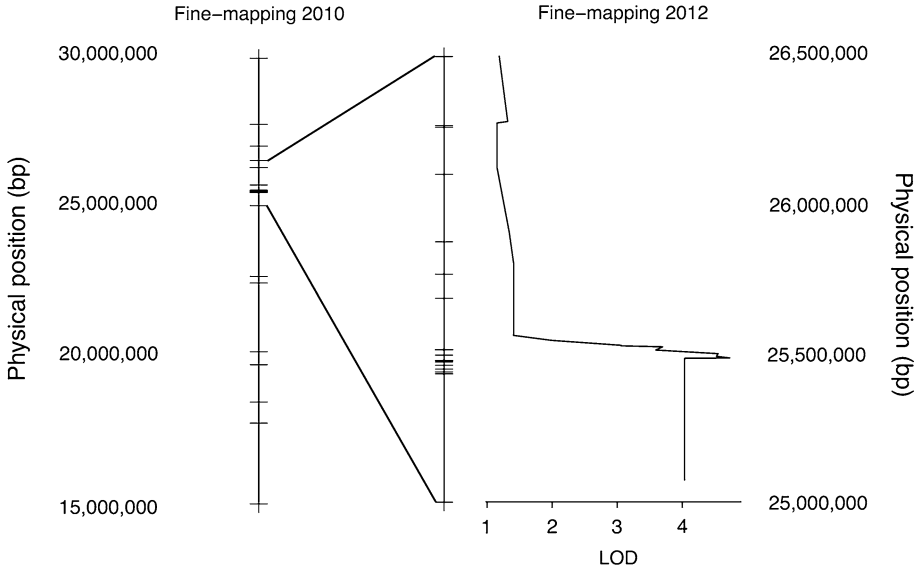

Fig. 1 Northern leaf blight (NLB) fine-mapping breakpoint analysis. Breakpoint analysis for NLB for 2010 and 2012 is shown. *On the left* is the 2010 fine-mapping where the interval was delimited to a 1.28 Mb region. Recombinants were evaluated in 2012 and the interval was narrowed to a 243-kb region. Genes within the most significant 22 kb were focused upon for detailed investigation

TBBC3 (Tx303 x B73 Backcross 3) introgression lines (Chung et al. 2010b), and there was a QTL in the nested association mapping population at PZB00718.5 (chr1: 17,666,998 bp) (Poland et al. 2011). In both of these studies, B73 was the resistance donor (Chung et al. 2010b). The Tx303 introgression in TBBC3 family 42_10E_02 spanned the interval from 6.1 (SYN6315) to 33.0 Mb (PUT-163a-18162870-1232) on chromosome 1 based on the Illumina MaizeSNP50 chip and conditioned an 18–38 % reduction in disease in the resistant NIL (Chung et al. 2010b). This interval, however, was too large to begin a fine-mapping study, so a narrowed region was selected based on prior findings (Chung et al. 2010b). The selected flanking markers, PZA02393.2 and chr1|29925693, delimited a 14-Mb fine-mapping interval from 16 to 30 Mb on chromosome 1.

High-resolution mapping of *qNLB1.02*$_{B73}$

A mapping population of 4959 plants was screened for recombinants. Screening 3328 plants with chr1|29925693 and PZA02393.2 in 2009, recombinants (n = 1239) were identified. Homozygous recombinants were evaluated in 2010 (n = 244) and in 2011 (n = 113). An additional 1631 plants were screened for recombinants in 2011 using SYN7041 and chr1|27735302, which led to the identification of an additional 230 recombinants. A total of 114 homozygous recombinants were screened in 2012. Recombinant lines were selected for evaluation based on whether breakpoints were within the interval of interest based on the previous years' delimitation of the interval and on seed availability. The NLB QTL was narrowed to an interval delimited by ss228821000 (25,156,553 bp) to ss228821424 (25,399,986 bp) in 2012 (Fig. 1).

Fine-mapping of broad-spectrum disease resistance

The locus *qNLB1.02*$_{B73}$ had been previously shown to be effective against Stewart's wilt and common rust (Chung et al. 2010b), in addition to NLB. To determine the genetic nature of multiple disease resistance at the locus, the fine-mapping population was evaluated for Stewart's wilt (n = 469) and common rust (n = 175). Only the 2010 and 2011 data were used for common rust, as there was insufficient disease pressure in 2012. The region comprising *qSw1.02* $_{B73}$ and *qRust1.02*$_{B73}$ was narrowed to 5.26 Mb (19,679,687–24,940,817 bp) and 2.56 Mb (22,379,568–24,940,817 bp), respectively, as shown in Fig. 2. The *qSw1.02* $_{B73}$ interval contained 451 genes, while the *qRust1.02*$_{B73}$ interval contained 218 genes.

To determine the specificity of the resistance conditioned by *qNLB1.02*$_{B73}$, multiple races of *S. turcica* were tested for their interaction with the locus. The 42_10E NIL carrying the B73 allele at *qNLB1.02*$_{B73}$ showed resistance to isolates representing races 0, 1, 23, and 23 N (Fig. 3).

Fine-mapping of NLB resistance

In 2012, the 95 % confidence interval spanned from ss228821000 (25,156,553 bp) to ss228821424 (25,399,986 bp). A total of 15 annotated genes were identified in this 243-kb interval, 10 of which were high confidence genes. Genes can be found in Table S3. We focused on the portion of the interval with the highest LOD scores, a 22-kb region from ss228821319 to ss228821424 including a putative pentricopeptide (GRMZM2G107805), a putative chaperonin (GRMZM2G069765), three uncharacterized genes (GRMZM2G070442, GRMZM2G107727, and GRMZM2G523621), and a putative remorin

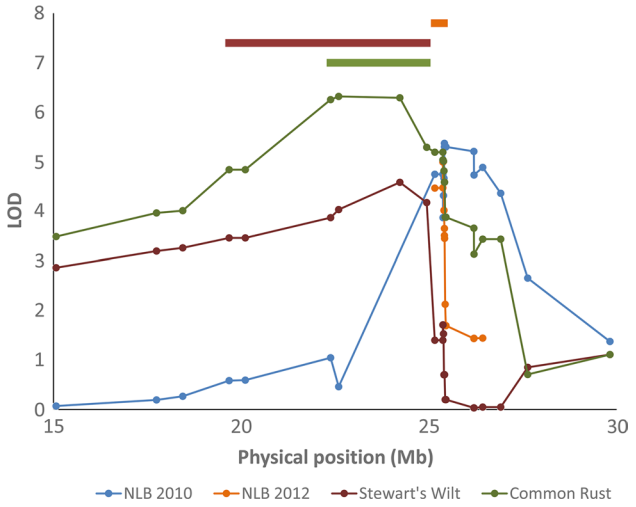

Fig. 2 Multiple disease resistance fine-mapping. The breakpoint analyses for NLB (year 2010 and 2012), Stewart's wilt and common rust are shown. Confidence intervals (95 % Bayes intervals) for each disease, are shown as *bars above*. The intervals for Stewart's wilt and common rust overlap while the interval for NLB (year 2012) is distinct

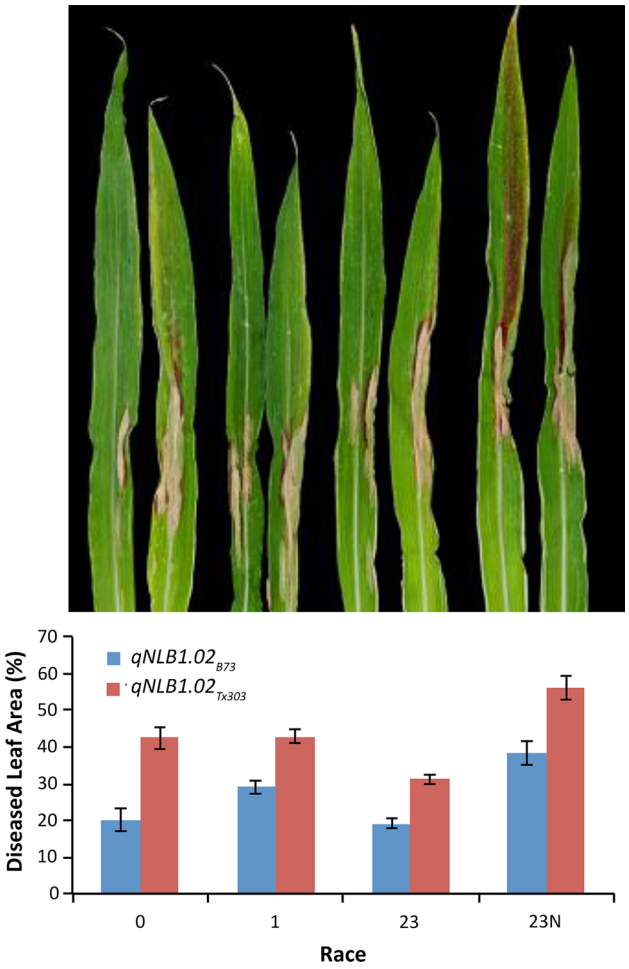

Fig. 3 Race testing for $qNLB1.02_{B73}$. The QTL provided resistance to all four of the *S. turcica* isolates tested. Phenotypes of near-isogenic lines (NILs) carrying the B73 allele are shown in *blue*, while those carrying the Tx303 allele are shown in *red*. Leaves are representative samples for each plant genotype × fungal isolate combination. The isolates represent the four commonly recognized pathotypes (races) of *S. turcica*

(GRMZM2G107774). A number of polymorphisms were identified between B73 and Tx303 within the fine-mapping region.

In the 243-kb interval of interest, two uncharacterized genes (GRMZM2G107727 and GRMZM2G523621) were found to overlap and to span a gap in the B73 reference sequence. One of the mRNAs (EU94788) from B73 that mapped to this locus shared homology (66 % identity) with a *Zea mays* F-box gene (GRMZM2G141332). The gap, which was <1 kb in length, was sequenced in Tx303 and B73, but no predicted F-box domain was found in the gap region in either B73 or Tx303. No significant associations for NLB were identified in the NAM within the fine-mapping region (Poland et al. 2011; Chia et al. 2012). Using NLB data (Wisser et al. 2011) for the 282-line Goodman diversity panel (Flint-Garcia et al. 2005), no significant associations were identified within the fine-mapping region.

Remorin nomenclature

The putative remorin within the fine-mapping interval was annotated with a remorin_C domain (PFAM 03763). In order to classify and assign suitable nomenclature to this gene, we conducted a phylogenetic analysis of all genes containing an annotated remorin domain in the maize genome, as well as previously classified remorin genes. Our remorin of interest grouped with other known group 6 remorin genes and was thus named *ZmREM6.3,* as shown in Fig. 4. Group 6 remorins have a conserved C-terminal region and are classified as long remorins (Raffaele et al. 2007). There is a potential role for group 6 remorins in plant–microbe interactions. AtREM6.1 has a putative recognition site for the bacterial effector AvrRpt2 (Chisholm et al. 2005).

Expression analysis of fine-mapping candidate genes

We tested the expression of the chaperonin, uncharacterized, F-box and remorin genes at 72 h after infection (Fig. 5). The uncharacterized gene had very low expression. The chaperonin gene was expressed but did not show differential expression between alleles or between pathogen-inoculated and mock-inoculated samples. However, both the putative F-box gene and *ZmREM6.3* were more highly expressed in the resistant NIL carrying the B73 allele (Fig. 5). A trend of lower expression in inoculated

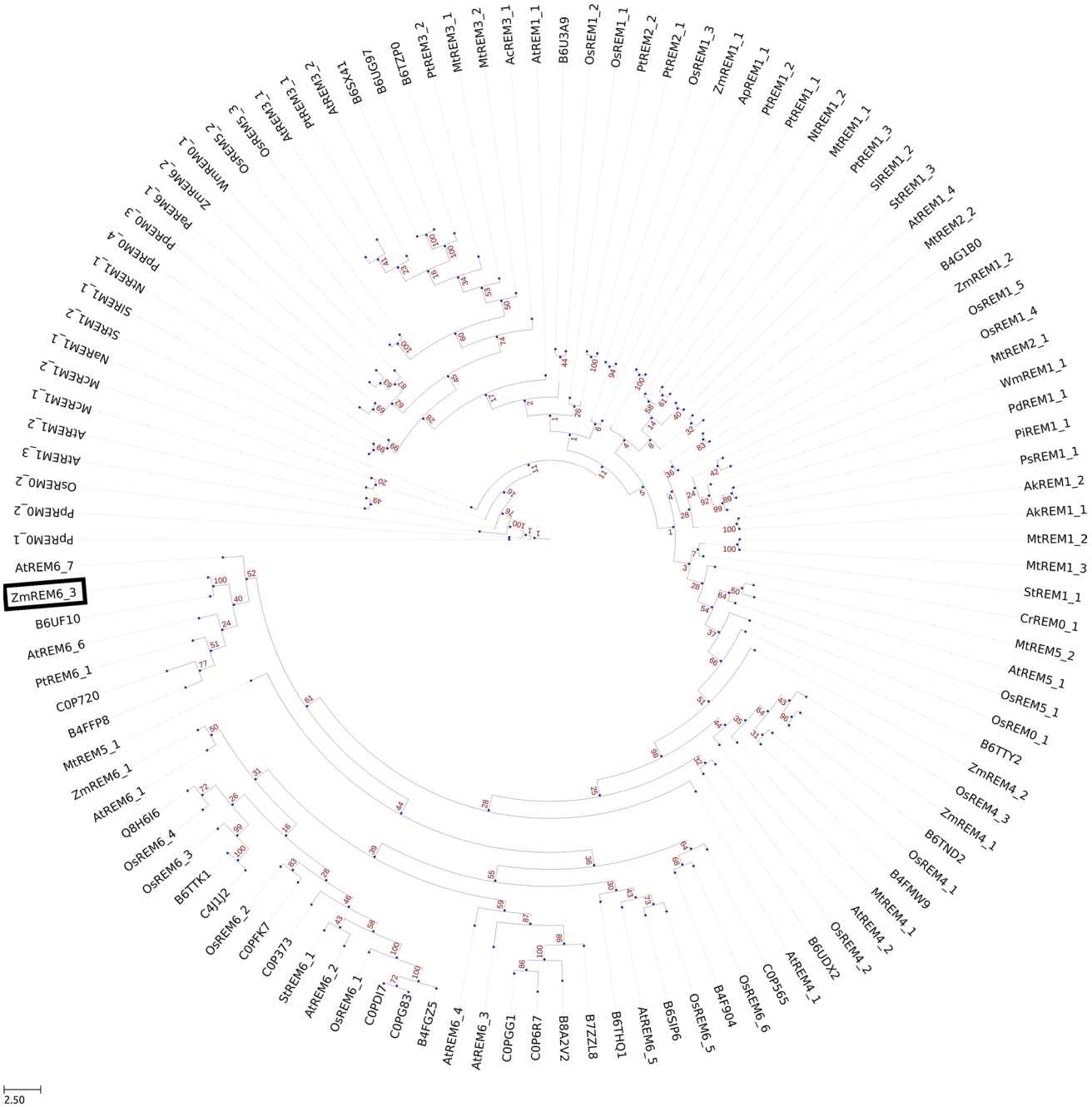

Fig. 4 Phylogenetic analysis of maize remorin genes. A total of 34 maize genes were identified with a remorin C domain. Also included were REM proteins previously classified by Raeffele et al. 2007. The remorin of interest (*ZmREM6.3*) grouped with other group 6 remorins

samples was observed in the qRT-PCR experiment for *ZmREM6.3*.

Mutant analysis

UniformMu lines, carrying Mu transposon insertions in a 'W22' background, corresponding to the candidate genes were selected and evaluated. Three UniformMu families with insertions in or near the candidate genes were selected: one with an insertion (mu1058569 in UfMu-07948) in the chaperonin (GRMZM2G069765), one with an insertion (mu1046469 in UfMu-06509) in *ZmREM6.3* (GRMZM2G107774), and one with an insertion (mu1051418 in UfMu-06505) ~1000 bp downstream of *ZmREM6.3* (GRMZM2G107774) and ~26 bp upstream of the pentatricopeptide (GRMZM2G107805). No mutant lines with insertions in the uncharacterized gene with homology to F-box genes were available. The UniformMu families with an

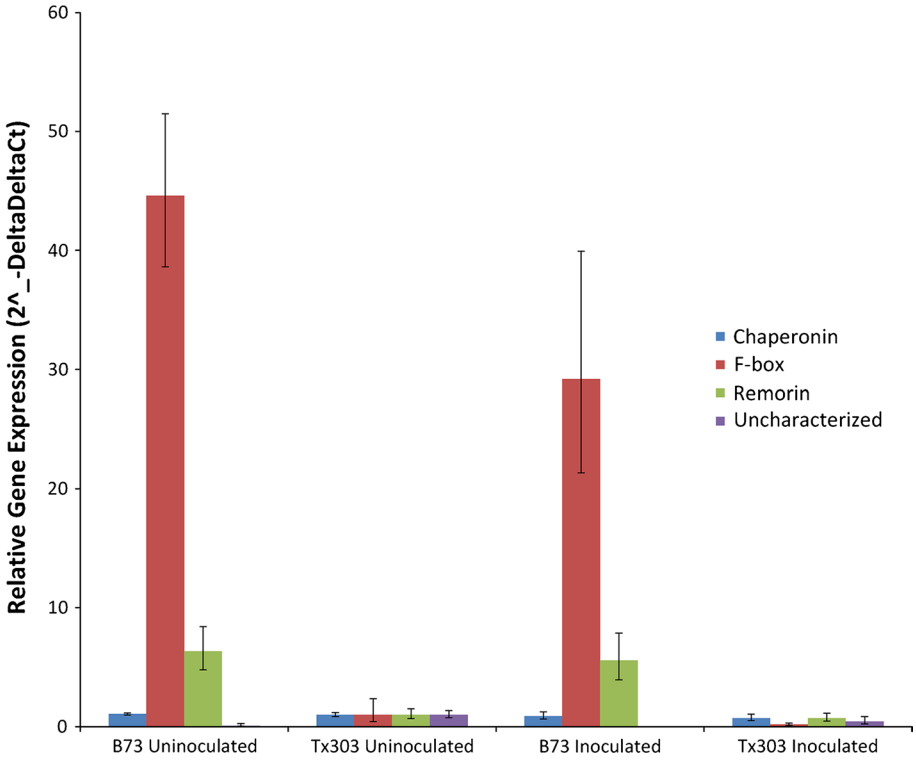

Fig. 5 Expression of candidate genes. Candidate gene expression in resistant (B73 allele) and susceptible (Tx303 allele) NILs. To test for expression differences, two NILs were selected, one carrying the B73 allele and one carrying the Tx303 allele, from a single family of F_4 plants. Tissue (80 mg/sample) was collected from field-grown mature leaf tissue in 2011 three days after inoculation with *S. turcica* isolate NY0001. Three biological replications were included. Each biological replicated was pooled from six plants. Three technical replications were performed. Results were analyzed on a ViiA7 (Life Technologies) by comparing expression of the four candidate genes to that of ubiquitin. *Error bars* represent standard deviation

insertion downstream of *ZmREM6.3* (UfMu-06505) and an insertion in the chaperonin were tested (UfMu-07948), but lines homozygous for the insertion of interest were not identified. When a mixed model with AUDPC as the response and experimental design factors and genotype was fitted for UfMu-06505 and UfMu-07948, genotype was not significant with p values of 0.20 and 0.92, respectively. The insertion mu1046469 in UfMu-06509 was located in the second exon of GRMZM2G107774_T01. Five lines were developed from the UfMu-06509 family: two lines homozygous for the mu1046469 insertion and three lines heterozygous for the mu1046469 insertion. No segregants were identified from the UfMu-06509 family that lacked the mu1046469 insertion.

When a mixed model including experimental design factors was fitted for AUDPC, genotype was found to be significant for UfMu-06509. The homozygous mutants had a 31 % increase in AUDPC as compared to the heterozygotes, while the heterozygotes had 32 % higher AUDPC relative to W22, as shown in Fig. 6. When a similar model was fitted for days to anthesis as the response variable, genotype was not significant. Thus, *ZmREM6.3* appears to have a specific effect on NLB disease development.

Discussion

Despite its utility, the genetic and mechanistic basis of quantitative disease resistance is not well understood. In this study, we examined a region of the maize genome that has been associated with resistance to two fungal pathogens with distinct pathogenic strategies, as well as one bacterial pathogen with a similar strategy to one of the fungal pathogens. One of our objectives was to determine whether multiple disease resistance is due to linkage or pleiotropy. Pleiotropy between traits is rare in maize (Wallace et al. 2014b). One notable exception, however, is disease resistance (Jamann et al. 2014; Wisser et al. 2011), potentially including resistance to common rust and Stewart's wilt at *qNLB1.02*. The fine-mapping confidence intervals for common rust and Stewart's wilt overlapped, but different markers were the most significant for each disease. We were, therefore, unable to distinguish tight linkage from pleiotropy in this case.

Tx303 carries a rare susceptible allele in the NAM at this locus, and this may have impeded the power of joint linkage mapping and genome-wide association mapping. An allelic series was identified using joint linkage mapping in the NAM populations at the $qNLB1.02_{B73}$ QTL (Poland et al. 2011). Alleles more resistant and more susceptible than the common parent B73 were identified and Tx303, a moderately resistant line, was found to be the only founder line that carried a susceptibility allele relative to B73 (Poland et al. 2011; Chung et al. 2010b). Even as a moderately susceptible line, B73 contains resistance alleles; the locus studied here includes such an allele (Poland et al. 2011; Chung et al. 2010b). Confirming the joint linkage mapping

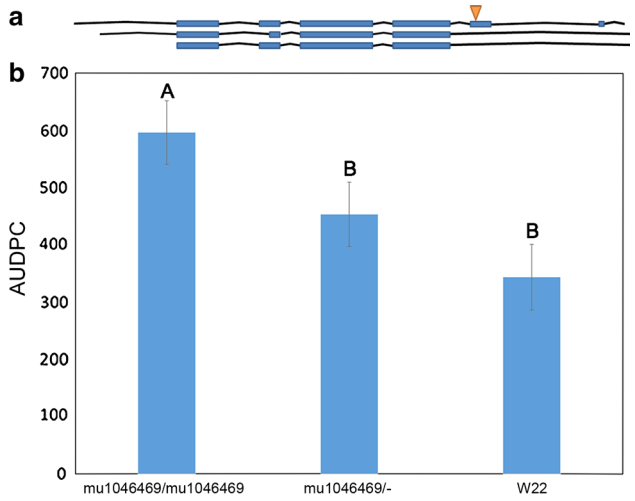

Fig. 6 Phenotype of UfMu-06509 lines with *Mu* insertion mu1046469 in a remorin candidate gene (GRMZM2G107774). *A* Gene models for *ZmREM6.3*. Exons are represented by *blue boxes*. The *Mu* insertion is located in second exon of the first transcript in the C domain of the remorin. Note that the gene is on the reverse strand. *B* The homozygous remorin UniformMu mutant lines are significantly more susceptible than the heterozygotes or the homozygous wild type lines ($P < 0.05$). *Errors bars* indicate standard error

results, Tx303 was the source of the susceptible allele in the TBBC3 NILs. Interestingly, the fine-mapping interval did not fall within the NAM joint linkage mapping confidence interval (Poland et al. 2011), consistent with the hypothesis that there are multiple QTL at the region and supporting the interpretation that Tx303 has a rare susceptible allele. Joint linkage mapping may be problematic when multiple QTL are present in a region, although a denser NAM genetic map may be able to distinguish QTL more effectively.

We focused on the QTL interval defined in 2012 to identify candidate genes. Association mapping in both the NAM and the diversity panel did not reveal any significant associations in the region. There are several possible reasons for this; the causal polymorphism might not have been included in the genotypic datasets; structural variation might underlie the QTL; or the allele frequency might not have allowed for detection. Given the low frequency of the susceptible allele in the NAM, it seems likely that the allele frequency may have been too low to be detected with association mapping.

Expression of the F-box gene and *ZmREM6.3* were higher in the resistant line, and *ZmREM6.3* was down-regulated upon infection with a *S. turcica* race 1 isolate in both the susceptible and resistant NILs. These observations are consistent with an independent genome-wide expression profiling experiment of the maize (B73)-*S. turcica* (St28A; race 23N) interaction, in which *ZmREM6.3* was found to be significantly down-regulated at 3, 7 and 10 days after infection (Condon, Wiesner-Hanks, Saha, Mideros, and Turgeon, personal communication) (Nordberg et al. 2014). The down-regulation of *ZmREM6.3* may indicate that it is an important part of the defense response and thus targeted by the pathogen. Amino acid sequence differences may also be important. Four amino acid differences, seven intronic, and two synonymous polymorphisms were identified in the *ZmREM6.3* between B73 and Tx303 in the HapMap dataset (Chia et al. 2012). One of the polymorphisms (PZE0125488574) causes an arginine to glycine amino acid change within the REM domain, but this polymorphism is not unique in the NAM to the susceptibility allele of Tx303.

We tested UniformMu lines with insertions in or near *ZmREM6.3* and the chaperonin gene, and found that the UniformMu line UfMu-06509, which was homozygous for an insertion in the second exon of *ZmREM6.3,* was significantly more susceptible than the corresponding line with one copy of the insertion. The inbred line W22, the genetic background for the UniformMu lines, is highly susceptible to NLB, and when *ZmREM6.3* was interrupted by a transposon insertion, the line with two copies of the insertion was more susceptible than the heterozygote with only one copy of the insertion and the background parent W22. This demonstrates that a null allele in a susceptible background can be informative. The NLB reactions of two other UniformMu lines were tested for the fine-mapping region, one with an insertion downstream of *ZmREM6.3* and upstream of the pentatricopeptide, and one with an insertion in the chaperonin. Genotype was not significantly associated with NLB for either of these lines. While the F-box gene is a strong candidate gene, no mutant lines were available to test this gene.

REMs are involved in cell surface signaling and have previously described roles in plant–microbe interactions, including symbiotic bacterial interactions and pathogenic viral and oomycete interactions (Bozkurt et al. 2014; Kim and Delaney 2002; Lefebvre et al. 2010; Raffaele et al. 2009; Perraki et al. 2014; Tóth et al. 2012). REM proteins are general regulators of the plasmodesmatal size exclusion limit and have been shown to physically interact with a viral movement protein and receptor-like kinases (Lefebvre et al. 2010; Raffaele et al. 2009; Perraki et al. 2014). REM proteins have been characterized for their roles in plant–microbe interactions. For example, REM1.3 functions as a susceptibility factor for the oomycete pathogen *Phytophthora infestans* (Bozkurt et al. 2014). In a contrasting study, transgenic tomato lines overexpressing REM were found to be more resistant to potato virus X and the REM altered viral cell-to-cell movement in this case (Raffaele et al. 2009). Previously, it had been shown that $qNLB1.02_{B73}$ prevents entrance into the vasculature (Chung et al. 2010b), consistent with this known function of REM proteins. We hypothesize that the resistance allele of *ZmREM6.3* restricts fungal movement between maize cells. This will be tested using histopathology.

A multi-faceted approach was needed to identify genes underlying this quantitative disease resistance locus. Mutant analysis may or may not confirm candidate disease resistance genes because of extreme null alleles and in some cases a susceptible background that may not allow for a discernible increase in susceptibility. While expression analysis provided evidence for candidate genes from fine-mapping, the fine-mapping was needed to delimit QTL intervals and distinguish allelic effects. In this case, the region identified by fine-mapping had not been implicated by association mapping, but did correspond to differentially expressed genes between resistant and susceptible NILs and mutants of one gene were significantly more susceptible. At this locus, multiple disease resistance is controlled by multiple genes. Our results suggest tight linkage of genes influencing Stewart's wilt, rust, and NLB, although there may be a role for pleiotropy for resistance to Stewart's wilt and common rust. We demonstrate a role for *ZmREM6.3* in disease resistance, a gene previously unassociated with plant-fungal interactions and QDR.

Author contribution statement TMJ, CC and RJN conceived of the study. TMJ, XL, LM, CC and JMK conducted the research. TMJ performed the analysis. TMJ and RJN wrote the paper.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

References

Adipala E, Lipps P, Madden L (1993) Occurrence of *Exserohilum turcicum* on maize in Uganda. Plant Dis 77:202

Bozkurt T, Richardson A, Dagdas Y, Mongrand S, Kamoun S et al (2014) The plant membrane-associated REM1.3 remorin accumulates in discrete perihaustorial domains and enhances susceptibility to *Phytophthora infestans*. Plant Physiol 165:1005–1018

Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890

Canaran P, Buckler ES, Glaubitz JC, Stein L, Sun Q et al (2008) Panzea: an update on new content and features. Nucl Acids Res 36:D1041–D1043

Chia JM, Song C, Bradbury PJ, Costich D, de Leon N et al (2012) Maize HapMap2 identifies extant variation from a genome in flux. Nat Genet 44:803–807

Chisholm ST, Dahlbeck D, Krishnamurthy N, Day B, Sjolander K et al (2005) Molecular characterization of proteolytic cleavage sites of the Pseudomonas syringae effector AvrRpt2. Proc Natl Acad Sci 102:2087–2092

Chung CL, Jamann T, Longfellow J, Nelson R (2010a) Characterization and fine-mapping of a resistance locus for northern leaf blight in maize bin 8.06. Theor Appl Genet 121:205–227

Chung CL, Longfellow JM, Walsh EK, Kerdieh Z, Van Esbroeck G et al (2010b) Resistance loci affecting distinct stages of fungal pathogenesis: use of introgression lines for QTL mapping and characterization in the maize–*Setosphaeria turcica* pathosystem. BMC Plant Biol 10:103

Cook DE, Lee TG, Guo X, Melito S, Wang K et al (2012) Copy number variation of multiple genes at *Rhg1* mediates nematode resistance in soybean. Science 338(6111):1206–1209

De Boeck P, Bakker M, Zwitser R, Nivard M, Hofman A et al (2011) The estimation of item response models with the lmer function from the lme4 package in R. J Stat Softw 39:1–28

den Boer E, Zhang NW, Pelgrom K, Visser RG, Niks RE et al (2013) Fine mapping quantitative resistances to downy mildew in lettuce revealed multiple sub-QTLs with plant stage dependent effects reducing or even promoting the infection. Theor Appl Genet 126:2995–3007

Doyle JJ, Dickson EE (1987) Preservation of plant samples for DNA restriction endonuclease analysis. Taxon 36(4):715–722

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl Acids Res 32:1792–1797

Esker P, Nutter F (2002) Assessing the risk of Stewart's disease of corn through improved knowledge of the role of the corn flea beetle vector. Phytopathology 92:668–670

Felsenstein J (1989) PHYLIP—phylogeny inference package (version 3.2). Cladistics 5:164–166

Fininsa C, Yuen J (2001) Association of maize rust and leaf blight epidemics with cropping systems in Hararghe highlands, eastern Ethiopia. Crop Prot 20:669–678

Flint-Garcia SA, Thuillet AC, Yu J, Pressoir G, Romero SM et al (2005) Maize association population: a high-resolution platform for quantitative trait locus dissection. Plant J 44:1054–1064

Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L et al (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323:1357–1360

Fukuoka S, Saka N, Koga H, Ono K, Shimizu T et al (2009) Loss of function of a proline-containing protein confers durable disease resistance in rice. Science 325:998–1001

Gore M, Chia J, Elshire R, Sun Q, Ersoz E et al (2009) A first-generation haplotype map of maize. Science 326:1115

Groth JV, Zeyen RJ, Davis DW, Christ BJ (1983) Yield and quality losses caused by common rust (*Puccinia sorghi* Schw.) in sweet corn (*Zea mays*) hybrids. Crop Protection 2(1):105–111

Huerta-Cepas J, Dopazo J, Gabaldón T (2010) ETE: a python environment for tree exploration. BMC Bioinformatics 1(1):24

Jamann T, Poland J, Kolkman K, Smith L, Nelson R (2014) Unraveling genomic complexity at a quantitative disease resistance locus in maize. Genetics 198(1):333–344

Jennings P, Ullstrup A (1957) A histological study of three *Helminthosporium* leaf blights of corn. Phytopathology 47:707–714

Johnson EB, Haggard JE, St Clair DA (2012) Fractionation, stability, and isolate-specificity of QTL for resistance to *Phytophthora infestans* in cultivated tomato (*Solanum lycopersicum*). G3 Genes Genomes Genet 2:1145–1159

Khan A, Ries S, Pataky J (1996) Transmission of *Erwinia stewartii* through seed of resistant and susceptible field and sweet corn. Plant Dis 80:398–403

Kim HS, Delaney TP (2002) Arabidopsis SON1 is an F-box protein that regulates a novel induced defense response independent of both salicylic acid and systemic acquired resistance. Plant Cell 14:1469–1482

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J et al (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363

Kump KL, Bradbury PJ, Wisser RJ, Buckler ES, Belcher AR et al (2011) Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat Genet 43:163–168

Lefebvre B, Timmers T, Mbengue M, Moreau S, Herve C et al (2010) A remorin protein interacts with symbiotic receptors and regulates bacterial infection. Proc Natl Acad Sci 107:2343–2348

Leonard K, Levy Y, Smith D (1989) Proposed nomenclature for pathogenic races of *Exserohilum turcicum* on corn. Plant Dis 73:776–777

Levy Y, Pataky J (1992) Epidemiology of northern leaf blight on sweet corn. Phytoparasitica 20:53–66

Manosalva PM, Davidson RM, Liu B, Zhu X, Hulbert SH et al (2009) A germin-like protein gene family functions as a complex quantitative trait locus conferring broad-spectrum disease resistance in rice. Plant Physiol 149:286–296

McMullen MD, Kresovich S, Villeda HS, Bradbury P, Li H et al (2009) Genetic properties of the maize nested association mapping population. Science 325:737–740

Mukhtar MS, Carvunis AR, Dreze M, Epple P, Steinbrenner J et al (2011) Independently evolved virulence effectors converge onto hubs in a plant immune system network. Science 333:596–601

Nordberg H, Cantor M, Dusheyko S, Hua S, Poliakov A et al (2014) The genome portal of the department of energy joint genome institute: 2014 updates. Nucl Acids Res 42:D26–D31

Nurmberg PL, Knox KA, Yun BW, Morris PC, Shafiei R et al (2007) The developmental selector *AS1* is an evolutionarily conserved regulator of the plant immune response. Proc Natl Acad Sci 104:18795–18800

Perkins JM (1987) Disease development and yield losses associated with northern leaf blight on corn. Plant Dis 71:940

Perraki A, Binaghi M, Mecchia M, Gronnier J, German-Retana S et al (2014) StRemorin1.3 hampers *Potato virus X* TGBp1 ability to increase plasmodesmata permeability, but does not interfere with its silencing suppressor activity. FEBS Lett 588:1699–1705

Poland JA, Balint-Kurti PJ, Wisser RJ, Pratt RC, Nelson RJ (2009) Shades of gray: the world of quantitative disease resistance. Trends Plant Sci 14:21–29

Poland JA, Bradbury PJ, Buckler ES, Nelson RJ (2011) Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. Proc Natl Acad Sci 108:6893–6898

R Core Development Team (2013) R: a language and environment for statistical computing. Vienna

Raffaele S, Mongrand S, Gamas P, Niebel A, Ott T (2007) Genome-wide annotation of remorins, a plant-specific protein family: evolutionary and functional perspectives. Plant Physiol 145:593–600

Raffaele S, Bayer E, Lafarge D, Cluzet S, German Retana S et al (2009) Remorin, a solanaceae protein resident in membrane rafts and plasmodesmata, impairs potato virus X movement. Plant Cell 21:1541–1555

Raymundo AD (1981) Measuring the relationship between northern corn leaf blight and yield losses. Plant Dis 65:325

Roper MC (2011) *Pantoea stewartii* subsp. *stewartii*: lessons learned from a xylem-dwelling pathogen of sweet corn. Mol Plant Pathol 12:628–637

Schaefer CM, Bernardo R (2013) Genomewide association mapping of flowering time, kernel composition, and disease resistance in historical Minnesota maize inbreds. Crop Sci 53:2518–2529

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112–1115

Settles AM, Latshaw S, McCarty DR (2004) Molecular analysis of high-copy insertion sites in maize. Nucl Acids Res 32:e54

St Clair DA (2010) Quantitative disease resistance and quantitative resistance loci in breeding. Annu Rev Phytopathol 48:247–268

Suparyono, Pataky JK (1989) Influence of host resistance and growth stage at the time of inouclaiton on Stewart's wilt and Goss's wilt development and sweet corn hybrid yield. Plant Dis 73:339–345

Szalma SJ, Hostert BM, Ledeaux JR, Stuber CW, Holland JB (2007) QTL mapping with near-isogenic lines in maize. Theor Appl Genet 114:1211–1228

Todesco M, Balasubramanian S, Hu TT, Traw MB, Horton M et al (2010) Natural allelic variation underlying a major fitness tradeoff in *Arabidopsis thaliana*. Nature 465:632–636

Tóth K, Stratil TF, Madsen EB, Ye J, Popp C et al (2012) Functional domain analysis of the remorin protein LjSYMREM1 in *Lotus japonicus*. PLoS One 7(1):e30817

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC et al (2012) Primer3—new capabilities and interfaces. Nucl Acids Res 40:e115

Van Inghelandt D, Melchinger AE, Martinant JP, Stich B (2012) Genome-wide association mapping of flowering time and northern corn leaf blight (*Setosphaeria turcica*) resistance in a vast commercial maize germplasm set. BMC Plant Biol 12:56

Wallace JG, Larson SJ, Buckler ES (2014a) Association mapping across numerous traits reveals patterns of functional variation in maize. PLoS Genet 10:e1004845

Wallace JG, Bradbury PJ, Zhang N, Gibon Y, Stitt M et al (2014b) Entering the second century of maize quantitative genetics. Heredity 112:30–38

Wilcoxson R, Atif A, Skovmand B (1974) Slow rusting of wheat varieties in the field correlated with stem rust (*Puccinia graminis tritici*) severity on detached leaves in the greenhouse. Plant Dis Rep 58:1085–1087

Wisser RJ, Sun Q, Hulbert SH, Kresovich S, Nelson RJ (2005) Identification and characterization of regions of the rice genome associated with broad-spectrum, quantitative disease resistance. Genetics 169(4):2277–2293

Wisser RJ, Balint-Kurti PJ, Nelson RJ (2006) The genetic architecture of disease resistance in maize: a synthesis of published studies. Phytopathology 96:120–129

Wisser RJ, Kolkman JM, Patzoldt ME, Holland JB, Yu J et al (2011) Multivariate analysis of maize disease resistances suggests a pleiotropic genetic basis and implicates a *GST* gene. Proc Natl Acad Sci 108:7339–7344

Yu JM, Pressoir G, Briggs WH, Bi IV, Yamasaki M et al (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203–208